SICNA

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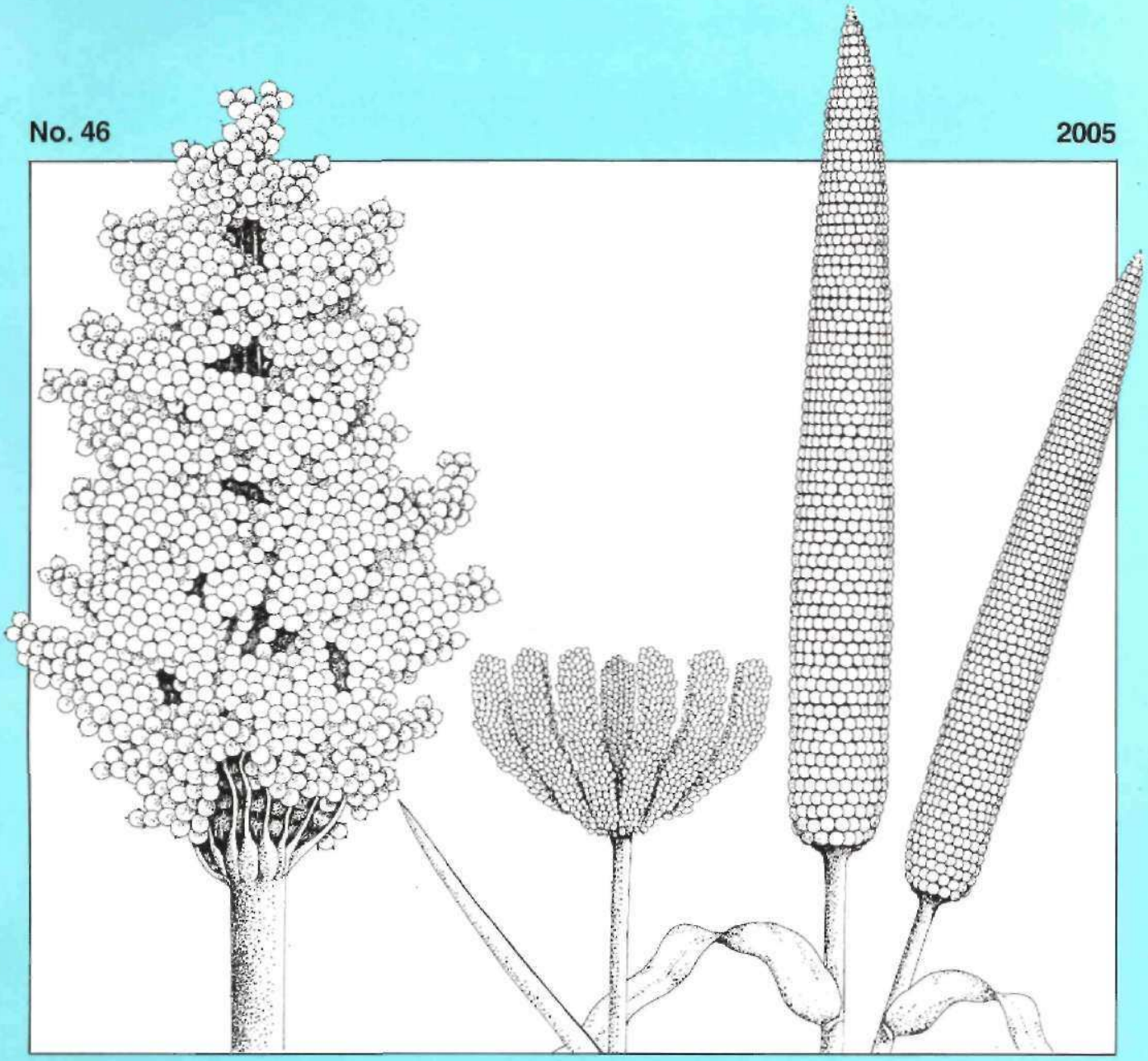

International Sorghum and Millets Newsletter

Co-publishers

SICNA
Sorghum Improvement Conference of North America
(www.sorghumgrowers.com)

ICRISAT
International Crops Research Institute for the Semi-Arid Tropics
(www.icrisat.org)

About SICNA

In 1947, sorghum breeders formed an informal working group to meet and review items of interest in sorghum breeding and genetics. This organization was named 'Sorghum Research Committee'. In the 1960s, with the advent of a number of severe disease and insect problems, special half-day sessions, particularly on diseases, became a part of the Sorghum Research Committee. In 1973, a concept was put forward that all sorghum workers, irrespective of discipline and employer, should meet twice a year to discuss mutual concerns with sorghum research and development. The Sorghum Improvement Conference of North America (SICNA) was that new organization. It is composed of eight disciplinary committees, dealing with genetics and breeding, pathology, entomology, chemistry and nutrition, physiology and agronomy, biotechnology, utilization and marketing, and agribusiness and commerce. SICNA meets formally once a year in conjuction with the National Grain Sorghum Producers Board. A general program of research, education, and developmental activities is prepared by the disciplinary committees. Funding is through membership participation and contributions from commercial donors. Essentially, SICNA represents the United States sorghum activities but accepts reports and encourages memberships from sorghum and millet researchers worldwide.

About ICRISAT

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) is a nonprofit, non-political organization that does innovative agricultural research and capacity building for sustainable development with a wide array of partners across the globe. ICRISAT's mission is to help empower 600 million poor people to overcome hunger, poverty and a degraded environment in the dry tropics through better agriculture. ICRISAT belongs to the Alliance of Future Harvest Centers of the Consultative Group on International Agricultural Research (CGIAR).

ISMN Scientific Editors 2005

J A Dahlberg SICNA, USA

R P Thakur ICRISAT, India

Contents

Agronomy/Physiology

Pathology

Entomology

Biotechnology

Utilization

Socioeconomics

Millets Research

Genetic Enhancement and Breeding

Agronomy/Physiology

Pathology

Entomology

Biotechnology

Selected Bibliography

Editorial

Dear Reader

This issue of International Sorghum and Millets Newsletter (ISMN) encompasses subject areas ranging from genetics and plant breeding, agronomy/physiology, pathology, entomology, biotechnology, utilization, and socioeconomics of sorghum and pearl millet. You will find some useful information of your interest in these articles.

The feedback survey conducted last year on your impression and interest in ISMN, its quality, and publication format provided some interesting results. About 20% of the 1500 recipients responded to the survey questionnaire. Of these respondents, 64% rated ISMN as excellent; 66% favored changing the name from Newsletter to Journal; 62% wanted to receive a printed copy; 46% agreed to pay subscription, while 40% did not favor payment; and 75% favored the idea of converting it to E-Journal of SAT Agriculture. SICNA and ICRISAT are considering these results for implementation in the future.

Several events have taken place during the year that will have tremendous effect on sorghum globally. First of all, there has been continued consolidation of the US private seed industry and today there are fewer private companies working on sorghum. This has serious implications for research on sorghum that is one of the most valuable crops for farmers having limited water availability and who plant on marginal lands. Consequently, research funding from both the public sector and federal agencies will become more important to sorghum in the future. Efforts are being made to emphasize the importance of sorghum, both in the US and internationally, to agencies that fund crop research and hopefully funding for sorghum research will continue to grow, even as the commitment from the private industry declines. Unfortunately, these realities are true for millets also.

Secondly, a recent workshop in Africa has highlighted the risks of both aflatoxin and mycotoxins in the food chain. Maize, which has been widely pushed in Africa as a crop of the future has some very serious issues with these mycotoxin producing pathogens and this is again an opportunity to promote the benefits of sorghum and pearl millet in areas that were traditionally sorghum and millet regions. We have the opportunity to again find these "lost crops of Africa."

Thirdly, some really exciting news came out this year. Sorghum will become the second cereal crop to be sequenced. The Joint Genome Institute, within the US Department of Energy, has announced that it will work on sequencing the genome of sorghum. This has real implications in future genetic and genomics research and should renew some interest within the private industry to fund research on sorghum.

Lastly, discussions have begun between SICNA and ICRISAT on the feedback survey results and about the costs of the newsletter. The costs of the newsletter have increased considerably from the past years and it is getting more expensive to publish a hardcopy of the newsletter. Our thoughts have moved to publishing the newsletter on a CD and distributing the CD to the recipients. This would allow us to reduce cost and not detract from the newsletter.

We would like to thank the reviewers for their time and efforts for their critical comments in a timely manner to facilitate the publication of this volume. They include: Belum VS Reddy, AG Bhasker Raj, S Chandra, PM Gaur, AG Girish, L Krishnamurthy, VN Kulkarni, JVDK Kumar Rao, P Lavakumar, S Pande, P Parthasarathy Rao, S Ramesh, VP Rao, SMH Rizvi, HC Sharma, HD Upadhyaya and V Vadez (all ICRISAT, Patancheru, India); S Indira, SS Rao and N Seetharama, (National Research Centre for Sorghum (NRCS), Hyderabad, India); K Mathur (Rajasthan College of Agriculture, MPUA&T, Udaipur, India) and Jurg Blumenthal, Yinghua Huang, Scott Bean, Tom Isakiet, Cleve Franks, Bonnie Pendleton and Rich Kochenower [Sorghum Improvement Conference of North America (SICNA), Texas, USA].

We also like to thank B Shubha Rao and G Ashwathama, Technical Editors for their efforts; to VS Reddy, Senior Newsletter Officer, Communication Office, ICRISAT for his efficient coordination in handling, processing and typesetting the manuscripts; and to ICRISAT library for timely compilation of the publication lists of sorghum and millets for inclusion in this volume.

Authors are requested to read carefully the "Information for ISMN Contributors" on the inside back cover of this volume and follow these guidelines while preparing the manuscripts. **The due date for submission of manuscript for ISMN 47 is 15 August 2006.**

Enclosed is a mailing update form for you to complete it and return to us either electronically or by post in time.

We look forward to your input and expect to see a continued growth in submission to the newsletter in the future. Wishing you a joyful festive season and prosperous New Year.

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News

Sorghum - the Second Cereal Crop to be Sequenced

LUBBOCK, TEXAS - The National Sorghum Producers (NSP) announced that sorghum will be the second cereal crop genome to be sequenced. Citing information from the Department of Energy Joint Genome Institute (JGI) Computation Genomics Program Head, Dr Daniel Rokhsar, sorghum has been targeted for sequencing in 2006. The JGI was instrumental in sequencing the human genome.

According to NSP Research Director Dr Jeff Dahlberg, the project will engage an international consortium led by Dr Andrew Paterson from the University of Georgia. Dahlberg said the project is a logical outgrowth of long-term research efforts that have been supported by NSP to enhance the knowledge of the hereditary information of the sorghum plant. In the past, genomics research has been funded by sources including the National Science Foundation Plant Genome Research Program, the United States Department of Agriculture National Research Initiative, and the International Consortium for Sugarcane Biotechnology.

"This is as important as the advent of sorghum hybrids 50 years ago," said Dahlberg. "Sequencing sorghum is a critical a step in building our knowledge base on how plants function and, like the use of hybrids, will allow us to make significant advancements in crop improvement for the next 50 years. This project will be valuable as we move from fundamental studies of genome organization and gene discovery to applied efforts in sorghum."

Rice was the first cereal grain to be sequenced and Dahlberg said that sorghum is the most logical choice for the next sequencing project because the crops are so complementary. "Sorghum is an important bridge to closely-related large-genome crops in its own tribe such as maize and sugarcane. Analysis of the levels and patterns of genomic diversity within and between sorghum, sugarcane, rice, and maize promises to advance our understanding of the biology and evolution of Poaceae grain and biomass crops, and create new opportunities for their improvement. Sorghum is one of the world's leading grain crops, and is an important model for tropical grasses worldwide."

NSP represents US sorghum producers nationwide. Headquartered in Lubbock, Texas, in the heart of the US Sorghum Belt that stretches from the Rockies to the Mississippi River and from South Texas to South Dakota, the organization works to ensure the profitability of sorghum production through market development, research, education and legislative representation.

Consultation Meeting on Hybrid Parents Research

ICRISAT-Patancheru organized a one-day consultation meeting on *Hybrid parents research in sorghum, pearl millet and pigeonpea at ICRISAT* on 30 August 2005. The partners of the Hybrid Parents Research Consortia decided to strengthen their continuing efforts to improve productivity, seed quality and disease resistance in sorghum, pearl millet and pigeonpea. According to Director General William Dar, the Consortia is guided by the vision of improving the well-being of the poor of the semi-arid tropics through partnership-based agricultural research for impact.

ICRISAT's model of the Consortia is unique and the first of its kind among the CGIAR Centers. It was launched in 2000, when the Consortia for sorghum and pearl millet were formed. ICRISAT's hybrid parents have led to the development of 50 hybrids in sorghum and 60 in pearl millet. ICRISAT scientists made presentations on the status and future prospects of sorghum (Belum VS Reddy) and pearl millet (KN Rai) hybrids parents' research at ICRISAT.

Promoting Improved Pearl Millet in Niger

A pearl millet improvement team of ICRISAT-Niamey participated in a seed fair organized by the Catholic Relief Service on 26 June 2005 at Famale (near Ayorou, 170 km from Niamey).

A range of improved ICRISAT cultivars adapted to the target region were presented to around 400 farmers who participated in the fair. A total of 270 packets (small 50 gm packets) were sold, the majority containing seed of the early-flowering cultivars GB 8735, SOSAT-C88, and ICMV IS 99001.

Project Launched to Increase the Use of Sorghum and Pearl Millet Grain for Poultry Feed

ICRISAT, along with its partners from various R&D sectors, has launched a collaborative project in May 2005 to enhance the utilization of sorghum and pearl millet in the poultry feed industry in India, China and Thailand. Global production of sorghum and pearl millet has been

declining for the last two decades. Within Asia India, China and Thailand are the major producers of sorghum and pearl millet. The market demand for food uses of sorghum and pearl millet grain has declined with growth in incomes and subsequent changes in consumer preferences.

The demand for poultry feed is increasing due to fast growth (by 15-20%) of poultry sector, while the growth rate in maize, the usual energy source in the poultry feed, is limited to only 2-4% annually. ICRISAT, along with its partners from various R&D sectors, launched a project funded by the Common Fund for Commodities (CFC), the Netherlands, in partnership with the Food and Agriculture Organization of United Nations. The three-year project, which commenced on 1 May, 2005 has a total funding of $2.1 million. The project with the following objectives brings together partners from the agricultural research institutes, universities, NGOs, poultry feed manufacturers, poultry growers, and farmers groups from three countries.

- Mobilize groups of small-scale farmers in order to improve crop productivity and enhance skills in harvesting, bulking, storage and handling practices of grain
- Provide the information on the improved production packages and seeds of improved cultivars by involving private seed companies
- Provide other inputs such as credits, fertilizer, etc., by organizing farmers in to groups for effective input delivery mechanisms
- Link farmer groups with poultry feed manufacturing companies and poultry producers so as to enable the farmers to sell the grain to feed manufacturers

The project will be operational in Andhra Pradesh and Maharashtra, India; in three counties Beizen, Heishan, and Yi of Liaoning province in China; and in Suphan Buri, Kanchana Buri, and Nakon Sawan provinces in Thailand.

ICRISAT Sorghum Scientist Felicitated

The Federation of Farmers Association of Andhra Pradesh (FFA-AP) felicitated Belum VS Reddy (sorghum breeder) as *Outstanding Scientist* in recognition of his significant contribution to improving sorghum productivity in India. Mr. Arnold Prazer, Counsellor in the Dutch Embassy honored him with a Certificate of Merit and shawl.

ICRISAT Publication Wins Award

During the 57th Annual meeting of the Indian Phytopathological Society held at Marathwada Agricultural University, Parbhani, from 12 to 14 January 2005, an ICRISAT paper was selected for the " MJ Narasimhan Medal Award" for the best research paper published during 2003. The paper titled *Pathogenic and genetic diversity among Indian isolates of Scelerospora gramnicola from pearl millet,* authored by S Sivaramakrishnan, RP Thakur, Seetha Kannan and VP Rao, was published in Indian Phytopathology 56(4): 392-397.

The Doreen Mashler Award Goes to Sorghum Research

The 2004 Doreen Mashler Award was given jointly to Dr Belum VS Reddy of ICRISAT, Dr N Seetharama of the National Research Centre on Sorghum, Dr P Sateesh Kumar of Prabhat Agri-Biotech and Dr ST Borikar of the Marathwada Agricultural University.

Obituary

Dr Leland R House passed away at his home at Bakersville, NC, USA, on 25 January 2005. He was 75 years old. His wife, Fadia, and three children (Ralph, Alan and Paul) survive him. Dr Leland House, fondly called by his friends and colleagues as "Lee" was a great human being and a world renowned plant breeder.

Dr Lee House joined the Rockefeller Foundation in India and contributed immensely to provide direction to the sorghum improvement research in India. He trekked the present ICRISAT site several times prior to the visit of the Feasibility Study Team, which led to **the** establishment of ICRISAT in 1972 at Patancheru. Later, he joined ICRISAT in November 1976 and led the global sorghum program for over 12 years with distinction.

He revolutionized ICRISAT's and the India's sorghum program by initiating research on hybrid parents development and releasing hybrids through networking. As a result of his efforts, sorghum hybrid Hageen Durra was released for the first time in 1983 in Sudan for general cultivation. He was instrumental in training a large number of breeders in sorghum, who are now leading sorghum programs in various countries—both in public and private sectors—including USA.

Genetic Enhancement and Breeding

Ten Myths about Tannins in Sorghums

L Rooney (Department of Soil & Crop Science, Texas A&M University, College Station, TX 77843-2474, USA) Corresponding author: lrooney@tamu.edu

Myth #1: Tannins are present in all sorghums

Fact: 99% or more of all sorghums in the USA do not contain tannins. Tannins are present in sorghums with a pigmented testa layer (Fig. 1). The presence of the testa layer is controlled by B_1 _B_2_ genes. When B_1_$B2$_ is dominant, a pigmented testa is present. Sorghums without a pigmented testa do not contain tannins.

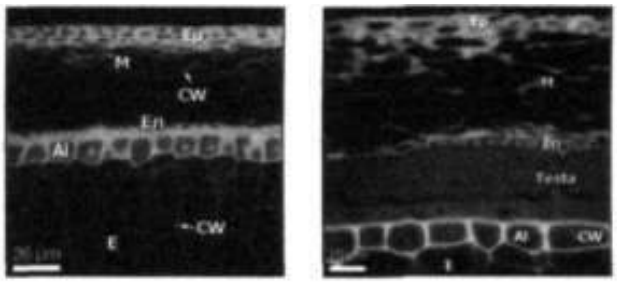

Figure 1. Fluorescence photomicrograph of cross-sections of a non-tannin (left) and a tannin sorghum kernel (right, adapted from Earp et al. 2004).

Myth #2: Tannin sorghums are toxic

Fact: Tannin sorghums have erroneously been reported to contain tannic acid. Tannin sorghums have condensed tannins, which are not toxic. Many foods such as grapes, blueberries, cranberries, dark chocolate, and carobs have condensed tannins. These foodstuffs are consumed without any adverse effects and are now considered as health foods because of the antioxidant properties of the tannins. Tannin sorghums are consumed as human food extensively in Africa and Asia without problems.

Myth #3: Birds and animals will not eat tannin sorghums

Fact: In a field with white, red, and tannin sorghums, birds will first eat white sorghum and then red sorghums before eating the tannin sorghums. Birds and animals consume tannin sorghums but prefer other sorghums when given a choice.

Myth #4: Tannins are measured by total phenol analysis

Fact: The total phenol analysis measures phenolic acids, ondensed tannins, and tyrosine. All plants contain phenolic compounds.

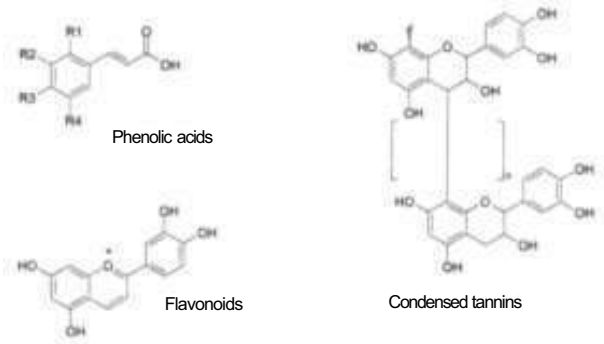

Figure 2. Structures of phenolic compounds.

Myth #5: Tannin sorghums prevent the digestion of nutrients

Fact: Tannins will decrease efficiency of growth in poultry and livestock; however, the amount depends on the animal species, processing the grain before feeding and the diet fed. In general, animals consume more feed to produce about the same or slightly less weight gains. In general, the feed efficiency is reduced by 5 to 10%.

Tannin sorghums do slow and reduce the digestibility of nutrients especially proteins. However, Elkin et al. (1996) demonstrated that sorghums containing equivalent amounts of tannins have different digestibilities. This suggests that tannins are only partially responsible for lower protein digestibility.

Myth #6: It is difficult to test for tannins

Fact: The chlorox bleach test is a good tool to identify sorghum with tannins. For tannin sorghums, bleaching dissolves the pericarp and turns the pigmented testa of tannin types black; non-tannin sorghums do not turn black (Fig. 3). However, the bleach test can yield false-positives on samples that have been molded and weathered. Care must be used when evaluating the bleached samples since some nontannin kernels might have some dark spots (Dykes et al. 2002, Taylor 2001, Waniska et al. 1992).

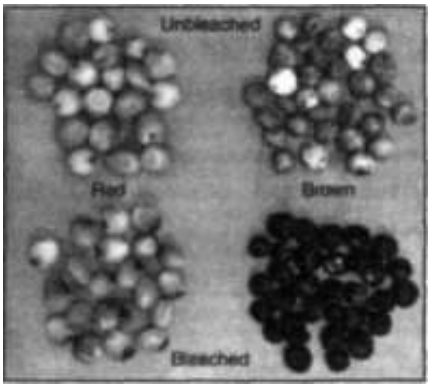

Figure 3. Chlorox bleach test of non-tannin and tannin sorghums.

Colorimetric methods have been used for many years to measure sorghum tannins. These include the Vanillin/ HCl assay and the HCl/Butanol assay. These methods are quick and economical to perform and give an estimate of tannin content.

Normal-phase HPLC analysis with fluorescence detection efficiently separates tannins according to their degree of polymerization (Gu et al. 2002, Awika et al. 2003). This research will provide significant new information on sorghum tannins.

Myth #6: All red sorghums have tannins

Fact: Grain color is not a reliable indicator of tannins in sorghum (Fig. 4). Only sorghums with a pigmented testa layer contain tannins. The presence of tannins in sorghums is controlled by the $B_1\_B_2\_$ gene. Sorghums with a white, red, or yellow pericarp may or may not have tannins. The grain in Fig 4 with a testa has condensed tannins and cannot be distinguished from the ones without pigmented testa.

Figure 4. Variation in appearance of sorghum tannins. (Adapted from Rooney and Miller 1982).

Myth #7: Tannic acid is present in tannin sorghums

Fact: Tannic acid has never been found in sorghum even though tannic acid has been used as a reference (standard) in some of the analyses. Only condensed tannins are present in tannin sorghums. Early experiments used tannic acid in feeding trials to evaluate the effect of tannins on feeding value. This information was prior to our current understanding that sorghum does not contain tannic acid.

Myth #8: Sorghum tannins are unhealthy for humans and animals

Fact: Tannin sorghums are an outstanding source of antioxidants (Table 1) that can be used in a wide variety of applications including preservation of ground meat (Jeschke 2004). Recent evidence strongly indicagtes that tannins are of benefit to human health. Tannins are known to bind to proteins making them indigestible since some animal studies have shown that they are excreted in the feces intact. However, in vitro data indicate that the microflora in the colon can degrade polymeric tannins into low molecular phenolic acids which could be absorbed through the colon. Tannins are nontoxic and may slow digestibility in humans, which is an advantage to type II diabetics.

Myth #9: Tannin sorghums make unacceptable food products

Fact: Many acceptable products, such as porridges and alcoholic beverages, have been developed from tannin

Table 1. Antioxidant activity (ORAC) levels of tannin sorghum brans compared to common fruits. (Adapted from Awika 2003).

Commodity	ORAC (dry wt.)
Tannin sorghum bran	2400-3100
Blueberries	87-870
Strawberries	356-400
Plums	452-600
Grapes	100
Watermelon	15
Orange	80-150

sorghums in Africa (Awika and Rooney 2004). Good-quality breads containing tannin sorghum bran have high antioxidant and dietary fiber levels with a natural dark brown color and excellent whole grain flavor (Gordon 2001). In addition, healthy bread mixes containing tannin sorghum bran, barley flour, and flaxseed have been developed (Rudiger 2003). Tannin sorghums are often preferred for production of sorghum beers and alcoholic beverages because of their dark color (Rooney and Awika 2004). The tannins affect malt enzyme activity but brewers avoid problems by using alkaline treatments during malting.

Myth #10: There are NO uses for tannin sorghum

Fact: Tannin sorghums have been used in the production of good-quality breads, malt, beer, and distilled beverages (Maltai). Tannin sorghum brans have higher antioxidant activity in vitro than fruits (Table 1). Sorghum tannins can be used as antioxidants in meat systems (Jeschke 2004) and they may retard oxidative damage due to high-energy irradiation (McDonough et al. 2004).

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Forging Research and Development Partnerships with Private Sector at ICRISAT-Sorghum as a Case Study

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ICRISAT - its mission and strategy

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), established in 1972 with its headquarters at Patancheru, Andhra Pradesh, India, is a non-profit, non-political, international research organization for science-based agricultural development. It belongs to the Alliance of Future Harvest Centers of the Consultative Group on International Agricultural Research (CGIAR). ICRISAT conducts research on its mandate crops - sorghum, pearl millet, chickpea, pigeonpea and groundnut - which support the livelihoods of the poorest of the poor in the Semi-Arid Tropics (SAT). The mission of ICRISAT is to help empower 600 million poor people to overcome hunger, poverty and a degraded environment in the dry tropics through better agriculture. ICRISAT pursues an integrated genetic and natural resource management (IGNRM) strategy to improve the livelihoods of the poor in the semi-arid crop-livestock-tree production systems. The strategic focus in the SAT is to attain impact while maintaining a global level of scientific excellence in agricultural research.

Public-private partnership research - the way forward

Private sector (PS) investment in agribusiness - to provide quality seed, food, and feed and enhanced livelihoods- has increased recently in developing countries. This is in response to the market-friendly government policies in India and many other developing countries. It is now widely recognized that, in the next decade, international efforts to apply science to the problems of the world's poorest people will be characterized by the joint efforts of both public and the PS by exploiting complementarities and synergies between them (Dar 2001). Research-for-development institutes, therefore, need to forge partnerships with the PS to complement research efforts to transfer their technologies as well as to elicit research-funding support and feedback on the adoption and impact of the technologies.

Public-private partnership at ICRISAT

ICRISAT has taken a proactive approach to develop partnerships with PS seed companies to jointly deal with the main constraints to agribusiness development through the identification of priorities and joint investments in key research areas. The partnerships/arrangements are developed considering the synergies and complementary expertise between ICRISAT and the PS. In this paper, we describe briefly the objectives, nature and mode of ICRISAT's partnership arrangements with the PS in the areas of hybrid parents and sweet sorghum improvement research and development, and discuss the expected impacts of these partnerships on the livelihoods of farmers and consumers.

A. Hybrid parents development

ICRISAT's research in the development of sorghum hybrid parents has contributed several parental lines that are international public goods (IPGs) and freely accessible to both public and PS research organizations. The business-oriented PS organizations, and the farmers they serve, have derived immense economic benefits from ICRISAT-generated research products (breeding materials, hybrid parental lines and research information) in sorghum. Over the years, the PS in India has emerged as a major channel for delivering ICRISAT's seed-based technologies to poor farmers in India, and other SAT of the world.

Objectives

The primary objective of the ICRISAT-PS partnership has been to enhance the pace of impact of ICRISAT-developed research products where both parties play complementary roles in the areas of their expertise to generate the synergy for more effective research and development. Thus, ICRISAT concentrates on strategic research emphasizing germplasm evaluation and its genetic improvement, including the development of parental lines of potential hybrids; while the private sector emphasizes the development and testing of hybrids, seed production and marketing of hybrids, continued assessment of farmers and consumer preferences, and changing market demands. In recent years, one more dimension of this partnership has emerged that relates to resource mobilization from the PS to provide partial funding support to ICRISAT's hybrid parents' research.

Evolution, nature and mode of partnerships

The partnership between ICRISAT and PS seed companies has evolved over time. In the early years, ICRISAT played a nurturing role to the fledgling PS seed industry and provided the breeding material, often through informal networks. However, ICRISAT scientists realized the significant research and developmental capabilities of the PS, particularly in the larger companies and soon recognized that the Institute's traditional relationship with public sector breeding programs, though important, was no longer the sole route to farm-level adoption of the hybrids developed based on ICRISAT-bred research products. This realization was all the more pertinent following the succession of funding shocks in ICRISAT and other CGIAR centers accompanied by increased scrutiny of the value and impact of international agricultural research efforts (Reddy et al. 2001).

All these considerations led to conceptualization and initiation of Sorghum and Pearl Millet Hybrid Parents Research Consortia during 2000 at ICRISAT, the first of its kind in the entire CGIAR system (Reddy et al. 2001). This partnership envisaged development of hybrid parents, hybrid seed production and dissemination to the clientele and partial funding support to ICRISAT's hybrid parents research with an explicit understanding that the products from this research will still remain in the public domain and ICRISAT will retain the exclusive rights on its research products. This consortium was later restructured in 2004 with expanded participation of PS companies and higher levels of funding support from each company. In the new structure, the research products are in the public domain with free access by both the public and private sector. The non-member PS companies have access to parental lines of released hybrids, three years later.

Results and impacts

Channelizing research products to end-users. ICRISAT's partnerships with the PS and public sector had significant impact on developing and disseminating large sets of hybrid parents. For example, ICRISAT supplied 93,985 sorghum seed samples of improved hybrid parents to the public and the PS in India as well as other countries between 1986-2000, and the PS received 41% of these samples. After the formation of the consortium, ie, from 2001 to 2004, ICRISAT supplied a total of 25,479 seed samples of improved breeding lines to both public and PS scientists in India, of which the PS share was 56%. Using the ICRISAT-bred materials, seed companies developed and marketed the most promising hybrids and derived immense economic benefits in India (and other countries in Asia).

ICRISAT regularly organizes field days to enable partners to observe and select appropriate breeding materials. Twenty-eight public sector scientists from 16 organizations and 29 PS scientists representing 16 companies in sorghum participated in the Scientists' Field Day organized at ICRISAT, Patancheru, during 2000. A total of 4678 sorghum seed samples belonging to more than 1600 distinct lines were supplied to 28 scientists (15 from public and 13 from PS organizations) based on their selection during the field days. Nearly 55% of these were provided to the PS (Gowda et al. 2003). Similarly, 22 public sector scientists and 16 PS scientists participated and selected several lines in the Sorghum Scientists' Field Days at ICRISAT-Patancheru during 22-23 September 2004. Based on the seed requests received after the field day, a total of 1209 seed samples were supplied to seven scientists in public sector organizations and 535 seed samples to nine scientists of PS seed companies (Table 1). In terms of distinct sorghum hybrid parents, 171 female lines and 339 restorers were supplied to the public sector and 102 female lines and 97 restorers to the PS. Thus, the public sector received 72% and the PS 28% of the hybrid parents supplied. The number of distinct hybrid parents supplied to the public and PS together are 200 female lines and 398 restorers.

Common platform to assess promising hybrids. PS seed companies often develop many promising hybrids, but they are permitted to contribute only 1-2 hybrids to the All India Coordinated Trials for multilocational evaluation. The consortium partners requested ICRISAT to coordinate a multi-locational trial of hybrids from consortium members, thus providing a common platform for the evaluation of promising hybrids.

Table 1. Number of improved distinct lines and sorghum seed samples supplied to public and PS scientists in India upon specific requests after 2000 and 2004 sorghum Scientists' Field Days.

	Scientists		No. of distinct lines selected		No. of seed samples supplied	
Sector	2000	2004	2000	2004	2000	2004
Public	15	07	720	510	2102	1209
Private	13	09	880	199	2576	535
Total	28	16	1600	709	4678	1744

Reduced time lag in developing hybrids. ICRISAT develops hybrid parents, while the PS makes hybrid combinations from selected hybrid parents. Promising hybrids identified from preliminary in-house testing of large number of hybrids are evaluated in ICRISAT-coordinated multi-location trials to identify hybrids suitable for marketing. These complementary roles of ICRISAT and the PS help reduce time required for developing and marketing new hybrids by about three to four years.

Cultivar adoption. The ICRISAT-PS partnership has greatly contributed to the development and marketing of improved hybrids and varieties in Asia. In India, more than four million ha of rainy season sorghum (80% of the total rainy season sorghum area) and one million ha of the summer season sorghum are planted with about 70 PS-based hybrids, of which 54 are based on ICRISAT-derived parental lines or their derivatives. An ICRISAT-PS partnership hybrid, JKSH 22, known for its high grain yield potential, large grain and earliness (5-10 days compared to the most popular hybrid CSH 9) showed remarkable adoption covering 210,000 ha in 2002 (about 0.5% of the total rainy season sorghum area) (Reddy et al. 2004). The adoption of another ICRISAT-PS partnership high yield potential hybrid, VJH 540, increased from 650 ha in 1997 to 1,42,000 ha in 2003 (Figure 1) in rainy season in major sorghum growing areas in India, as evidenced from the increased seed sales of this hybrid from 6.5 t in 1997 to 1420 t in 2003 (personal communication from Dr Yogeshwara Rao, Executive Director, Vikki's Agro-Tech Ltd. Hyderabad).

These are only illustrative examples of the power of partnership to exploit the complementary expertise between ICRISAT and the PS to develop and deliver desired products to the farming community. Apart from these, several other private sector hybrids, such as MLSH 296, GK 4009 and GK 4013, are widely adopted in India. High rate of adoption of ICRISAT-based hybrids is due to large grain, higher grain and fodder productivity. These hybrids have made substantial contributions to enhance cultivar diversity, productivity, yield stability, and also improved the livelihoods of poor farmers in the dry areas (Gowda et al. 2003).

Benefits from seed production. Seed production regimes were developed and started in mid-1960s by the public sector organizations when public sector-bred hybrids were released initially in early 1960s in India. The popularity of PS hybrids, most of which are based on ICRISAT-developed parental lines or their derivatives has further expanded seed production activity in several villages in Andhra Pradesh and Karnataka states of India. It is estimated that on an average, hybrid seed production fetches US$630 ha^{-1}, about three times the income from commercial crop. Between 1994 and 2002, for example, seed production of JKSH 22 (an ICRISAT-PS partnership hybrid) earned farmers, on an average, US$0.3 million

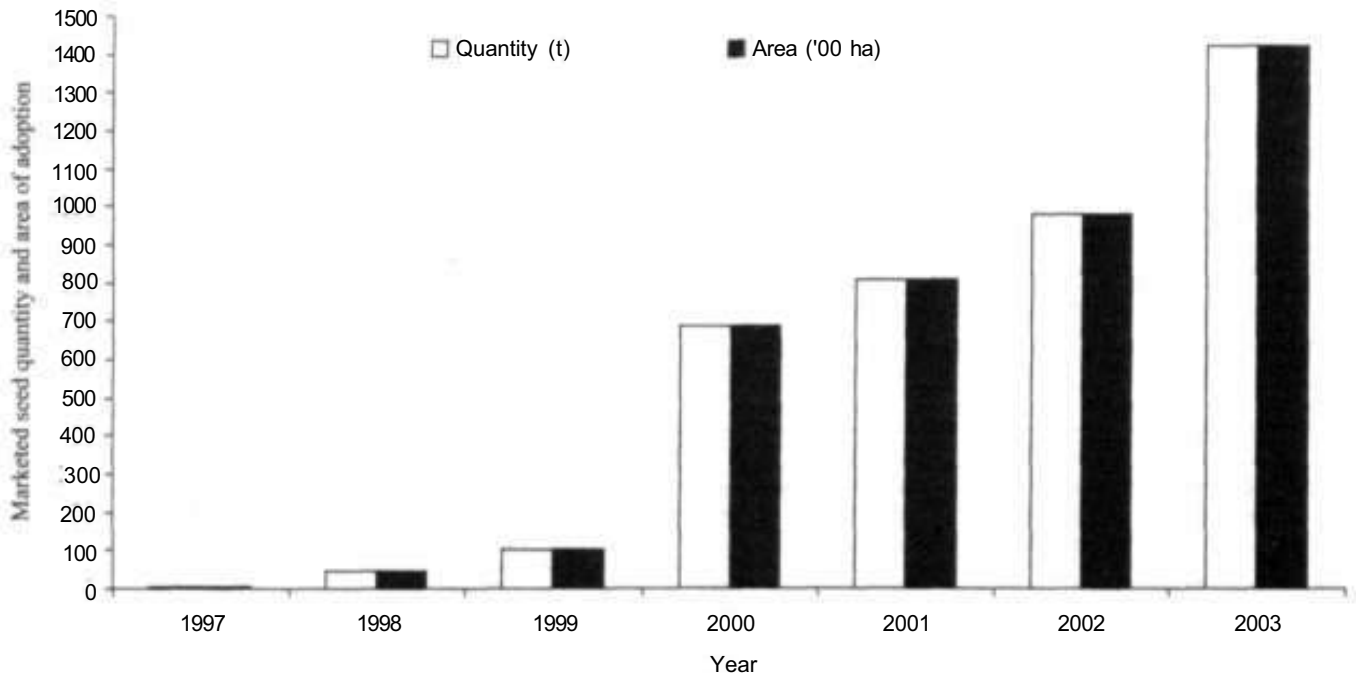

Figure 1. The area of adoption and seed sales of VJH 540, an ICRISAT-PS partnership hybrid in India.

per year in Andhra Pradesh and Karnataka, and US$2.7 million per year from commercial cultivation of JKSH 22 in Maharashtra and other sorghum growing areas in India (Reddy et al. 2004). Several seed villages in Andhra Pradesh and Karnataka became prosperous by taking large scale hybrid seed production (C Ramakrishna, JK Seeds, Hyderabad, personal communication). In the last three years, a total of 29,800t of certified seed of ICRISAT-PS sorghum hybrids was produced contributing about 65% to the total hybrid seed production (Reddy et al. 2004), which gave a total income of US$18.8 million to seed growers in India, and has led to improved livelihoods as a result of higher income accrued from hybrid seed production.

Resource mobilization. During the first phase of the consortium (2000-2003), ICRISAT generated US$0.2 million for sorghum hybrid parents research. As of May 2005, 17 PS seed companies (13 primary and 4 promotional members) have enrolled as members in the revised Sorghum Hybrid Parents Research Consortium for a five-year period. Through this consortium, ICRISAT expects to generate funds of US$0.75 million over a five-year period. The funds generated will augment the core funds to support sorghum improvement research at ICRISAT for developing elite sorghum hybrid parents to serve the public and private sectors. This resource mobilization is particularly significant at the crucial time of diminishing core funding to crop improvement research at ICRISAT.

Feedback on research and cultivar adoption. Scientists' Field Days and meetings provided opportunity to elicit feedback on the utility of ICRISAT-bred hybrid parents, more specifically the number of hybrids developed and marketed, extent of farm-level adoption of hybrids, and constraints for their adoption. Feedback received suggested that the development of both grain and forage type A-/B-lines and diversification of CMS-base of A-/B-lines and molecular-assisted breeding for resistance to drought, grain mold and shoot fly are important. These feedbacks are in agreement with those reported by Umakanth and Seetharama (2003) through an extensive survey seeking scientists from All India Coordinated Sorghum Improvement Project (AICSIP) centers and randomly selected PS seed companies to score on the economic importance of rainy season sorghum production constraints. The feedbacks from PS and the national agricultural research systems (NARS) have helped set priorities (such as farmer or trade or industry preferences) for future global sorghum improvement research at ICRISAT.

B. Sweet sorghums for ethanol production

With the Government of India's policy to blend petrol and diesel with 5% ethanol (and likely to increase this proportion to 10% gradually), the requirement of ethanol in India is around 5000 million L. The current ethanol production from sugarcane molasses and other sources is estimated to be 2000 million L, leaving a deficit of 3000 million L, which can be readily made good by promoting the use of sweet sorghums in ethanol production

Objectives

ICRISAT is following a two-pronged strategy: (1) Development of sweet sorghum cultivars through partnership and (2) facilitation of the ethanol production technology using newly developed sweet sorghum cultivars.

Mode of partnerships

In collaboration with public sector scientists in India, sweet sorghum cultivars will be developed. Through memorandum of agreement and business work plans, the ethanol production technology will be transferred to the private sector distilleries under the Agri-Business Incubator (ABI) at ICRISAT.

Results and impacts

ICRISAT renewed a program for the identification and development of sweet-stalk and high-biomass sorghum hybrid parents and varieties in 2002. Promising lines such as ICSB 631 and ICSB 264 among the seed parents; and Seredo, ICSR 93034. S 35, ICSV 700, ICSV 93046, E 36-1, NTJ 2 and Entry 64 DTN among the varieties/male parents were identified for their high stalk sugar content at ICRISAT, Patancheru. The sugar percentage in these seed parents and varieties ranged from 16.8 to 21.6%. Four of these lines, S 35, ICSV 700, ICSR 93034 and Entry 64 DTN, are being evaluated in AICSIP centers and two of these (ICSV 700 and ICSR 93034) with high stalk-sugar content and juice yield (kL ha^{-1}) have been promoted for advanced testing.

A sweet sorghum hybrid, NSSH 104, developed from ICSA 38 Ian ICRISAT-bred male-sterile (seed) parent] and SSV 84 [a male parent bred in Indian program] is being recommended for release for commercial cultivation. The Indian national sorghum program.

through extensive testing, released a sweet-stalk sorghum variety SSV 84 in 1992/93 for general cultivation. Several promising sweet-stalk hybrids developed at ICRISAT, Patancheru, have been contributed for multi-location testing.

ICRISAT has signed a Memorandum of Agreement (MOA) with Vasanthadada Sugar Institute (VSI), Pune, for identification/development of improved sweet sorghum varieties, characterizing the juice, and ethanol quality and quantity. The ABI has signed another MOA with Rusni Distilleries Private Limited of Hyderabad, to incubate the ethanol production technology using these sweet-stalk sorghum lines.

ICRISAT is hopeful that private seed companies in India would complement the efforts of the national program in the development of location-specific hybrids with sugar-rich high stalk yield (using hybrid parents developed in ICRISAT and the national program) to meet the expected increased demand for raw material for ethanol production in the years to come.

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Prospects of Breeding for Micronutrients and ß-Carotene-Dense Sorghums

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Introduction

Micronutrient malnutrition, primarily the result of diets poor in bio-available vitamins and minerals, causes blindness and anemia (even death) in more than half of the world's population, especially among women and pre-school children (Underwood 2000). Two micronutrients, iron (Fe) and zinc (Zn) and pro-vitamin A (ß-carotene) are recognized by the World Health Organization (WHO) of the United Nations as limiting. Deficiency for Fe, Zn and ß-carotene is highest in South and Southeast Asia and sub-Saharan Africa (SSA). These are also the regions [typified as semi-arid tropics (SAT)] where sorghum (*Sorghum bicolor*) is cultivated and consumed as a staple food by millions of people. The introduction of crop varieties selected and/or bred for increased Fe, Zn and pro-vitamin A contents through plant breeding approach will complement the existing approaches (such as fortified foods and food supplementation while processing) to combat micronutrient deficiency. The plant breeding approach would avoid dependency on behavioral changes in farmers or consumers unlike other programs.

In this paper, we report and discuss the results of pre-breeding research carried out at ICRISAT, Patancheru, as a part of the short-term strategy of HarvestPlus, [the Consultative Group on International Agricultural Research's (CGIAR's) challenge program seeking to reduce micronutrient malnutrition by developing micronutrient-rich crop varieties in high-yielding background] and their implications on the prospects of breeding for micronutrients and ß-carotene-dense sorghums.

Materials and Methods

The material for the study consisted of a set of 84 diverse sorghum lines involving parental lines of popular hybrids, varieties, yellow endosperm lines, germplasm lines, high protein digestible lines, high lysine lines and waxy lines. The lines were evaluated at ICRISAT, Patancheru, during 2003-04 postrainy season following Randomized Complete Block Design (RCBD) with three replications. Each entry was grown in 4 rows of 4 m length with a row-to-row spacing of 0.75 m and 0.1 m between plants within a row. All the recommended production practices were followed to raise a healthy crop with protective irrigation. The randomly selected five plants from the middle two rows of each entry were used for recording data on agronomic traits, such as days to flowering, plant height, grain yield, stover yield; and grain traits, such as grain size (g 100^{-1} seeds) and grain hardness. The grain hardness (breaking strength) was determined as force (in kg) required to break the grain, using Kiya grain hardness tester. The panicles from five selfed plants of each entry from only two replications were hand-threshed and utmost care was exercised to avoid contact of any metal particles with grains while cleaning them. The grain samples were collected in clean cloth bags and sent to National Institute of Nutrition, Hyderabad, India, for estimation of micronutrients (grain Fe and Zn) and ß-carotene contents and anti-nutritional constituents (phytates). The Fe, Zn and phytate contents were estimated using Inductively Coupled Plasma Spectrometry (Houk 1986). The ß-carotene content was estimated spectro-photometrically and was confirmed by High-Performance Liquid Chromatography (HPLC) in selected samples.

Statistical analyses. The computed mean values of data recorded on sample plants in three replications for agronomic traits and mean values of estimates of micronutrients and anti-nutritional contents (phytates) from grain samples collected from two field replications were used for statistical analysis. Analyses of variance were carried out to assess the genetic variability (Steel and Torrie 1980). The phenotypic and genotypic variances were estimated and were standardized as phenotypic coefficient of variability (PCV) and genotypic coefficient of variability (GCV), respectively, to compare the extent of variability for grain Fe, Zn and phytates, which were expressed in different units of measurements. The broad-sense heritability was estimated as the ratio of genotypic variance to phenotypic variance. The correlation coefficients of micronutrients and phytate contents with agronomic and grain traits and among themselves were estimated.

Results and Discussion

Genetic variability. The analysis of variance revealed significant genetic differences for Fe, Zn and phytate contents (Table 1), and for agronomic and grain traits. While the grain Fe content ranged from 20.1 ppm (ICSR 93031) to 37.0 ppm (ICSB 472 and 296 B) with an average of 28 ppm, grain Zn content ranged from 13.4 ppm (JJ 1041) to 31.0 ppm (IS 1199) with an average of 19 ppm (Table 1). Wehmeyer (1969) reported a much larger range of grain Fe (25 to 115 ppm) and Zn contents (15 to 65 ppm) among the 79 sorghum cultivars, which might be partially due to native and managed soil fertility and laboratory protocol used. Nevertheless, it is worth re-testing the lines used by Wehmeyer (1969) to confirm Fe and Zn contents, and subsequently using those having high Fe and Zn contents in breeding programs. Thus, it is evident that substantial genetic variability exists for grain Fe and Zn, and phytate contents and this variation does not appear to be significantly influenced by environment as reflected from narrow differences between PCV and GCV, and high heritabilities (Table 1). The substantial variability coupled with higher heritability offers good prospects of breeding Fe- and Zn-dense sorghum cultivars under low phytate background.

Micronutrients contents and the kind of genetic material. When the grain Fe and Zn contents were compared between different categories of genetic material [maintainer (B-) lines, varieties/restorer (R-) lines and germplasm lines], the mean grain Fe and Zn

Table 1. Estimates of mean, and variability parameters and heritability for grain Fe, Zn, phytates contents in sorghum, 2003 postrainy season, ICRISAT-Patancheru, India.

Micronutrient	F-test	Mean±SE	Range	PCV	GCV	Heritability (%)
Fe (ppm)	**	28.0 ± 0.9	20.1-37.0	0.12	0.11	84.99
Zn (ppm)	**	19.0 + 0.8	13.4-31.0	0.15	0.14	**85.73**
Phytates (mg g^{-1})	**	7.6 ± 0.1	3.8-13.5	0.21	0.20	**99.22**

** - Significant at P=0.01; PCV: Phenotypic coefficient of variability; GCV: Genotypic coefficient of variability

contents in germplasm lines were significantly higher than those in other categories of genetic material (B-lines and varieties/R-lines), although the differences were not large (Table 2). While mean grain Fe content was slightly higher in B-lines compared to that in varieties/R-lines, there were no significant differences in grain Zn contents between B-lines and varieties/R-lines.

A critical examination of grain Fe, Zn and ß-carotene contents in individual lines in different categories of materials (data not shown), revealed encouraging results. Several ICRISAT, Patancheru-bred high-yielding *milo* (A_1) cytoplasm-nuclear male sterility (CMS)-based maintainer (B-) lines such as ICSB 37, ICSB 38, ICSB 39, ICSB 52, ICSB 74 and ICSB 101, shoot fly resistant line ICSB 418, and stem borer resistant line ICSB 472 had grain Fe contents more than 30 ppm. The varieties/ R-lines bred at ICRISAT, Patancheru, such as ICSV 745 (high-yielding, midge-resistant variety released in midge endemic areas in northern Karnataka state in India), PVK 801 (high-yielding, grain mold resistant variety released in Maharashtra state in India), IRAT 204 (high-yielding variety released in Burkina Faso in western Africa), ICSR 89058 (male parent of high-yielding hybrid released in Maharashtra, India) and ICSV 21005 (high-yielding, stay-green restorer line) also had grain Fe contents more than 30 ppm. Among germplasm/landraces, which had more than 30 ppm Fe content, paccha Jonna is a highly popular variety in Andhra Pradesh state in India and IS 7776 is a yellow-endosperm line with high ß-carotene content.

Similarly, the hybrids parents with higher Zn content (more than 20 ppm) include ICSB 472 (also high Fe content) and ICSB 484 among the CMS lines and ICSR 90017 and IRAT 204 (also high Fe content) among the R-lines/varieties. B-lines and R-lines/varieties with higher Fe and Zn contents along with several R-lines such as ICSR 93031, ICSR 91027, ICSR 94489, ICSR 94035 and ICSR 89001, and varieties such as JJ 1041, PVK 801 and ICSV 93046 with lower Fe and Zn contents are good for inheritance studies as well as developing mapping populations for identification of quantitative trait loci (QTL) for high Fe and Zn contents.

Table 2. Category-wise performance of the sorghum lines for mean grain Fe and Zn, and phytates, 2003 postrainy season, ICRISAT-Patancheru, India.

Material	No. of lines	Iron (ppm)	Zinc (ppm)	Phytates (mg g^{-1})
Maintainer (B-)Lines	19	29.5	18.9	7.6
Varieties/restorer (R-) lines	47	26.8	18.1	7.8
Germplasm lines	18	30.0	21.9	7.2
F-test	-	**	**	NS
LSD (5%)	-	0.15	0.18	-

** - Significant at P=0.01; NS = Non-significant.

Table 3. Estimates of correlation coefficients of micronutrients (Fe and Zn) and phytates with agronomic traits in sorghum, 2003 postrainy season, ICRISAT-Patancheru, India.

Grain micronutrient/ agronomic trait	Phytate	Iron	Zinc	Days to 50% flowering	Plant height	Stover yield	Grain yield	Grain size	Grain hardness
Phytate	1.00								
Iron	0.02	1.00							
Zinc	0.12	0.55**	1.00						
Days to 50% flowering	-0.06	0.18	0.12	1.00					
Plant height	-0.28*	-0.02	0.30**	0.18	1.00				
Stover yield	0.02	-0.29**	-0.54**	0.13	-0.19	1.00			
Grain yield	0.04	-0.32**	-0.54**	0.06	-0.22*	0.98**	1.00		
Grain size	-0.16	-0.18	-0.11	-0.15	0.32**	-0.12	-0.22*	1.00	
Grain hardness	0.23*	-0.10	-0.09	-0.14	-0.27*	0.03	0.05	0.02	1.00
Grain lustre	0.28*	0.32**	0.15	-0.18	-0.31	-0.20	-0.18	-0.19	0.26*

N-2 = 82 degrees of freedom

* - Significant at 5% level; ** - Significant at 1% level.

Association between grain Fe and Zn contents. Significant and fairly higher positive correlation (r = 0.55) between grain Fe and Zn contents (Table 3) suggested the possibilities of combining both the micronutrients in single agronomic background. It is interesting to note that seeds rich in Fe and Zn contents show several agronomic advantages such as higher seedling vigor, especially in low-fertile soils, higher levels of resistance to diseases, and empowering plants with higher water-use efficiency, all of which are decisive and critical advantages in SAT (Graham and Welch 1996).

Association of grain Fe and Zn contents with agronomic and grain traits. In order to realize maximum impact of micronutrient-dense cultivars, the micronutrients must be delivered in top-yielding cultivars with farmer-preferred grain quality evident traits, such as pearly white, lustrous and bold grains. Under this premise, correlation of grain Fe and Zn contents with desirable agronomic and grain quality traits were estimated. Though statistically significant (negative) a rather weaker correlation of grain Fe content with grain (-0.32) and stover yields (-0.29) (Table 3) indicated the possibility of breeding for high Fe content in high yielding background, significant negative and relatively strong correlation of grain Zn content with grain (-0.54) and stover yields (-0.54) suggested the need for compromising optimization of Zn content and grain and stover yields. The poor correlation of agronomic traits such as days to 50% flowering and plant height with grain Fe and Zn contents indicated the possibility of developing micronutrient-dense lines in desired maturity and height background, with little compromise in grain and/or stover yields. While grain luster, one of the most important farmer-preferred attributes, had significant positive association with grain Fe content, it had a very weak relationship with grain Zn content. However, other farmer-preferred grain traits such as grain size and grain hardness appeared to have poor correlation with grain Fe and Zn contents. These results suggest that it is possible to deliver high Fe and Zn contents in cultivars with farmer's preferred traits such as early maturity, high yield potential, bold grain and lustrous grain.

Variability for ß-carotene content. The grains of non-yellow endosperm lines had only traces of ß-carotene content. However, in 11 yellow endosperm germplasm lines the grain P-carotene content ranged from 0.56 (IS 24724) to 1.132 ppm (IS 26886) with six lines (IS 7684, IS 7776, IS 24703, IS 24868, IS 24883 and IS 26886) having higher ß-carotene contents than the average of 0.85 ppm. The grain samples analyses of 20 yellow endosperm sorghum germplasm lines by Kapoor and Naik (1970) also revealed similar range of ß-carotene contents (0.2 to 1.4 ppm).

Association of grain ß-carotene content with Fe and Zn contents and grain yield: It appeared that the genes controlling grain Fe and Zn contents are independent of those controlling P-carotene content as indicated by the poor and negative association of P-carotene content with grain Fe (r = -0.24) and Zn (r = -0.31). However, it may be noted that these correlation coefficients are only indicative, as the number of genotypes on which the correlations are estimated are rather fewer for arriving any conclusions on selection scheme. Fairly higher grain Fe and Zn and ß-carotene contents in IS 26886 provide strong evidence to support breeding for all the three vital nutrients. Enriching sorghum cultivars with all the three nutrients - Fe, Zn and ß-carotene - is highly desirable as there are potential synergistic interaction among these for their absorption, transport, and functioning and hence results in increased bioavailability in the human body (Graham and Rosser 2000).

Micronutrients vs. phytates. Sorghum grains contain phytic acid or the phytates, which are recognized as anti-nutritional factors as they form complexes with micronutrients such as Fe, Zn and ß-carotene, thus interfering with their bioavailability. In the present study, the absence of significant differences between improved genetic materials (B- and R-lines) and un-improved germplasm lines for phytates (Table 2) indicated that genetic enhancement for agronomic traits did not result in concomitant variation in phytates contents providing a clue that they are under independent genetic control.

The narrow differences between PCV and GCV for phytates contents, which are amply reflected in high heritability (Table 1), suggest that selection for low levels of phytates contents would be highly effective. The weak correlations of phytates contents with grain Fe (0.02), and Zinc contents (0.12) (Table 3) indicate that it is possible to breed Fe and Zn-dense cultivars with low phytate contents.

Conclusions

Significant genetic variability was evident for grain Fe and Zn contents and anti-nutrients (phytates). While grains of non-yellow endosperm lines had only traces of grain ß-carotene content, those of yellow endosperm germplasm lines had ß-carotene content ranging from 0.56 to 1.13 ppm with six lines having higher ß-carotene contents than the experimental average of 0.85 ppm.

Several trait-based hybrid parents bred at ICRISAT had grain Fe (> 30 ppm) and Zn contents (> 22 ppm), fairly higher than the trial average levels (Fe=28 ppm; Zn=19 ppm). Substantial genetic variability coupled with high heritability and weak association of Fe and Zn contents with ß-carotene and phytate contents suggest that it is possible to breed Fe and Zn and ß-carotene-dense cultivars with low phytate contents. Further, significant and fairly higher positive correlation between grain Fe and Zn contents and their poor correlation with agronomic traits such as days to 50% flowering and plant height and with farmer-preferred grain traits such as grain size and grain hardness indicated the possibility of delivering high Fe and Zn contents in cultivars with farmer's preferred traits such as early maturity, high yield potential, bold grain and lustrous grains.

Considering that the grain sorghum is grown in different soil types with varying levels of native soil fertility with/without farmer's managed fertility in India, it is necessary to examine the stability of micronutrient-dense cultivars across different soil types and soil fertility levels typical to the areas for which these cultivars are targeted.

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Response of Selected Sorghum Lines to Soil Salinity-Stress under Field Conditions

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Introduction

The demand for sorghum to meet the food and non-food requirement of an ever-growing population necessitates sorghum production in marginal and problematic soils such as acidic and saline soils. Soil acidity, and its associated Al^{3+} toxicity and salinity are probably the most important constraints to sorghum productivity in tropical environments. Saline and sodic soils cause mineral stresses on approximately 0.9 billion ha of land in the world (Gourley et al. 1997). There are vast areas in India, Yemen, Saudi Arabia, and Iran with salinity-affected soils. Extension of the cultivation of sorghum to these salinity-affected soils would not only help meet increased demand, but also ensure sustainable and eco-friendly management of such problematic soils. Soil salinity reduces germination and seedling emergence, retards leaf area expansion and ultimately affects partitioning of photosynthates to harvestable economic parts, thus reducing both grain and fodder yield potentials. Salinity tolerance could be empirically defined as the ratio of economic yield (grain/fodder) at a given salinity stress to that under salinity-free conditions. The extent of yield reduction may change with the degree of the salinity-stress. Although sorghum possesses higher salinity-stress tolerance (Igartua et al. 1994) compared to maize, the development of high-yielding salinity-tolerant sorghums is the best option to increase the productivity in such soils. In this paper, we report and discuss the responses of sorghum cultivars (previously selected for tolerance to induced salinity-stress in pot-culture experiments) to salinity-stress under field condition.

Materials and Methods

Forty-two entries were selected based on the biomass production of a diverse set of 100 breeding lines at 39 days after sowing under induced salinity-stress (ECe 23.4 dS m^{-1}) relative to biomass production of the plants in the salinity-free soils in pot-culture experiments at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. These entries included 24 hybrid parents f 15 maintainer (B-) lines, nine

restorer (R-) lines], 16 varieties, one hybrid, and one salinity-stress sensitive check. These 42 entries were evaluated for yield potential using a randomized complete block design with three replications in saline-affected soils (ECe 8.0 dS m^{-1}) at the Agricultural Research Station (ARS), Gangavathi, Karnataka, and in normal (salinity-free) soils at ICRISAT, Patancheru, during the 2004 rainy season as a part of the International Center for Biosaline Agriculture (ICBA), Dubai, and ICRISAT collaborative research project. Each entry was planted in two rows of 2 m length at both the locations. The spacing between rows was 75 cm at ICRISAT-Patancheru, while it was 45 cm at ARS, Gangavathi. The data on days to 50% flowering, plant height, plant agronomic and stay green scores at maturity and grain yield were recorded on five randomly selected plants in each entry at both the locations. Apart from these traits, stover yield was also recorded at ARS, Gangavathi.

Statistical analysis: The computed mean values of the data recorded from sample plants on all the traits were used for statistical analysis. Analyses of variance were carried out to assess the genetic variability as per Steel and Torrie (1980). The phenotypic and genotypic variances were estimated and were standardized as phenotypic coefficient of variability (PCV) and genotypic coefficient of variability (GCV), respectively, to compare the extent of variability for different traits under soil salinity-stress and salinity-free conditions. The broad-sense heritability was estimated as the ratio of genotypic variance to phenotypic variance.

Results and discussion

The results indicated significant genetic variability for days to 50% flowering, plant height and grain yield in salinity-affected (ECe 8 dS m^{-1}) soils at ARS, Gangavathi. The grain yield of the entries ranged from 0.5 t ha^{-1} to 3.9 t ha^{-1} with an average of 2 t ha^{-1}; days to 50% flowering ranged from 70 to 92 with an average of 75 days; plant height ranged from 1.0 to 2.7 m with an average of 1.7 m; plant agronomic score ranged from 2.3 to 4.7 (on a 1-5 scale, where, l=very good and 5=poor) with an average of 3.4; stay-green score ranged from 2.0 to 4.7 with an average of 3.6 (on a 1-5 scale, where, 1= most green and 5=least green) (Table 1). In general, delayed flowering, reduced plant height, poor plant agronomic and stay-green scores and reduced grain yield (by nearly 40%) were some of the responses of the cultivars to soil salinity-stress compared to those in salinity-free conditions at ICRISAT, Patancheru, although the responses varied with the cultivar. For example, while 50% flowering was delayed by 11 days in PSH 1, it was delayed by only one day in S 35 under salinity-stress; similarly, while grain yield of ICSV 112 was reduced by 48%, the reduction was 38% in SPV 1022 under salinity-stress (Table 1). However, these responses cannot be attributed solely because of salinity-stress as other environmental factors inherent in different locations could be confounded with the differences in salinity-stress levels. The evaluation of diverse cultivars in different salinity-stress levels and salinity-free soils in similar environments is necessary to confirm and understand these cultivar-dependent responses to salinity-stress.

Components of variability: The large differences between the estimates of PCV and GCV indicated significant influence of environment on grain yield and other traits under salinity-stress conditions at ARS, Gangavathi, which are amply reflected in lower magnitude of heritability estimates (Table 1). It appears therefore, that progress of genetic enhancement of sorghum for grain yield and other traits under salinity-stress might slow down. The results from the evaluation of diverse set of lines in multi-environments under a broad range of salinity-stress levels would provide reliable and dependable estimates on the extent of variability, heritability and genotype x salinity stress level interaction. These, along with nature of gene action, would be helpful in designing a most effective breeding program for genetic enhancement of sorghum under salinity-stress.

Cultivar performance and selection environment strategy: Some of the high-yielding popular varieties such as ICSV 112 (3.4 t ha^{-1}), S 35 (3.1 t ha^{-1}) and JJ 1041 (2.9 t ha^{-1}) and a hybrid PSH 1 (3.4 t ha^{-1}) (Table 1) bred and released earlier for salinity stress-free conditions performed better for grain yield under salinity-stress conditions at ARS, Gangavathi. These varieties, JJ 1041 (6.9 t ha^{-1}), ICSV 112 (6.5 t ha^{-1}), S 35 (5.6 t ha^{-1}) and a hybrid PSH 1 (5.9 t ha^{-1}) (Table 2) were among the top yielders under salinity-free conditions at ICRISAT, Patancheru, proving again their superior genetic potential. -It is interesting to note that two of these varieties, ICSV 112 and S 35, were among the best performers for fodder yield under salinity-stress soils (ECe 10 dS m^{-1}) in farmers' fields at Oman (Personal communication from Dr John Stenhouse, Plant Genetic Resources Specialist, ICBA, Dubai). Further, the line ICSB 406 (0.9 t ha^{-1}) identified earlier as sensitive to salinity-stress based on pre-anthesis biomass production in pot culture experiments conducted at ICRISAT, Patancheru, (Krishnamurthy et al. 2003) produced significantly lower grain yield. These results indicated that there is a certain degree of corroboration between performance of the entries evaluated under salinity-free and salinity-stress conditions, which is also adequately supported by fairly high correlation coefficient (0.53). However, the correlation is only indicative, as it is based on the results from two locations

Table 1. The mean performance of best sorghum lines and estimates of genetic parameters under salinity-stress (at ARS, Gangavathi, India) and salinity-free soils (at ICRISAT-Patancheru, India), 2004 rainy season.

Sl No.	Genotype	Days to 50% flowering		Plant height (m)		Plant agronomic score[1]		Stay green score[2]		Grain yield (t ha^{-1})	
		S	SF	S	SF	S	SF	S	SF	S	SF
1	GD 65008 Brown	79	71	2.7	3.0	2.7	1.0	3.0	3.0	3.9	6.0
2	PSH 1	78	67	2.0	2.5	3.0	1.3	3.7	3.0	3.4	5.9
3	ICSV 112	78	70	1.5	2.1	2.3	1.0	3.7	2.7	3.4	6.5
4	S 35	71	70	2.0	2.5	3.3	2.0	3.7	3.3	3.1	5.6
5	JJ 1041	76	69	2.1	2.7	2.7	1.3	4.0	3.0	2.9	6.9
6	SPV 1022	78	66	2.1	2.5	3.0	2.7	3.3	3.0	2.9	4.7
8	ICSB 406 **(Sensitive check)**	75	63	2.0	1.0	3.7	2.0	3.7	2.0	0.9	2.9
	F-test	**	**	**	**	**	**	**	**	**	**
	Mean	77	70	1.7	2.1	3.4	1.9	3.6	2.7	2.0	4.1
	Range	70-92	57-83	1.0-2.7	1.0-3.4	2.3-4.7	1.0-3.3	2.0-4.7	1.7-4.3	0.5-3.9	0.6-6.9
	LSD (5%)	4.0	2.4	0.4	0.3	1.4	0.8	1.3	0.8	1.4	1.3
	PCV	4.7	7.3	28.7	27.0	28.6	45.9	25.9	24.8	55.7	42.7
	GCV	3.5	7.1	23.9	25.8	11.5	37.2	14.3	15.3	34.0	38.0
	Heritability (%)	0.55	0.92	0.70	0.91	0.16	0.66	0.31	0.38	0.37	0.79

SF: Salinity-free soils at ICRISAT-Patancheru; S: Salinity-stress soils (ECe 8 dS m^{-1}) at ARS, Gangavathi

1. Agronomic score taken on a 1 to 5 scale where 1=very good and 5=poor
2. Stay green score taken on a 1 to 5 scale where 1=most green and 5=least green

** Significant at P=0.01

Table 2. Category-wise performance of the sorghum lines for grain yield and agronomic traits, under salinity-stress (ECe 8 dS m^{-1}) soils at ARS, Gangavathi, India, 2004 rainy season.

Category of lines	No. of lines in the category	Days to 50% flowering	Plant height (m)	Agronomic score at maturity[1]	Stay green score at maturity[2]	Grain yield (t ha^{-1})	Stover yield (t ha^{-1})
B-lines	15	76	1.4	3.5	3.7	1.88	5.39
R-lines	9	80	1.8	3.3	3.6	1.48	6.94
Varieties	16	78	1.9	3.2	3.5	2.26	8.39
Hybrid[3]	1	78	2.0	3.0	3.7	3.4	10.80
ICSB-406 (Check)	1	75	2.0	3.7	3.7	0.9	2.8
Mean	-	77	1.7	3.4	3.6	2.00	6.90
F-test	-	**	**	NS	NS	**	**
LSD (5%)	-	1.13	0.13	0.41	0.38	0.41	0.93

** Significant at P=0.01; NS: Non-significant;

1. Agronomic score taken on a 1 to 5 scale where 1=best and 5=poor;
2. Stay green score taken on a 1 to 5 scale where 1= most green and 5= least green;
3. Hybrid data was not included for analysis of variance, as there was only one hybrid.

differing in several environmental factors (other than salinity levels) and that the differential cultivar response could be due to the factors other than soil salinity levels. Further, the magnitude and direction of correlation might change with changes in salinity-stress levels. Therefore, it is imperative to evaluate the lines at the same location under salinity-stress and salinity-free conditions to preclude the influence of other edaphic and environmental factors (such as photoperiod, temperature and rainfall distributions) for meaningful comparison of the relative performance of lines under salinity-stress and normal conditions. From plant breeding and agronomic points of view, cultivars performing better under both stress and non-stress conditions are desirable (Reddy 1986; Calhoun et al. 1994). Theoretical investigations (Rosielle and Hamblin 1981) indicating general increase in mean yield in both stress and stress-free environment if selection is practised for mean productivity (average yield in stress and stress-free environments) would lend adequate support to these practical considerations.

Cultivar options: Comparison of the performance of different categories of lines revealed interesting results. While B-lines and R-lines were comparable for grain yield, varieties were significantly superior to hybrid parents (Table 2). The only hybrid with higher grain yield among the entries was the best. Comparative evaluation of large number of hybrids vs. varieties would validate the present results. The superiority of hybrids over varieties under soil salinity-stress environment has been demonstrated previously by Peng et al. (1994), therefore, hybrids should be target cultivars to enhance productivity in saline-affected soils.

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Modeling Male Fertility in Sorghum

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Introduction

Environmental variation can affect pollen viability in sorghum. Temperatures below 13°C during sensitive stages can induce male-sterility (Downes and Marshall 1971; Brooking 1976) and high temperatures appear to have a similar effect (Dhopte 1984). Significant relationships between reduced pollen viability and reduced seed set and increased ergot severity in sorghum have been documented (Ogunlela and Eastin 1984; McLaren 1997). Therefore, it is desirable to develop lines with improved male fertility characteristics to avoid seed losses and ergot epidemics in hybrid seed production fields. The objective of this study was to determine relationships between pollen production, time to shed, and pollen viability in sorghum and various weather variables.

Materials and Methods

Blocks of eight sorghum hybrids including 'Wheatland x TX 2737', 'Wheatland x TX 430', 'SA3042 x TX 2737', TX 2752 x TX 430', 'OK 11 x TX 2737', 'OK 11 x TX 2741', 'Wheatland x TX 2783' and 'Wheatland x TX 2862', were planted every two weeks for five planting dates beginning 8 May 2000 in Manhattan, KS, so that pollen could be collected across a wide range of environmental conditions. At mid-bloom, peduncles were struck and rated for pollen production on a scale of 1 (sparse pollen cloud) to 5 (a dense pollen cloud). Time to pollen shed was recorded in hours after sunrise. Differences in pollen viability were quantified by pollen germination. Pollen grains were germinated on culture medium containing 1% agar, 0.9 M sucrose, 2.43 mM boric acid, and 2.12 mM calcium nitrate for 4 h as described by Tuinstra and Wedel (2000). Pollen germination was evaluated by observation of 200 random pollen grains for each hybrid and was quantified as the percentage of germinated pollen grains. Weather parameters were collected in the plot each day. Triad values (mean of 3 consecutive days) of maximum temperature, minimum temperature, maximum relative humidity, minimum relative humidity, total solar radiation, and precipitation were used in correlation analyses to evaluate the relationship between each male fertility characteristic and weather variables. Multiple regression analyses were conducted to model the relationship between each male fertility characteristic and correlated weather variables.

Results

The male fertility characteristics of sorghum were evaluated on 31 different days during the summer and fall of 2000. Several different weather variables were found to be useful for predicting specific components of male fertility (Table 1). Multiple regression analysis yielded the model $Y = -37.86 + -0.792\ X_1 + 0.865\ X_2 + 0.163\ X_3 + -0.286\ X_4 + 0.371\ X_5$ for pollen viability, where Y = expected pollen viability in a genetically diverse set of sorghum hybrids, X_1 = mean maximum temperature 18-20 days before anthesis, X_2 = mean maximum relative humidity 12-14 days before anthesis, X_3 = mean minimum relative humidity 1-3 days before anthesis, X_4 = mean minimum relative humidity 12-14 days before anthesis, and X_5 = mean total precipitation 15-16 days before anthesis. This model accounted for 65% of the variation in pollen viability ($R^2 = 0.655$). The analysis for time to shed yielded the model $Y = 13.87 + -0.115\ X_1 + -0.0864\ X_2$, where Y = expected time to shed in a genetically diverse set of sorghum hybrids, X_1 = mean maximum temperature 0-2 days before anthesis and X, = mean maximum relative humidity 9-11 days before anthesis. This model accounted for 58% of the variation in time to shed ($R^2 = 0.582$). The analysis for pollen production yielded the model $Y = -5.93 + 0.0964\ X_1 + -0.0465\ X_2 + -0.158\ X_3 + 0.164\ X_4 + 0.0200\ X_5 + 0.066\ X_6 + 0.023\ X_7$, where Y = expected pollen production rating in a genetically diverse set of sorghum hybrids, X_1 = mean maximum temperature 0-2 days before anthesis, X_2 = mean minimum temperature 0-2 days before anthesis, X_3 = mean minimum temperature 5-7 days before anthesis, X_4 = mean minimum temperature 6-8 days before anthesis, X_5 = mean minimum temperature 7-9 days before anthesis, X_6 = mean maximum relative humidity 10-12 days before anthesis and X_7 = mean total precipitation 13-15 days before anthesis. This model accounted for 86% of the variation in pollen production rating scores ($R^2 = 0.863$).

Discussion

Mean maximum temperature, maximum and minimum relative humidity, and precipitation before anthesis were all highly correlated with pollen viability at flowering. Negative correlations between mean maximum temperatures and pollen viability suggest a critical stage for heat stress. High temperatures just before and during microsporogenesis seem to significantly decrease pollen viability. Although precipitation has not been correlated with pollen viability in previous studies, positive correlations between pollen viability and maximum and minimum relative humidity

and precipitation during pollen development suggests that adequate rainfall during this period improves pollen viability at anthesis.

Time to pollen shed appears to be influenced by mean maximum temperature 0-3 days before anthesis. As temperatures increase, time to pollen shed decreases. This is consistent with anecdotal observations by sorghum researchers. The high negative correlation between time to shed and mean maximum relative humidity 9-11 days before anthesis is difficult to explain, especially since no significant correlations were found between time to shed and mean minimum relative humidity and precipitation.

The model for pollen production included all weather variables except mean minimum relative humidity and total daily solar radiation. This suggests that the factors contributing to pollen production are complex. The positive relationship between mean maximum and minimum temperatures and total solar radiation 0-2 days

Table 1. Significant correlations between pollen viability, time to shed, and pollen production; and triad values of maximum and minimum temperature, maximum and minimum relative humidity, solar radiation and precipitation for 8 sorghum hybrids grown at Manhattan, KS, in 2000.[1]

Pollen Viability (% Germination)		Time to Shed (hours)		Pollen Production (1-5)[2]	
Days before anthesis	*r*	Days before anthesis	*r*	Days before anthesis	r
		Mean maximum temperature (°C)			
17-19	-0.39*	0-2	-0.62**	0-2	0.67**
18-20	-0.48**	1-3	-0.50**	1-3	0.55**
19-21	-0.39*				
		Mean minimum temperature (°C)			
0-2	0.40*	0-2	-0.52**	0-2	0.58**
6-8	0.43*	5-7	-0.41*	1-3	0.47**
7-9	0.42*	6-8	-0.46**	5-7	0.46**
		7-9	-0.43*	6-8	0.54**
		7-9	0.53**		
		Mean maximum relative humidity (%)			
9-11	0.37*	2-4	-0.38*	2-4	0.42*
11-13	0.51**	3-5	-0.49**	3-5	0.52**
12-14	0.60**	8-10	-0.53**	9-11	0.61**
		9-11	-0.68**	10-12	0.76**
		11-13	0.58**		
		Mean minimum relative humidity (%)			
0-2	0.45**			9-11	0.46**
1-3	0.46**			10-12	0.47**
2-4	0.38*			11-13	0.49**
11-13	0.41*			12-14	0.40*
12-14	0.49**				
13-15	0.49**				
		Solar Radiation (Langleys)			
3-5	0.39*	0-2	-0.46**	0-2	0.49**
5-7	0.46**	1-3	-0.46**	1-3	0.49**
6-8	0.60**	2-4	-0.40*	2-4	0.44*
7-9	0.48**			3-5	0.40*
9-11	0.42*			6-8	0.42*
10-12	0.45**				
		Precipitation (mm)			
15-17	0.42*			13-15	0.48**
19-21	0.37*				

1. * - Significant at 0.05% level; ** - Significant 0.01% level.
2. On a scale of 1-5. 1 represents light pollen cloud and 5 represents dense pollen cloud.

before anthesis may be related to rapid anther dehiscence. A relationship between mean minimum temperatures 5-9 days before anthesis and pollen production also exists, but the cause of this relationship is not clear. The relationships between pollen production, maximum relative humidity and precipitation suggests that significant rainfall during panicle development increases pollen production.

Conclusions

The models developed in this study may be useful for predicting male fertility characteristics under environmental conditions that commonly occur on the Central Great Plains of the US. Previous studies have evaluated the effects of cool temperature stress, high humidity and rainfall on male fertility; however, only a few studies have evaluated the effects of high temperature and drought stress on these traits. Heat and drought stress are much more common than cool temperature stress in most sorghum-producing environments. These models should provide some guidance for predicting male fertility responses of sorghum under hot dry conditions.

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Timing of Anthesis in the Sorghum Hybrid MR Buster and the Elite Line 31945-2-2

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Introduction

The flowering biology of sorghum [*Sorghum bicolor* (L.) Moench] is an important factor in hybrid seed production. A possible resistance mechanism to sorghum midge [*Stenodiplosis sorghicola* (Coquillett)] consists of resistant genotypes completing their daily flowering before sorghum midge begin ovipositing (Diarisso 1997). Flowering biology is important in interactions with sorghum ergot (caused worldwide by three species of *Claviceps*), because a successfully pollinated spikelet is no longer able to be infected by ergot (Bandyopadhyay et al. 1998). Additionally, both Frederickson et al. (1993) and Ryley (personal communication) have trapped airborne conidia of *C. africana* and found peak conidial release occurred during daylight hours. Flowering outside this period may result in avoidance of peak times of airborne spores.

Preliminary studies were undertaken to determine the nature of flowering in two sorghum genotypes, sorghum midge-resistant MR Buster® (Pacific Seeds Australia Pty Ltd), a sorghum hybrid grown widely in Australia, and 31945-2-2, an elite inbred line used in the sorghum breeding program of the Department of Primary Industries.

Materials and Methods

In January 2001, preliminary experiments were conducted in a controlled environment cabinet (12 h photoperiod; day/night temperature 26°C/21°C; relative humidity 70%) to simulate mid-summer conditions in Australia. Because of space limitations, plants were grown in a glasshouse until the flag-leaf stage, and then moved into the controlled environment cabinet. The timing of the cabinet day-night cycle was set the same as the glasshouse to avoid potentially interfering with the true flowering behavior.

A preliminary investigation was conducted to determine the best method of observing flowering behavior, therefore the results on these nights are not presented. On the other nights, insufficient numbers of spikelets flowered to interpret flowering behavior. The data presented in this paper is a composite of the flowering behavior of each genotype during two nights.

Observations were made on the flowering behavior on individual spikelets, so on the afternoon before each of the nights, several rachis branches were tagged in that part of the panicle that were expected to flower. Spikelets that had previously flowered were removed. The handling of panicles was minimized and the target spikelets were not touched, because any disturbance was found to artificially stimulate flowering.

Observations were taken hourly from 0100h to 0800h. The flowering stage of each spikelet was assessed at hourly intervals, using the following six categories [modified from the 10-stage scale of Ayyangar and Rao (1931)]:

FS1: Glumes begin to open
FS2: Staminal column visible
FS3: Stamens separate
FS4: Anthers tilt down, and then become pendent
FS5: Glumes begin to close
FS6: Glumes completely closed

A number of spikelets at flowering stages 1 through 5 were combined to determine the percentage of spikelets that had commenced flowering but not finished. Flowering stage 6 is presented separately as the percentage of spikelets that had completed flowering.

Both the number of spikelets assessed per panicle and the number of panicles per night varied because of the availability of flowering material and limitations in the number of spikelets that could be assessed at any one time. Not all spikelets on all marked panicles flowered within the period of observation. The total numbers of assessed spikelets were 291 for MR Buster* and 133 for 31945-2-2.

Results and Discussion

MR Buster® started flowering from 0100-0300h (Fig.1). All spikelets that flowered that night opened in this two-hour period, gradually closed during the next five hours. More than 90% of the spikelets had closed by 0600h.

By contrast, the line 31945-2-2 started flowering before 0100h (Fig. 1), and less than 10% had closed by 0800h. Although the end of flowering (stage FS6) was not observed for this line it is possible that these flowers would still be open for some time after ()8()0h.

Analysis of the flowering data using ANOVA (Genstat v6) found highly significant differences between the genotypes for flowering hour, as well as highly significant interactions for both beginning and end of

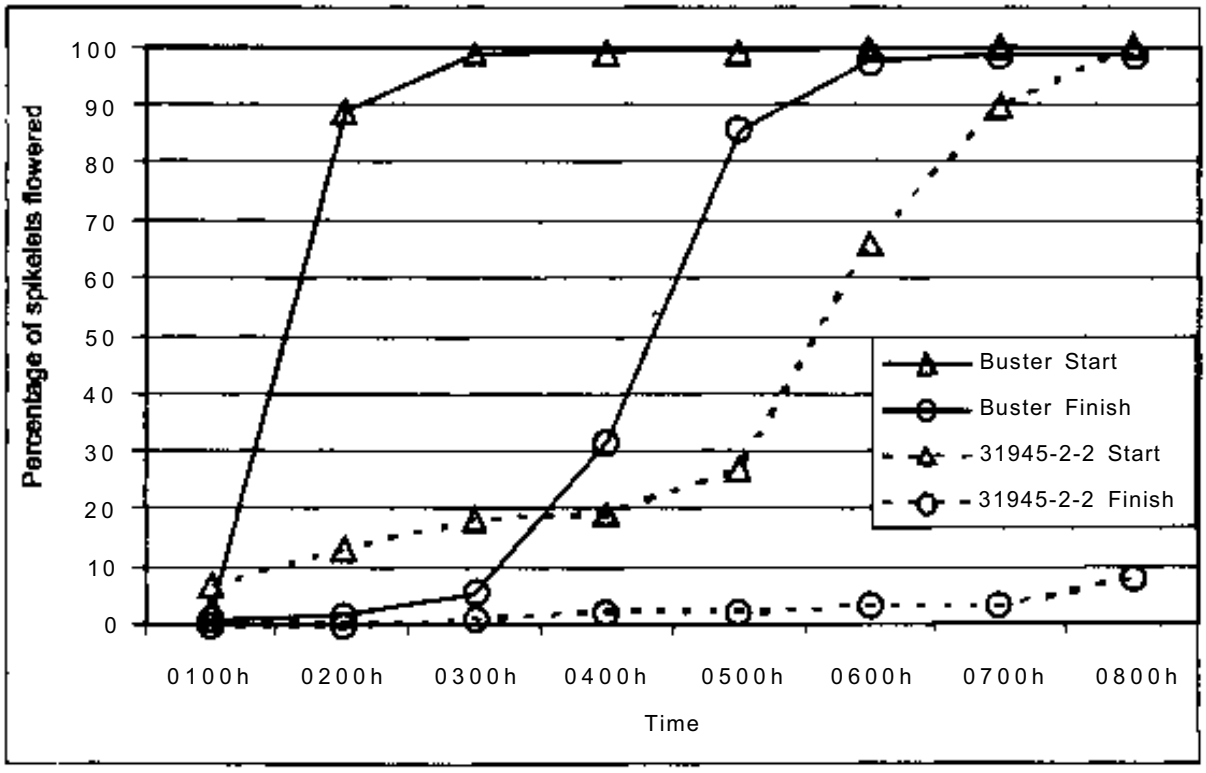

Figure 1. Average hourly percentage of flowering spikelets (FS1-5) and spikelets that had finished flowering (FS6) for two sorghum genotypes.

flowering time. These results indicated that the flowering behavior of the two genotypes over time were markedly different.

In a separate study more than 75% of the pollen grains of MR Buster® were trapped between 0300h and 1000h during the Australian mid-summer (MJ Ryley, unpublished data), which corroborates the Findings of these experiments. In the controlled environment cabinet, anther dehiscence was generally observed after the anthers had been completely exserted, at FS4. This stage corresponds to a pollen peak occurring from 0200 to 0400h for MR Buster® under the conditions of the experiments. There would theoretically be a less obvious pollen peak for 31945-2-2, which commenced flowering during a much longer period.

The results of this investigation demonstrate that flowering behavior differs between sorghum genotypes, and therefore must be considered in studies on the biology of pollen, ergot and sorghum midge. In particular, the relationship between flowering biology and resistance to the latter two pests needs to be recognised.

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Evidence for Apomixis and its Inheritance in the Sorghum Line SSA-1

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Introduction

Apomixis is a method of asexual reproduction in plant seeds that is of great significance in the evolution and formation of species and in plant breeding. Because apomixis can fix hybrid vigor, it has aroused wide attention among plant breeders (Zhao Shixu 1990). Poaceae, to which the sorghum species belongs, is a major family possessing this trait. Rao and Narayana (1968) were the first to report apomixis in the sorghum [*Sorghum bicolor* (L.) Moench] line R473 and it was considered to be apospory. Hanna et al. (1970) identified a facultative apomictic line PGY in sorghum. Because of the low frequency of apomictic progeny, cross infertility, and male sterility in PGY, it was difficult to evaluate genetically and consequently has seen little use in breeding programs. Niu Tiantang et al. (1994) have developed the apomictic line SSA-1 and made detailed studies of its embryology. The line showed a high frequency of apomixis with no cross infertility and has been a good basic material to research inheritance of apomixis and for use in fixing heterosis.

Evidence for apomixis. During 1992-1993, SSA-1 was evaluated for its apomictic behavior through hybridization and emasculation for four generations based on the methods suggested by Murty (1982) in Yuci, Shanxi province and Huangliu, Hainan province. The results showed SSA-1 could set seeds autonomously both in Yuci, Shanxi Province and Huangliu, Hainan Province, when it was emasculated and bagged (Table 1), indicating the evidence for apomixis. However, there were large differences in the frequency of apomictic seed-set in different locations and years. After analyzing experimental conditions, it was hypothesized that this differential frequency of apomixis could be due to temperature variations in different locations and years. A perusal of results clearly indicated a large influence of temperature on the apomictic frequency. High temperature appeared to increase the frequency of apomictic seed-set in SSA-1. These results are in line with those of Murty et al. (1982) in apomictic line R473 in sorghum.

In order to further confirm apomictic seed-set in SSA-1, experiments were conducted involving emasculation and

Table 1. Seed-set (%) of the emasculated SSA-1 plants with or without artificial pollination.

Year	Location	Temperature at the initial stage of megaspore cell formation		No. of plants	Emasculation and bagged seed-set			Emasculation and pollinated seed-set		
		Day °C	Night °C		No. of florets	No. of seeds	(%)	No. of florets	No. of seeds	(%)
1992 Summer	Yuci Shanxi	29.5	21.5	10	300	98	32.7	300	184	61.0
1992 Winter	Huangliu Hainan	25.1	16.3	8	400	65	16.3	400	188	47.0
1993 Summer	Yuci Shanxi	24.3	16.4	9	270	36	13.3	270	61	22.6
1993 Winter	Huangliu Hainan	24.1	15.3	9	450	62	13.8	450	252	56.0

Table 2. Seed-set (%) upon emasculation and removal of stigma with or without pollination in apomictic lines in comparison with sexual lines.

	Variety	Emasculation without pollination			Emasculation with pollination			Emasculation and removal of stigma with pollination			Emasculation and removal of stigma without pollination		
		No. of florets	No. of seeds	(%)	No. of florets	No. of seeds	(%)	No. of florets	No. of seeds	(%)	No. of florets	No. of seeds	(%)
Sexual	A_1TX623B	150	1	0.7	150	86	57.3	150	0	0	150	0	0
Lines	A_2V4A	150	0	0	150	81	54.0	150	0	0	150	0	0
	Sanchisan	150	0	0	150	40	26.7	150	0	0	150	0	0
	HM65	150	1	0.7	150	55	36.7	150	1	0.7	150	0	0
	Total	600	2	0.3	600	262	43.7	600	1	0.017	600	0	0
Apomictic Lines	SSA-1-1	150	15	10	150	73	48.7				150	19	12.7
	SSA-1-2	150	34	22.7	150	116	77.3				150	13	8.7
	SSA-1-3	150	13	8.7	150	99	66.0				150	23	15.3
	SSA-1-4	150	10	6.6	150	80	53.3				150	2	1.3
	Total	600	72	12.0	600	368	61.3				600	57	9.5

stigma removal in SSA-1 using sexual lines, A_1 TX623B and A_2 V4B (maintainers), and Sanchisan and HM65 (restorer lines) as controls during 1993 in Hainan Province. The seed set was 0.3% in sexual line material after emasculation and bagging, while it was zero when both stamens (emasculation) and stigma were removed (Table 2). On the other hand, seed-set was 12% and 9.5% on an average in four lines of SSA-1, confirming apomixis in SSA-1.

Apomictic trait. In 1992 summer, SSA-1 was crossed with ten restorer lines (male parent) in Yuci and the resulting F_1s were planted during 1992 winter in Hainan Province. Of the ten combinations, pseudo-hybrids (maternal plants) appeared in nine, with the frequency of 0-100%. During 1992 winter, 23 combinations with restorer lines and maintainer lines (of Sudan grass cultivars, Sweet Sorghum cultivars, TX623B and A_2V4B) were used to test frequency of SSA-1 in Hainan Province; frequency of maternal plants was 0-66%. When crossing was done, SSA-1 produced pseudo-hybrids, indicating that it possessed the typical trait of facultative apomixes. It showed both sexual reproduction (forming real hybrid) and apomixis (forming seed without fertilization) when crossed with other cultivars.

Frequency of apomixis. The frequency of apomixis in SSA-1 was as high as 52.5% (Table 3), which is much higher than that reported in R 473 (33.76%)(Murty et al. 1982). The studies on embryology (Wu Shubiao et al. 1994) showed apomictic embryo in SSA-1 during the formation of megasporocyte and it existed in two forms, apospory and diploid sporogony. Evaluation of embryology indicated that the frequency of apomixis was 42%, calculated as percentage of the ovule of initial cell of apomictic embryo in the total ovule.

Table 3. Frequency of apomictic plants in SSA-1.

Combination	No. of combinations	Year	Location	F_1 phenotype: Total No. of plants	F_1 phenotype: No.of maternal type plants	F_1 phenotype: No. of hybrid type plants	Frequency of apomixes (%)
SSA-1 x R lines	8	1992	Huangliu Hainan	61	32	29	52.5
SSA-1 x R or B lines	23	1993	Yuci Shanxi	145	37	108	25.5
SSA-1 x Sudangrass	1	1994	Yuci Shanxi	46	12	34	26.1

Table 4. Seed-set (%) in emasculated F_1 plants without pollination.

Combination	No.of combinations	Year	Place	No.of plants	No. of florets emasculated	No. of seeds	Seed-set (%)
SSA- 1 x R lines	8	1992	Hainan	16	480	1	0.2
SSA- 1 x R or B lines	23	1993	Yuci	46	2300	4	0.17

Table 5. Segregation for number of apomictic plants set and number of plants without seed-set (%) in the $F_2$of crosses between SSA-1 and Sexual lines.

Combination	No.of combinations	Total no. of plants	No. of plants without seed-set upon emasculation without pollination: No. observed	No. of plants without seed-set upon emasculation without pollination: No. expected	No. of plants with apomictic seed-set: No. observed	No. of plants with apomictic seed-set: No. expected	x^2
Sexual lines x SSA-1	7	397	379	372	18	25	1.713
SSA-1 x Sexual lines	4	239	221	224	18	15	0.970
SSA-1 x Sexual lines	2	338	312	317	26	21	1.460

Inheritance of apomixis. The apomictic seed-set in SSA-1 was not observed in F_1 (Table 4), indicating that apomixis might be under the control of recessive gene(s). The seed-set in some of the combinations might be due to the incompleteness of emasculation (Table 2).

The emasculation without pollination in 13 F_2 populations derived from the crosses between SSA-1 and sexual lines showed segregation of ratio of 15 plants without seed-set: 1 apomictic plant with X^2 <(0.05; 1=3.84) (Table 5). These results suggested that inheritance of apomixis is governed by two pairs of genes with duplicate dominant gene interaction and the apomixis is under the control of two recessive alleles in SSA-1.

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Pollen Release in the Australian Commercial Grain Sorghum Hybrid Cultivar, MR Buster®

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Introduction

There are few published reports on the timing of pollen release of grain sorghum [*Sorghum bicolor* (L.) Moench]. Not only is this information important in understanding the flowering biology of sorghum and its implications in hybrid seed production, but it is also critical in gaining an insight into the interactions between pollen and floral diseases such as sorghum ergot (caused in Australia by *Claviceps africana* Frederickson, Mantle, & de Milliano). This disease is now endemic in Australia, and has had considerable economic impact in the hybrid seed production and livestock industries (Ryley et al. 2002). Infection occurs when airborne secondary conidia deposited on the stigmas, and occasionally between the floral elements and the ovary, germinate and the hypha grow into the ovary, replacing it with a fungal mass, the sphacelium. The weather conditions which are conducive for development of ergot epidemics are becoming better understood (Ryley et al. 2002), but there is little published research on the interactions between weather and flowering biology of sorghum.

The timing of deposition of both pollen and *C. qfricana* secondary conidia on stigmas plays an important role in determining if ovaries are fertilized by pollen or colonised by the pathogen. Frederickson et al. (1993) in Zimbabwe and Ryley in Australia (unpublished data) have trapped airborne conidia of *C. africana* in infected plots of *S. bicolor* and found that maximum numbers are found during daylight hours; Frederickson's data indicated daily peaks in the late afternoon, while Ryley's results identified major peaks in the mid-day period. Both workers found that the numbers of conidia rose dramatically after rainfall events. By contrast, no publications on the timing of pollen release of *S. bicolor* under field conditions, or on its interaction with weather conditions, could be found. Field trials were conducted in 2005 in Queensland, Australia, to obtain some of this information.

Materials and Methods

Two trials were conducted in the summer of 2005 at Wellcamp Field Station, Wellcamp, Queensland, Australia (approx. 27°34' S 151°52' E), to monitor the release of pollen from the Australian hybrid grain sorghum cultivar, MR Buster® (Pacific Seeds Pty Ltd, Australia). This cultivar is grown in all sorghum-growing areas of Australia and comprises approximately 35% of the grain sorghum market (N Muller personal communication). Both trial plots consisted of 20 x 20 m rows, 1 m apart. The first trial was planted on 20 November 2004, and the second on 23 December 2004.

When 50% of panicles had emerged, and before anthesis, an area of approximately 1.5 m x 1.5 m in the middle of each plot was slashed, and a Quest™ volumetric spore trap (PO Box 3448, Smuts, Republic of South Africa) was placed on a portable, metal stand, adjusted so that the orifice of the spore trap was

approximately 0.25 m above the top of the panicles. The monitoring disks were changed after 7 days, and were transported to the DPI&F Plant Pathology laboratories in Toowoomba, Qld, where they were stored at 8°C until examined. The monitoring disk was gently immersed in 100mL of 90% ethanol + 10 drops of a saturated aqueous acid fuchsin solution for 60 seconds prior to examination, to aid in counting. The stained disk was placed on the stage of a Nikon SMZ1500 dissecting microscope with a HR ApoPlan x 1.6 WD 24 mm lens (total magnification x 160) and examined under a combination of incident and transmitted light. The numbers of pollen grains trapped every hour were counted and recorded.

To distinguish pollen of *S. bicolor* cv. MR Buster® from that of other grasses in the vicinity of the trial, flowering panicles of each of the grasses within 100 m of the trial site were collected, the anthers excised and were gently squashed in two drops of lactophenol-1.5% acid fuchsin on a glass slide. The anthers were removed, a coverslip was placed on the staining solution then sealed with several layers of nail polish. The morphology (including diameter) of 100 pollen grains of each of the grasses was determined by examination under a compound microscope at x 100.

During the course of the trials, hourly measurements of air temperature, relative humidity (RH), wind speed, rainfall and leaf wetness were recorded using Tinytag™ dataloggers (Gemini Data Loggers, United Kingdom) which were set up within 2 m of the trial plots. Hourly vapour pressure deficit (VPD) values were calculated from air temperature and relative humidity using the formulae of Wang et al. (2004). Correlation coefficients between mean hourly pollen counts and mean hourly temperature, relative humidity, VPD and rainfall were calculated (Genstat v 6.1; Lawes Agricultural Trust, Rothamsted Experimental Station).

Results and Discussion

The pollen grains of all the grasses were spherical, smooth- and thin-walled. However, those of *S. bicolor* cv. MR Buster® were larger [20-(25.3 ± 4.3)-35 µm] than pollen of the other grasses, *Panicum maximum* [20-(23.1±1.8)-25 µm], *Brachiaria* sp. [17.5-(20.8±2.6)-25 µm] and *Cynodon dactylon* [15-(17.1±3.0)-22.5 µm]. There were <100 flowering plants of *P. maximum* and *Brachiaria* sp. within 100 m of the trial site, compared to the approximately 4,000 and 2,000 *S. bicolor* plants within 15 m of the spore trap in the first and second trials, respectively. Consequently, despite the potential for misidentification of *S. bicolor* pollen, particularly with those of *P. maximum,* it could be assumed that during flowering period of *S. bicolor* there were considerably more pollen grains of *S. bicolor* in the air than those of the other grasses.

Pollen counts were made between 22 and 30 January 2005 and 9-14 February 2005. During the first period, the hourly air temperature ranged from 14.9 to 35.3°C (mean 23.5°C) and RH from 32.2 to 100% (mean 80.5%), and there were rainfall events on 22 January (7.8 mm), 23 January (1.6 mm), 24 January (3.6 mm), and 25 January (3.4 mm). During the second period, the respective values were 14.5-33°C (mean 22.5°C) and RH 38.5-100% (mean 76%), with rain on 9 February (0.6 mm) and 10 February (13.8 mm). Pollen of *S. bicolor* was trapped for 17-24 hours per day during the first trial, and 14-24 hours per day during the second trial. In the first trial the highest daily count was on 23 January (2298 pollen grains m^{-3}) with daily totals gradually dropping on the following days. The highest hourly total (880 pollen grains m^{-3}) was recorded on 24 January. In the second trial, the greatest daily and hourly totals were recorded on 13 February (2763 and 1512 pollen grains m^{-3} respectively). On these two days of highest daily pollen counts, the highest proportions of panicles at 50% flowering were also recorded (data not shown).

In all days in both trials, over 75% of the total daily number of pollen grains was recorded between 0300h and 1000h, with the start, finish and length of this period differing from day to day (hereafter this period will be called the peak period). On eight of the 15 days, the maximum pollen counts occurred at 0400h, while on the other days it occurred once at 0300h, 0500h and 0700h,

Table 1. Relationships between hourly pollen counts of sorghum hybrid MR Buster® and weather parameters for two periods in 2005.

Weather parameter	Hour of daily maximum	Peak period	Other hours
Temperature (°C)	14.9-(19.8,20.9)-24.r	14.9-(19.8,20.9)-24.1	14.5-(22.7,27.4)-35.3
Relative humidity (%)	80.4-(98.8,98.8)-98.8	71.3-(97.7.98.8)-98.8	32.2-(78.7,96)-98.8
Vapour Pressure Deficit (hPa)	0.3-(0.5,1.6)-7.4	0.3-(0.8,4.4)-11.0	0.3-(8.4,19.3)-40.2
Wind speed (m s^{-1})	0.7-(3.2,4.6)-6.4	0.5-(3.3,4.9)-6.8	0.3-(4.3,5.6)-8.7
Rainfall (mm)	0-(0,0)-0.2	0-(0,0)-0.6	0-(0,0)-7.4

1. Minimum-(median, 75 percentile)-maximum values, data combined for both trials.

and twice at 0600h and 0800h. Table 1 presents combined data for both trials on various weather parameters for the hour with the highest pollen count for the day, the peak period, and hours outside this period. Hours with maximum counts in the peak period had lower temperatures, VPD and wind speed, and higher RH, than hours outside this period. The diurnal changes in pollen counts and selected climatic parameters between 1200h on 23 January and 1200h on 24 January are displayed Figure 1.

Keijer (1999) summarised the mechanisms of angiosperm anther dehiscence and emphasised the role of environmentally-linked hygroscopic absorption in the rupturing of the anther, and Matsui et al. (1999) suggested that rapid swelling of the pollen grains of rice contributed significantly to anther dehiscence. Our findings indicate that anther dehiscence in at least one grain sorghum hybrid is favoured by humid air during the early hours of the morning over summer in southern Queensland. The viability of the pollen of *S. bicolor* released during this period may also be prolonged, because Barnabas and Kovacs (1997) reported that, unlike other angiosperms, the viability of grass pollen is favoured by high relative humidity.

Rainfall has been demonstrated to have a considerable impact on the development of epidemics of *C. africana* (Ryley et al. 2002), particularly in the production and release of secondary conidia (Ryley unpublished data). Rainfall may also adversely affect the release of pollen grains. On all but one day when rain fell during the trials there was no decrease in the total daily pollen count. The exception was on 10 February, when rain fell between 0300h and 0800h (total 10.4 mm, 0.2-7.4 mm hr^{-1}), resulting in a large reduction in the daily total number of pollen grains that were trapped (711 m^{-3}) compared to the days before (9 February; 1763 m^{-3}) and after (11 February; 1381 m^{-3}). Pollen was trapped at low numbers in hours when rain fell on 10 February.

Pollen tubes reach the ovary much faster than hyphae from germinated secondary conidia of *C. africana,* and fertilised ovaries are resistant to infection by the pathogen (Bandyopadhyay et al. 1998). Consequently, for successful infection to take place, anther dehiscence and pollination must be delayed sufficiently long enough for the infection processes of *C. africana* to occur, and for the ovary to be colonised. Rainfall and/or low temperatures are conducive to the production and germination of secondary conidia (Bandyopadhyay et al. 1998; Ryley et al. 2002), and to the suppression of pollen release (present study) and viability (Bandyopadhyay et al. 1998; Ryley et al. 2002). Further research on the relative contributions of these aspects in the dynamics of ergot epidemics is warranted.

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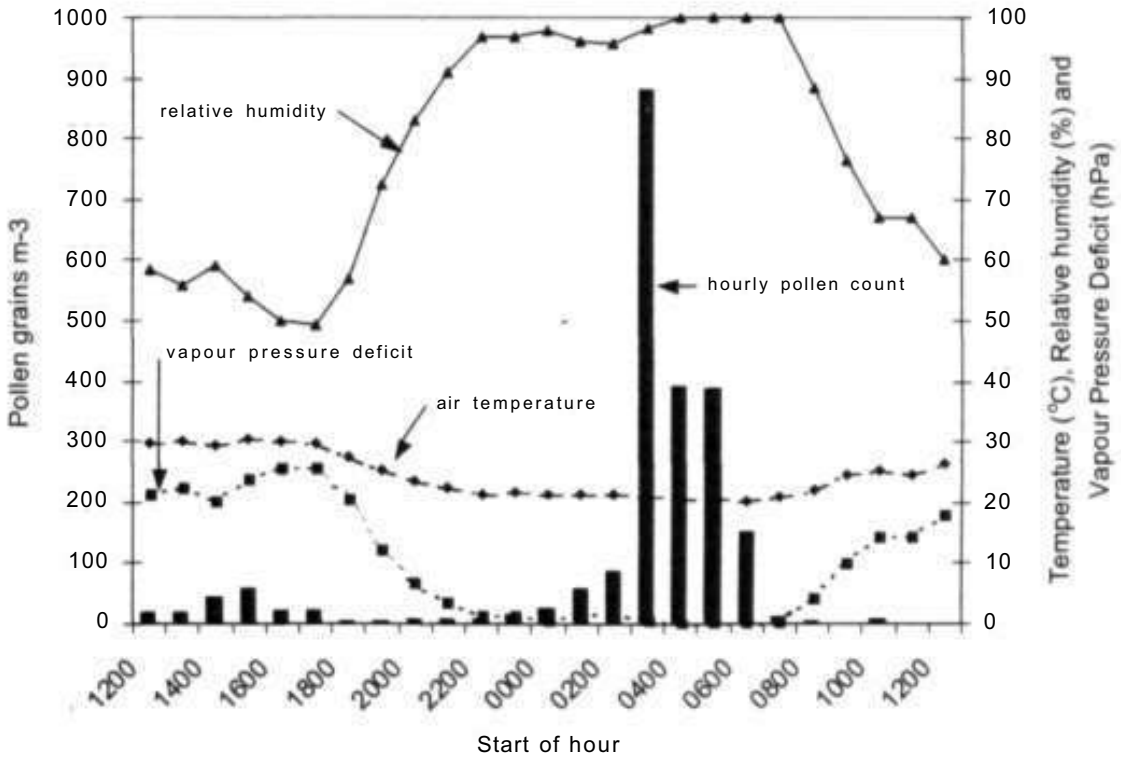

Figure 1. Diurnal changes in hourly pollen counts of sorghum hybrid MR Buster® and selected weather parameters between 1200h on 23 January 2005 and 1200h on 24 January 2005.

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Phosphorus and Potassium-Based Osmotic Hardening Seed Treatments and the Germination of Sorghum ICSV 745 (*Sorghum bicolor* L.) after Four Weeks of Storage

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Introduction

The osmotic conditioning of seeds involves soaking in high osmotic potential solutions, which permit partial hydration of seed without radical protrusion (Heydecker 1978). This has not found application in field crops on a large scale due to economic constraints of using osmotica like polyethylene glycol. This experiment investigated the use of fertiliser-based seed soaking treatments as the osmotic factor and their influence on germination in sorghum *[Sorghum bicolor* (L.) Moench] variety ICSV 745 after storing treated seed. Work was conducted at the Institute of Crop Science, University of Kassel, Germany.

Materials and Methods

Seven seed treatments with phosphorus and potassium fertilisers were investigated. A dry, untreated control and

Table 1. The influence of seed treatments on the germination percentage and speed of sorghum seed.

Seed Treatment	FGP (%)	MGT (day)
Dry Control	72.2 b	4.7 a
Wet Control	74.0 b	4.0 ab
Hyperphosphate (1 g L^{-1}) [1]	81.2 a	2.9 c
Superphosphate (1 g L^{-1}) [2]	71.0 c	3.4 bc
KC1 (1 g L^{-1}) [3]	56.8 d	3.6 b
K_2SO_4 (1 g L^{-1}) [4]	68.7 bc	3.3 c
1 + 2	57.7 d	3.3 c
2 + 3	54.3 d	3.7 b
3 + 4	52.2 d	3.9 b

Means in columns followed by similar letters are not significantly different (α< 0.05). FGP: Final Germination Percentage. MGT: Mean Germination Time (the higher the MGT value the longer it takes for a seed to germinate and, hence, the lower the overall germination index for that treatment. Lower MGT values denote a faster germination). Means tested using Duncan's Multiple Range Test.

a wet control were also tested. After being soaked in solutions containing KCl, K_2SO_4, superphosphate, hyperphosphate or mixtures of them for 3 days at 5°C in the dark, seeds were dried (25°C for 3 h) and stored at 25°C for 4 weeks.

Results

Post-storage germination tests at 39/29°C (day/night temp) and -3.0 bar moisture level revealed that soaking seeds in water or 1g L^{-1} hyperphosphate gave significantly higher final germination percentages and germination index values than untreated seeds (Table 1). Treating seeds with KCl either alone or in combination with another salt significantly reduced the germination percentage due to toxic effect of the Cl ion.

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Pathology

Grain Mold Resistance in Advanced Sorghum B-lines

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Introduction

Grain mold, caused by a complex of pathogenic and saprophytic fungi, is a highly destructive disease of sorghum [*Sorghum bicolvr* (L.) Moench] and is widely distributed in the semi-arid tropics of Africa and India. Annual global losses due to grain mold have been estimated at US$ 130 million (ICRISAT 1992). Improved cultivars, particularly hybrids bred for early to medium maturity to escape terminal drought stress in India are normally more vulnerable to the disease than late maturing cultivars (Bandyopadhyay et al. 1988). Major efforts in breeding cytoplasmic-nuclear male sterility-based sorghum seed parents (A/B-lines) for grain mold resistance at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India, and other locations in India as well as in the US have met with partial success (Reddy et al. 2000). Therefore, efforts were made to improve sorghum B-lines for grain mold resistance through a specific breeding approach and the outputs of these efforts, along with future perspectives of breeding for grain mold resistance, are reported here.

Materials and Methods

The material for the study consisted of 28 promising grain mold resistant B-lines developed at ICRISAT, Patancheru, during the past six years using pedigree selection among segregating progenies derived from crosses between grain mold resistant lines and high-yielding bold grain B-lines. These were evaluated along with three controls (IS 14384 as grain mold resistant, and Bulk Y and 296B as grain mold susceptible) during the 2003 rainy season in a randomized complete block design (RCBD) with 2 replications. Sprinkler irrigation was provided for 30 min per day on dry days during flowering to physiological maturity of sorghum to create high humidity (>90% relative humidity) congenial to the development of adequate and uniform disease pressure. Ten panicles of uniformly flowering plants were tagged in each replication for recording grain mold infection at

Table 1. Performance of advance sorghum B-lines for grain mold resistance and agronomic traits during the 2003 rainy season, ICRISAT, Patancheru.

Designation	DTF[a]	Plant heigh (m)[a]	Grain mass (g 100^{-1})	Grain yield (t ha^{-1})	PGMR[b]	TGMR[b]	Grain hardness[c] (kg $seed^{-1}$)	Panicle type[d]	Glume color[e]	Glume cover[e]	Grain color[e]
SGMR 03-1	74	1.9	2.37	1.54	5.6	6.4	5.1	SC	W	50	w
SGMR 03-2	72	1.9	2.13	1.57	4.0	4.3	4.8	SC	R	25	w
SGMR 05-1	71	1.7	2.20	2.35	6.4	6.0	3.9	SC	R	50	w
SGMR 05-2	72	1.7	2.13	1.52	6.7	7.3	3.7	SC	R	25	w
SGMR 07-1	71	1.9	2.35	2.29	4.4	5.2	3.9	SC	R	25	w
SGMR 07-2	74	1.8	2.22	1.83	4.9	5.4	4.3	SC	R	25	w
SGMR 08-1	79	1.7	2.34	1.09	7.4	8.4	4.8	SC	w	50	w
SGMR 08-2	77	1.9	2.55	1.14	7.7	8.4	4.9	SC	w	50	w
SGMR 09-1	77	1.7	2.20	0.58	6.1	8.1	4.9	SC	w	50	w
SGMR 09-2	77	1.8	2.21	0.92	7.3	8.2	5.9	SC	w	25	w
SGMR 10-1	75	1.9	1.99	1.56	5.1	4.9	4.6	L	w	50	R
SGMR 10-2	74	2.0	1.81	1.19	6.0	5.9	3.5	SC	R	50	R
SGMR 11-1	74	1.9	2.13	1.14	3.6	3.8	5.0	SC	R	50	R
SGMR 11-2	77	1.8	2.01	0.98	4.6	4.2	5.1	SC	w	50	R
SGMR 12-1	71	2.0	2.46	1.82	4.4	5.1	3.9	SC	R	50	R
SGMR 12-2	70	2.0	2.51	2.68	4.3	5.4	4.0	SC	R	25	R
SGMR 21-1	78	1.7	1.97	0.73	3.4	3.6	5.4	SC	R	50	R
SGMR 21-2	81	1.7	2.12	0.50	5.3	5.5	3.8	SC	w	50	R
SGMR 21-3	81	1.6	1.57	0.29	5.5	6.1	3.9	SC	R	50	R
SGMR 21-4	78	1.7	1.88	0.77	3.2	2.8	5.6	SC	B	50	B
SGMR 23-1	79	1.9	1.51	0.74	5.4	6.1	1.7	SC	R	50	B
SGMR 23-2	77	1.9	1.60	1.39	3.8	4.4	1.9	SC	R	75	R
SGMR 24-1	70	1.7	2.34	2.25	4.3	5.1	4.0	SC	w	50	B
SGMR 24-2	71	1.7	2.26	1.78	6.8	7.5	3.0	SC	w	50	B
SGMR 33-1	70	1.9	1.95	2.62	1.7	2.3	3.8	c	R	75	B
SGMR 33-2	70	2.0	2.01	2.69	1.8	2.2	3.3	c	R	50	B
SGMR 40-1	62	1.9	2.51	2.50	1.7	3.2	4.4	SC	B	25	B
SGMR 40-2	62	1.6	2.41	2.31	2.3	4.5	4.4	SC	B	25	B
IS 14384 (C)	71	2.8	1.99	3.63	1.2	1.3	8.3	L	B	25	R
Bulk Y (C)	56	1.3	3.63	2.09	8.2	9.0	7.6	L	B	25	w
296 B (C)	73	1.1	1.88	1.57	8.4	8.7	2.3	C	w	50	w
Mean	73	1.8	2.17	1.58	4.9	5.5	4.4	-	-	45	-
MSS	92.83**	0.22**	0.44**	1.96**	75.63**	81.72**	3.73**	-	-	-	-
LSD(*P*=0.05)	2.49	0.19	0.31	0.32	1.1	0.9	1.5				

[a]Mean of three replications. DTF=Days to 50% flowering

[b]PGMR=Grain mold reaction at grain physiological maturity and TGMR = Grain mold reaction on threshed grain. Mean of 2 replications, 10 panicles/rep., based on 1-9 scale where 1 = No mold, 2=1-5%, 3=6-10%, 4=11-20%, 5=21-30%, 6=31-40%, 7=41-50%, 8=51-75% and 9>75% mold infection

[c]Mean of two replications. 25 grains/rep

[d]C=Compact; SC=Semicompact; L=Loose

[e]W=White; R=Red; B=Brown

**Significant at P<0.01

Table 2. Influence of grain and glumes colors on grain mold reactions in advanced sorghum B-lines.

	Grain color			Glume color			Glume coverage (%)		
Moldreaction	White (10)	Red (10)	Brown (8)	White (10)	Red (15)	Brown (3)	25 (8)	50 (18)	75 (2)
PGMR	6.1	4.6	3.4	6.0	4.4	2.4	4.4	5.1	2.8
TGMR	6.8	4.9	4.2	6.7	4.9	3.5	5.4	5.6	3.2

Figures in parentheses represent the number of B-lines.
PGMR=Grain mold reaction at grain physiological maturity and TGMR=Grain mold reaction on threshed grain.

physiological maturity (PM) and on threshed grain (TG) using the 1-9 progressive scale where 1= no mold, 2=1-5%, 3=6-10%, 4=11-20%, 5=21-30%, 6=31-40%, 7=41-50%, 8=51-75% and 9>75% mold. Grain (20 g from each panicle) threshed from all the panicles were pooled and mold infection was recorded as TGMR using the same 1-9 scale.

A separate trial was conducted with the same set of entries during the 2003 rainy season at ICRISAT, Patancheru in RCBD for agronomic evaluation. The observations were recorded on 10 randomly selected plants in the middle two rows for growth and yield traits; days to 50% flowering (DTF), plant height, grain yield, panicle type (compact, semi-compact and loose), grain mass, glumes and grain color (white, red, and brown), glumes cover on grains, and grain hardness (at 7% grain moisture).

Results and Discussion

Significant differences were observed among the 28 lines for PGMR and TGMR scores, and DTF, plant height, grain hardness, grain mass, and grain yield (Table 1). None of the entries showed significantly higher levels of PGMR and TGMR than the resistant control IS 14384, which showed PGMR and TGMR scores of 1.2 and 1.3, respectively. Nevertheless, SGMR 33-1, SGMR 33-2, SGMR 40-1 and SGMR 40-2 had PGMR score less than 2.3 and were at par with the resistant check IS 14384, and produced significantly higher grain yield (2.31-2.691 ha^{-1}). These B-lines, besides flowering early (<70 days), had semi-compact to compact panicles and red/brown grains with 25-75% coverage by red/brown glumes. These lines had 1.95-2.51 g 100^{-1} grain mass and moderate grain hardness of 3.3-4.4 kg $seed^{-1}$ compared with 1.88 g 100^{-1} grain mass and 2.3 kg^{-1} grain hardness of 296 B, a susceptible control.

Relationship of PGMR and TGMR with grain and glumes traits. The results indicated that grain and glume color (red or brown) appeared to contribute to grain mold resistance (Table 2). The lines with brown grain and glumes showed higher grain mold resistance than those with red grain and glumes or white grain and glumes as evident from mean PGMR and TGMR scores (Table 2). The earlier reports on the contribution of grain color (Menkir et al. 1996) and glume color (Audilakshmi et al. 1999) to grain mold resistance in sorghum support the present findings. Three lines [SGMR 3-2 (PGMR: 4.0 and TGMR: 4.3) and SGMR 7-1 (PGMR: 4.4 and TGMR: 5.2) and SGMR 7-2 (PGMR: 4.9 and TGMR: 5.4)] in white grain background had moderate mold resistance levels, although they had pigmented (red) glumes. All white-grain B-lines with white glume color were susceptible (Table 1). The glume cover on the grain also appeared to provide some protection to grains from mold infection. Two lines (SGMR 23-2 and SGMR 33-1) with more than 75% glume coverage showed much higher grain mold resistance than those with 25 and 50% glume coverage (Table 2). However, no definite relationship was observed between the extent of glume coverage and mold resistance levels. For example, lines, such as SGMR 05-1, SGMR 08-1, SGMR 08-2, SGMR 09-1 and SGMR 24-2 with 50% glume coverage were more susceptible (PGMR and TGMR scores >6.0), than SGMR 40-1 and SGMR 40-2 with 25% glume coverage (PGMR< 2.0 and TGMR<4.5) (Table 1). The higher mold resistance levels in some of these lines with less glume coverage is encouraging considering farmers' preferences for cultivars with less glume coverage, which reduces postharvest processing cost.

A significant positive correlation between PGMR and early flowering and significant negative correlation between PGMR and TGMR scores with grain yield implied that it is necessary to maintain a balance between maturity, mold resistance levels, and grain yield. A weak correlation between mold resistance levels and grain mass and grain hardness was observed, which is contrary to earlier reports of significant negative correlation between mold resistance and grain mass (Reddy et al. 2000) and positive correlations between mold resistance and grain hardness

(Audilakshmi et al. 1999). These results indicate better prospects for developing mold resistant B-lines without compromising grain size (one of the important traits preferred by farmers). Concerted breeding efforts involving diverse sources of mold resistance and high-yielding bold grain lines are essential for the development of B-lines with enhanced mold resistance levels under desirable agronomic background.

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Variability in Target Leaf Spot Pathogen *Bipolaris sorghicola* of Sorghum in Rajasthan, India

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Introduction

Target leaf spot, caused by *Bipolaris sorghicola* (Lefebvre & Sherwin) Alcorn, is known to be prevalent and occasionally severe in the US since 1939 (Dalmacio 2000) and was reported from India by Munjal and Kapoor (1960). Limited information is available on this disease, probably due to its misidentification since its symptoms closely resemble those of other foliar diseases such as zonate leaf spot and gray leaf spot (Dalmacio 2000; Leslie 2002). Target leaf spot was observed to be widely prevalent in southern Rajasthan during 2001-2004, and critical studies of its symptoms and morphological and cultural characteristics were made to provide dependable identification of the disease in the field. The results of this study are presented and discussed here.

Materials and Methods

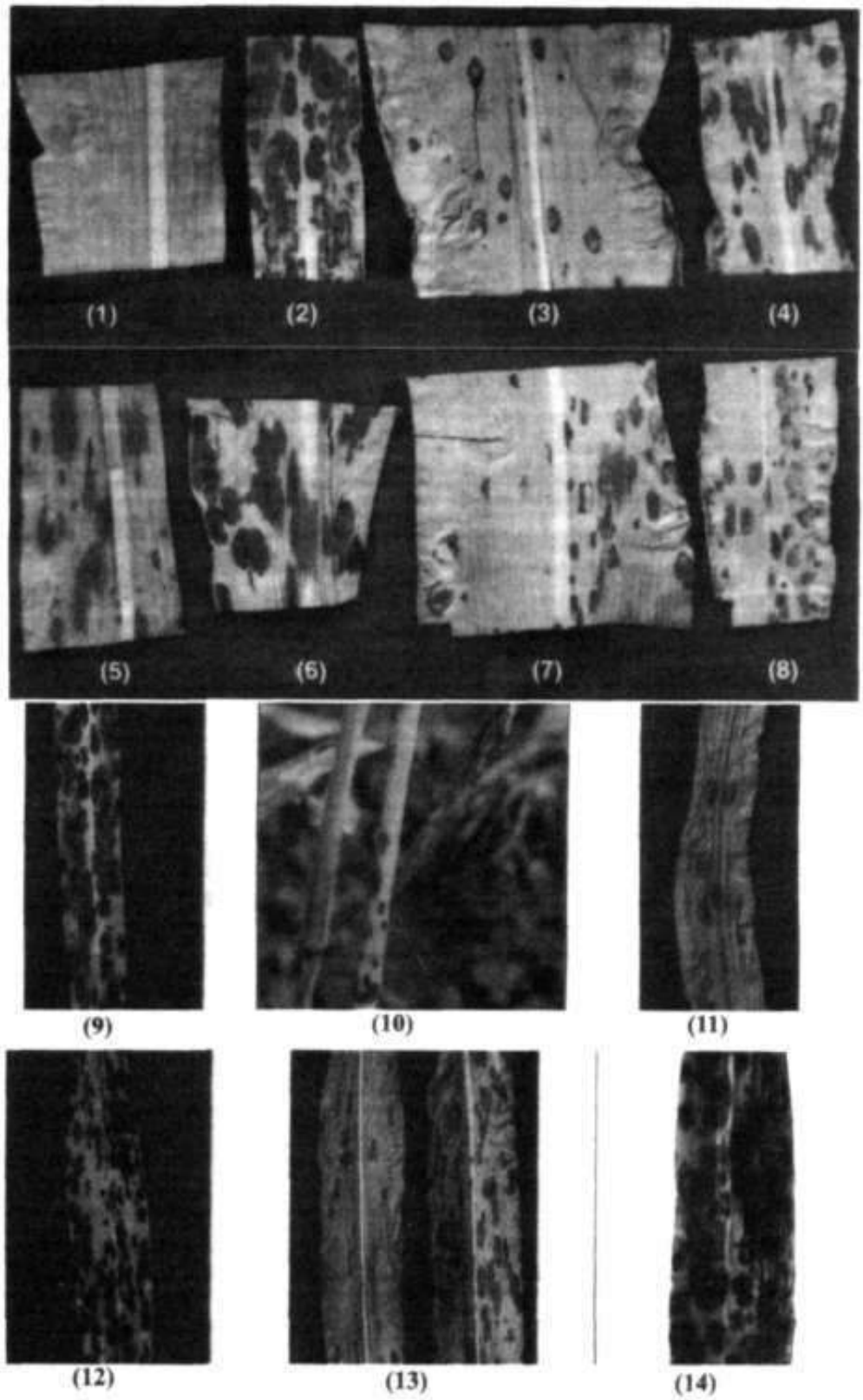
The leaves infected by symptoms of target leaf spot collected from different places and cultivars (Table 1) were studied in detail and the pathogen was isolated on potato dextrose agar (PDA) medium. Important morphological characteristics *viz.,* conidial development on 0.5% PDA (by slide-culturing method), germination of conidia (in slide-germination tests), and cultural characteristics were studied, and compared with the available literature (Dalmacio 2000). The pathogen collected from various regions was identified as *Bipolaris sorghicola.* The cultures were maintained on PDA slants at 4°C.

Comparative pathogenic potential of five isolates of *B. sorghicola* was studied by inoculating 28-day-old pot-grown plants of four cultivars *Kekri* local, S Path-97, IS-164 and SU-45 with spore suspensions (1 x 10^5 conidia ml^{-1}), prepared from 10-day-old cultures of *B. sorghicola* isolates. Each isolate was maintained in three replicated pots, each with five plants. Pots were separated by polythene sheets to avoid drift of inoculum from other pots while spray inoculating. The inoculated plants were kept in humid chambers for 24 hours and then transferred to the cagehouse and high humidity was maintained throughout the disease development period by regular irrigations. Observations were recorded 30 days after inoculation on a 1-5 disease rating scale. Number of plants in each score was recorded and percent disease index (PDI) was calculated as:

$$\text{Percent disease index} = \frac{\text{Sum of all disease ratings}}{\text{Total number of ratings} \times \text{maximum score}} \times 100$$

Results and Discussion

The symptoms of target leaf spot on different cultivars consisted of yellow, diffused spots on cultivars with tan reaction (Fig. 1) or straw-colored spots with or without red-brown margins, or bright red to purple brown (Figs. 4-6). The shape of the spots varied from elongated pointed or round, or cylindrical lesions (Figs. 3, 7, 8) to



Figures 1-14. Variable symptoms of target leaf spot of sorghum caused by *Bipolaris sorghicola:* (1) Tan reaction on IS 22545; (2) Typical target lesions on Kekri local; (3); Small, straw colored lesions with red margin on E 111; (4) Elongated bright red lesions on SU 45; (5) Large purple brown spots on local landrace in Farmer's field , Hoda village; (6) Elongated red-brown spots on local landrace in Farmer's field, Kathaar village; (7) Cylindrical and coalescing lesions with dark margins on E 118; (8) Small brown lesions with characteristic zonation on Kekri local after inoculation with isolate Bs-01; (9) Naturally occurring characteristic target lesions on Kekri local; (10) Lesiunson leaf sheath and stalk of Kekri local; (11) Roundish, scattered spots on IS 164; (12) Purple-red spots on leaves of S Path 97; (13) Bright red lesions without distinct margin on local landrace in Farmer's field in Kathaar, Rajsamand; (14) Larger, blackish-brown, round lesions with characteristic target spots on local land race in farmer's field in Bhatewar, Udaipur.

typical target spots showing characteristic concentric rings of straw color and dark margins (Fig. 2).

On *Kekri* local the lesions were oblong, with concentric zones, obtuse at the ends, and 8.2 x 3.5 mm (7.0-22.0 x 3.0-8.0 mm) in size (Fig. 9), and lesions were also formed on the leaf sheath (Fig. 10). On IS 164 the spots were roughly circular, delimited by veins, with dark red margins and straw colored centers (Fig. 11), and 7.5 x 6.7 mm (6.5-8.0 x 5.5-7.5 mm) in size. On S Path 97 (Fig. 12), the lesions were abundant, smaller, and purple-red. In samples collected from Kathaar (Fig. 13) the spots were dark reddish-brown, delimited by veins, slightly pointed at the ends, somewhat darker red-brown in the center and 8.0-22.0 x 3.0-8.0 mm (mean 14.1 x 5.2 mm). The size of lesions on S Path 97 was 2.0-10.0 x 2.0-3.0 mm (6.0 x 2.6 mm). On local sorghum in Bhatewar, the lesions were roughly circular, almost purple-brown with straw colored centers with very well developed zonation (Fig. 14), and measured 10.0-16.0 x 3.0-7.0 mm (12.6 x 4.2 mm) in size.

All the five isolates grew and sporulated well on PDA but there were significant (P = 0.05) differences in the rates of growth and sporulation. Isolate Bs-03 showed maximum growth (90.0 mm) after 7 days (Table 1), while the least growth (83.0 mm) was observed in Bs-02, followed by Bs-05 (83.6 mm). Bs-03 produced the maximum number of spores (29 x 10^4 mm^2) and minimum counts were observed (25 x 10^4 mm^2) in Bs-02 and Bs-05.

The size of conidia of different isolates ranged from 65 to 73 x 12 µm. Conidia of Bs-01 isolate were the longest and thinnest (73 x 12 µm) followed by those of Bs-03 (71 x 12 urn), while isolate Bs-04 produced the shortest and widest conidia (65 x 12 µm). Conidia of isolate Bs-05 were similar to those of Bs 04, but were wider and less variable (65 x 12 µm) among themselves. Maximum number of septa (6.0) per conidium was in isolate Bs-05 and the least (5.5) in Bs-04 (Table 1). In the slide germination test, conidia of all the five isolates germinated in bipolar mode, typical to the genus *Bipolaris.*

Table 1. Variation in growth, and morphology of five isolates *of Bipolaris sorghicola* collected from sorghum cultivars in Rajasthan.

Isolate designation	Cultivar	Location	Growth (mm)[a]	No. of conidia ($x10^4/mm^2$)[b]	Size of conidia (µm)					
					Length		Width		No. of septa[c]	
					Mean[c]	Range	Mean[c]	Range	Mean[c]	Range
Bs-01	Kekri local	Udaipur	84.8	26	73±3.3	64-76	12±1.6	10-14	5.8±1.6	3-9
Bs-02	Local land race	Bhatewar	83.0	25	67±6.8	52-76	12±1.5	10-16	5.8±1.8	3-10
Bs-03	IS-164	Udaipur	90.0	29	71±2.8	64-76	12±1.1	10-14	5.7±1.5	3-9
Bs-04	S.Path 97	Udaipur	86.2	27	65±4.7	52-72	12±1.3	10-14	5.5 + 1.6	3-9
Bs-05	Local land race	Kathaar	83.6	25	65±3.2	60-70	12±1.3	10-14	6.0±1.4	4-9
LSD (P = 0.05)			2.02	1.6						

a. Mean of five replications, b. Mean of 5 replications, 3 observations/replication, c. Mean of 60 conidia.

Table 2. Per cent disease index (PDI) due to inoculation of five isolates of *B. sorghicola* on different sorghum cultivars.

Isolates of B sorghicola	PDI on different sorghum cultivars"				Mean
	Kekri local	S Path-97	IS-164	SU-45	
Bs-01®	70.4 (57.0)[b]	60.9 (51.3)	53.6(47.1)	58.8 (50.1)	60.9
Bs-02	61.5 (51.7)	52.1 (46.2)	49.5 (44.7)	50.4 (45.2)	53.4
Bs-03	77.6(61.8)	75.8 (60.5)	51.9 (46.1)	62.7 (52.4)	67.0
Bs-04	73.3 (58.9)	73.4 (59.0)	55.6 (48.2)	59.1 (50.2)	65.3
Bs-05	66.2 (54.5)	54.3 (47.5)	56.9 (49.0)	50.9 (45.5)	57.1
Mean	69.8	63.3	53.5	56.4	

LSD (P<0.05) for isolate means 2.0; cultivar means = 1.8 and their interactions = 4.0

[a]Average of three replications, 5 plants in each replication.

[b]Arcsine √ per cent angular transformed values.

In the pathogenicity test, the maximum disease was caused by Bs-03 on *Kekri* local (PDI-77.6) followed by Bs 03 on S Path 97 (75.8) and Bs-04 on S Path 97 (73.4); the lowest levels of disease incidence was observed in Bs-02 on IS 164 (49.5) and SU 45 (50.4), by Bs-05 on SU 45 (50.9) (Table 2). The isolates varied in aggressiveness across the four cultivars, Bs-03 was the most aggressive (mean PDI 67.0) and Bs-02 the least (mean PDI 53.4). Across the isolates, *Kekri* local was the most susceptible, and IS 164 was the least susceptible. The percent disease index was significantly *(P* = 0.05) different among the isolates, host cultivars and their interaction.

This study revealed that although the symptoms of target leaf spot are somewhat similar to zonate leaf spot (*Gloeocercospora sorghi)* and gray leaf spot, the latter could be differentiated by critical observation. The lesions of zonate leaf spot are initially rectangular, then semi-circular or roughly circular, up to 7 cm long and with reddish-purple bands alternating with straw colored or tan areas that form a concentric or zonate pattern. The lesions of grey leaf spot are rectangular, 2-5 x 5-15 mm, with grey center, and brown, red or purple margin (Frederiksen and Odvody 2000). The target leaf spot lesions are initially small, red, rectangular to elliptical or oval, or even cylindrical. These soon enlarge and develop straw colored or light red center and dark red purple margin. The size of target leaf spot lesions has been variously reported as 5-15 x 4-6 mm by Munjal and Kapoor (1960); 1.5 to 4 mm long (5-10 mm on coalescence) and 0.75 to 2.25 mm broad (1.25-2.25 mm on coalescence) by Mishra and Mishra (1971) and 1-100 mm long (Dalmacio 2000). In our study, the lesion size on different cultivars ranged from 7-22 mm long and 3-8 mm wide, and always revealed different colored zones, as reported by Zummo and Gourley (1987). The target leaf spot pathogen has been variously reported as *Helminthosporium sorghicola* (Lefebvre and Sherwin 1948; Munjal and Kapoor 1960; Mishra and Mishra 1971 and Odvody and Dunkel 1975); or *Bipolaris sorghicola* (Dalmacio 2000). Based on spore-morphology, conidio-genesis and bipolar germination of conidia, the pathogen reported here is *Bipolaris sorghicola.*

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Entomology

Host Plant Resistance to Insects in Sorghum: Present Status and Need for Future Research

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Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is an important cereal crop in Asia, Africa, the Americas, and Australia. Grain yields on farmers' fields in the semi-arid tropics (SAT) are generally low (500 to 800 kg ha^{-1}) mainly due to insect pest damage. Nearly 150 insect species have been reported as pests on sorghum, of which sorghum shoot fly (*Atherigona soccata* Rond.), stem borer *(Chilo partellus* Swin.), armyworm (*Mythimna separata* Walk.), shoot bug *(Peregrinus maidis* Ashmead), aphid *(Melanaphis sacchari* Zehnt.), sorghum midge (*Stenodiplosis sorghicola* Coq.), earhead bug (*Calocoris angustatus* Leth.), and head caterpillars (*Helicoverpa, Eublemma,* and *Cryptoblabes*) are the major pests (Sharma 1993). Annual losses due to insect pests in sorghum have been estimated to be over $1089 million in the SAT.

Host plant resistance

Techniques to screen for resistance to insect pests. The ability to develop insect-resistant cultivars, use of marker-assisted selection, and development of transgenic plants with insect resistance depends on the precision of resistance screening techniques. Infester row, cage and leaf disc screening techniques have been standardized to evaluate sorghum germplasm, breeding material, and mapping populations for resistance to insect pests under field and greenhouse conditions (Sharma et al. 1992, 2003). However, several of these techniques are being used in the sorghum improvement programs only sparingly because of lack of resources or lack of enthusiasm on the part of the scientists involved. Lack of infrastructure for insect rearing could be an impediment to screening for resistance to stem borer, but infester row and no-choice cage techniques developed to screen for resistance to shoot fly, midge, and head bugs do not require much investment. There is a need to standardize the techniques to screen for resistance to aphids - an emerging pest problem. One of the problems in selecting for resistance to stem borer is the relative importance of foliar injury, deadhearts, stem tunneling, exit holes, and tiller production (Singh 2002). The effects of different damage parameters on grain yield loss are not fully understood. Another important question is whether the material should be screened in each generation, alternate generations, or only after the material has become homozygous in F_5 - F_6 generations. Extensive studies at ICRISAT have indicated that the material subjected to borer infestation in F_2 to F_5 generations had greater frequency of resistant progenies than the material exposed to borer infestation in the F_5 generation only. Such information needs to be generated for different insect pests.

Identification and utilization of sources of resistance to insect pests. Host plant resistance should form the backbone of pest management in sorghum. Over the past five decades, a large proportion of the world sorghum germplasm collection has been evaluated for resistance to insect pests, and a number of lines with resistance to major insect pests have been identified (Sharma et al. 1992, 2003). Large-scale screening of the sorghum germplasm at ICRISAT has resulted in identification of several lines with reasonable levels of resistance to shoot fly, stem borer, midge, and head bugs (Table 1). Sources of resistance to insects in sorghum have been used in the breeding program, and many varieties with resistance to insect pests have been developed (Table 2). However, cultivars with resistance to insect pests are cultivated by farmers only on a limited scale due to an overemphasis by national programs on grain yield as a criterion for release of cultivars. Since sorghum varieties and hybrids with a yield potential of over 10 t ha^{-1} are already available in the market, it is important that insect and disease resistance be used as a criterion to identify varieties and hybrids for use by farmers for sustainable crop production.

Diversification of the cytoplasmic male-sterile systems with resistance to insect pests. Most of the hybrids grown in India are based on *milo* cytoplasm (A_1 cytoplasm), which is highly susceptible to sorghum shoot fly (Dhillon 2004) (Fig. 1). Extensive use of the A_1 cytoplasm as a source of cytoplasmic male-sterility (CMS) has resulted in narrowing of the genetic base of sorghum hybrids currently being cultivated by farmers, and this might increase the vulnerability of this crop to biotic and abiotic stresses. In general, the CMS lines are more susceptible to sorghum shoot fly (*A. soccata*), sugarcane aphid (*M. sacchari),* shoot bug (*P. maidis*), and midge

(S. sorghicola) than the maintainer lines, suggesting that the maintainer lines harbor the factors that influence expression of resistance to insects (Sharma et al. 2004b). Therefore, there is a need to develop a range of CMS, maintainer, and restorer lines with resistance to insect pests, and diversify the CMS systems in sorghum. The A_4M cytoplasm is slightly less susceptible to shoot fly than the other CMS systems. Recovery from shoot fly damage is better in A_4M, A_3, and A_2 cytoplasms than the A_1 cytoplasm. Shoot fly survival and development is also poor on A_4M and A_4VzM CMS systems. The A_4M cytoplasm being less susceptible to shoot fly and having better recovery resistance, can be exploited for developing shoot fly-resistant hybrids in future. However, as a first step, it may be better to transfer the traits associated with resistance to shoot fly into the hybrid parents in A_1 cytoplasm. Another alternative would be to explore opportunities for using male gametocytes and/or temperature and photoperiod-induced male-sterility for sorghum hybrid seed multiplication as these might allow exploitation of the favorable effects of normal maintainer line cytoplasm(s) on expression of resistance to insects in this crop. Of course, the simplest alternative would be to focus on open-pollinated varieties that do not require use of male-sterility for seed multiplication.

Development of CMS and restorer lines for resistance to insect pests. Much of the area under high-yielding sorghum cultivars is sown to hybrids in Asia, Australia, and the Americas. Therefore, it is apparent that for host plant resistance to be an important component of pest management in sorghum, we need to transfer the insect resistance genes into male-sterile, maintainer, and restorer lines that can be used by the public institutions and private seed industry to develop insect-resistant hybrids. The CMS, maintainer, and restorer lines with resistance to shoot fly, stem borer, midge, and head bugs

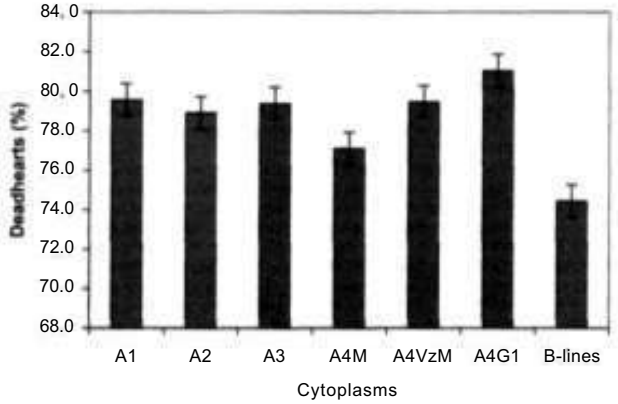

Figure 1. Relative susceptibility of different CMS systems to sorghum shoot fly, *Atherigona soccata.*

have been developed at ICRISAT (Table 3). Much of mis materia] has been shared with public institutions and private seed industry over the past decade for use in sorghum improvement, and for developing high-yielding hybrids with resistance to insects. To develop insect-resistant hybrids, the genes conferring resistance to insect pests need to be transferred into both CMS and restorer lines (Fig. 2a, b) (Sharma et al. 2004b). Hybrids based on resistant x resistant parents exhibit greater resistance than the hybrids based on other cross combinations (Sharma et al. 1996, Sharma et al. 2004b, Dhillon 2004). The hybrids based on susceptible x susceptible parents are highly susceptible. The CMS lines showed a greater influence on the expression of resistance/susceptibility to shoot fly and sorghum midge than the restorer lines. Therefore, efforts should be made to transfer insect resistance genes into a diverse array of both CMS and restorer lines for developing hybrids with resistance to insect pests.

Wild relatives of sorghum as sources of diverse genes for resistance to insect pests. Levels of resistance to sorghum shoot fly and stem borer in cultivated germplam are low to moderate (Sharma et al. 2003). Therefore, it may be important to identify wild relatives of sorghum with high levels of resistance to these pests. Wild relatives of sorghum have been evaluated for resistance to sorghum shoot fly at ICRISAT, and accessions belonging to *Parasorghum (S. australiense, S. purpureosericeum, S. brevicallosum, S. timorense, S. versicolor, S. matarankense,* and *S. nitidum)* and *Stiposorghum (S. angustum, S. ecarinatum, S. extans, S. intrans, S. interjection,* and *S. stipoideum)* did not show any shoot fly damage under multi-choice conditions in me field (Venkateswaran 2003) (Table 4). *Heterosorghum (S. laxiflorum)* and *Chaetosorghum (S. macrospermum)* showed very low damage, while the species belonging to section wild races of *S. bicolor* subsp. *verticilliflorum (aethiopicum arundinaceum, verticilliflorum,* and *virgatum)* were highly susceptible to shoot fly, as was *S. halepense.* Accessions belonging to *Heterosorghum, Parasorghum* and *Stiposorghum* showed little damage by the spotted stem borer under artificial infestation in the field, except for one accession of *Heterosorghum,* which showed *2%* deadhearts (Venkateswaran 2003) (Table 5). In contrast, section *Chaetosorghum (S. macrospermum)* was highly susceptible to stem borer damage. Within the section *Sorghum,* the four wild races of *S. bicolor* subsp. *verticilliflorum* (races *arundinaceum, aethiopicum, verticilliflorum,* and *virgatum)* were highly susceptible to stem borer damage. Sorghum midge females did not lay any eggs in the spikelets of S. *angustum, S. amplum,* and *S. bulbosum* compared to 30 eggs in spikelets of S. *halepense* under no-choice conditions. Odors from the panicles of S. *halepense* are more attractive to the

females of sorghum midge than the odors from panicles of *S. stipoideum, S. brachypodum, S. angustum, S. macropsermum, S. nitidum, S. laxiflorum,* and *S. amplum* (Sharma and Franzmann 2001). The accessions belonging to the secondary gene pool with diverse mechanisms of resistance to insects can be crossed with cultivated sorghum, while those belonging to the tertiary gene pool may require application of embryo rescue techniques to transfer resistance genes from the wild relatives into the cultivated sorghums.

Marker-assisted selection. It takes five to six generations to transfer a trait within a species into high-yielding locally adapted cultivars through conventional breeding, and one has to evaluate a large number of progenies to be able to select the plants with the appropriate combination

Table 1. Germplasm accessions identified to be resistant to insect pests in sorghum.

Insect pest	Germplasm accessions (IS numbers)
Shoot fly	923, 1032, 1034, 1037, 1044, 1054,1071, 1096, 1104, 1119, 2122. 2123, 2146, 2162, 2168, 2195, 2205. 2265, 2269, 2291, 2309, 2312, 2394, 2681, 3461, 3962. 4224, 4273, 4646, 4663, 4664, 4835, 4881, 4981, 5075, 5076, 5078, 5210, 5429, 5469, 5470. 5480, 5484, 5490, 5511, 5538, 5566, 5571, 5604, 5613, 5619, 5622, 5636, 5648, 8064, 8100, 8320, 8571, 8721, 8811, 8887, 8891, 8918, 8922, 8988, 9009, 9692, 6566, 10711, 10795, 12150, 13674, 14108, 15437, 15896, 16235, 16357, 7726, 17742, 17745, 17747, 17750, 17948, 17966, 18274, 18325, 18366, 18368, 18369, 18371, 18476, 18551, 18580, 18635, 18662, 18700, 18733, 19485, 19569, 19706, 20064, 21871, 21877, 21969, 22039, 22114, 22121, 22144, 22145, 22148, 22149, 22196, 25744, and 26789,
Stem borer	923, 1044, 1051, 1082,1096, 1104, 2122, 2123, 2146, 2162, 2195, 2263, 2265, 2269, 2290, 2291, 2292, 2312, 2375, 2376, 3962, 4546, 4637, 4646, 4663, 4664, 4756, 4757, 4776, 4835, 4995, 5072, 5210, 5253, 5268, 5469, 5470, 5480, 5484, 5490, 5511, 5566, 5571, 5579, 5585, 5604, 5613, 5619, 5648, 8811, 5658, 6566, 7224, 8165, 8189, 8549, 8671, 12308, 13100, 17745, 17948, 18333, 18371, 18551, 18573, 18577, 18578, 18579, 18581, 18584, 18585, 18662, 18677, 21883, 22039, 22091, 22113, 22114, 22121, 22129, 22144, 22148, 22196, 22778, 23411, 23962 ,and 24027.
Midge	2290. 2292, 2579, 2687, 2739, 2830, 3461, 6283, 7005, 7134, 7138, 7151, 8100, 8151, 8190, 8196, 8198, 8204, 8577, 8671, 8721, 8729, 8751, 8887, 8891, 8918, 8922, 8988, 10712, 14380, 15107, 8849, 8884, 8946, 9021, 9045, 9107, 9112, 9608, 9807, 18563, 18573, 18695, 18696, 18698, 18733, 19474, 19476, 19512, 19955, 19957, 21006, 21031, 21211, 21871, 21873, 21879, 21881, 21883, 21883-1, 22400, 22464, 22471, 22806, 23748, 26789, 27103, 27466, 31626, 31635,and 31636.
Head bugs	2761, 8064, 14108, 14317, 14334, 14380, 16357, 17610, 17618, 17645, 18579, 19455, 19945, 19948, 19949, 19950, 19951, 19955, 19957, 20024, 20059, 20068, 20638, 20643, 20664, 20740, 21006, 21211, 21443, 21444, 21485, 21525, 21574, 22284, 22507, 23748, 24357, 25069, 25098, 25760, 21512, 25733, 25766, 27329, 27397, 27452, 27466, and 27477.

Sharma et al. (1992. 2003).

Table 2. Sorghum varieties with resistance to insect pests, developed at ICRISAT.

Insect pest	Improved lines with resistance to insects (ICSV numbers)
Shoot fly	700, 701, 702, 705, 707, 708, 711 to 714, 717, 726, 89013, 89018, 89025, 93093, and 25001 to 25055.
Stem borer	700, 708, 711, 714, 717, 89008, 89010, 93046, and 25056 to 25162.
Midge	197, 239, 305, 313, 385 to 395, 563, 564, 573, 693, 729 to 758, 804, 830 to 832, 835, 836, 843, 88006, 88013, 88014, 88028, 88032, 88035, 88041, 89001, 89010, 89031, 89034 to 89039, 89042 to 89044, 89049 to 89054, 89057, 89058, 90001 to 90010, 90014, 90016, 90018, 91015, 91025, 92011 to 92013, 92015 to 92018, 92020, 92021, 92023, 92024, 93001 to 93026, 93035. 93046, 93057, 93059, 93065 to 93067, 93069, 95071 to 93093, 95080, 95123 to 95125, 96009 to 96011, 96027, 96031,96062 to 96082, and 25163 to 25244,.
Head bugs	25245 to 25263.

Agarwal BL, Sharma HC, Taneja SL, Rcddy BVS, and Stenhouse JH (unpublished).

of traits. The use of DNA markers for indirect selection offers great potential gains for quantitative traits with low heritability, as these are the most difficult characters to work with in the field using direct phenotypic selection. The effectiveness of a marker-assisted selection (MAS) can only be as good as the quality of the phenotypic data on which the development of the marker was based. At ICRISAT, mapping populations have been phenotyped and genotyped for sorghum shoot fly (296B x IS 18551 and BTx 623 x IS 18551), and spotted stem borer, sorghum midge, and aphid (ICSV 745 x PB 15881-3). Genetic linkage maps based on these populations have been constructed to identify quantitative trait loci (QTLs) associated with resistance to these insects. Polymorphic simple sequence repeat (SSR) loci associated with resistance to shoot fly and the traits associated with resistance to this insect have been identified (Folkertsma et al. 2003). These QTLs are now being transferred into locally adapted hybrid parental lines via SSR based MAS. The QTLs associated with antibiosis and antixenosis mechanisms of resistance to sorghum midge (Tao et al. 2003), and tolerance to green bug (Nagaraj et al. 2005) have also been identified (Table 6). It is hoped that MAS will allow for rapid introgression of the resistance genes, and ultimately gene pyramiding, into the high-yielding varieties and hybrids.

Table 3. Cytoplasmic maintainer and male-sterile lines with resistance to insect pests, developed at ICRISAT.

Shoot fly	Stem borer	Midge	Head bugs
ICSB 415, ICSB 416, ICSB 417, ICSB 418, ICSB 419, ICSB 422, ICSB 423, ICSB 425, ICSB 428, ICSB 429, ICSB 432. ICSB 433, ICSB 434, and ICSB 435	ICSB 464, ICSB 467, ICSB 468, ICSB 469, ICSB 472, ICSB 473, and ICSB 474	ICSB 488, ICSB 493, ICSB 494. ICSB 502, ICSB 505, ICSB 508, ICSB 512, ICSB 513, ICSB 516, ICSB 518, ICSB 520, ICSB 524, ICSB 527, and ICSB 541	ICSB 547, ICSB 548, ICSB 550, ICSB 552, ICSB 553, ICSB 555, ICSB 557, and ICSB 563

Reddy BVS, and Sharma HC (unpublished).

Table 4. Relative susceptibility of wild relatives of sorghum to shoot fly, *Atherigona soccata.*

Section	Species	Accession	Deadhearts (%)		Adult emergence (%)
			Field conditions	No-choice conditions	
Chaetosorghum	*Sorghum macrospermum*	TRC 24112	6.7	61.5	-
Heterosorghum	*S. laxiflorum*	IS 18958	0.0	7.4	6.2
Parasorghum	*S. australiense*	IS 18954	0.0	10.1	4.2
	S. matarankense	TRC 243576	0.0	0.0	-
	S. purpureosericeum	IS 18943	0.0	50.3	-
	S. nitidum	TRC 243514	0.0	9.7	-
	S. timorense	TRC 243498	0.0	21.1	-
	S. versicolor	IS 23177	0.0	6.2	-
Stiposorghum	*S. angustum*	TRC 243499	0.0	4.0	0.0
	S. ecarinatum	TRC 243574	0.0	3.5	-
	S. intrans	TRC 243571	0.0	1.1	-
	S. extans	TRC 243601	0.0	0.0	-
	S. interjectum	TRC 243461	0.0	1.2	-
	S. stipoideum	TRC 243399	0.0	0.0	-
Sorghum	*S. aethiopicum*	IS 27584	88.9	-	99.5
	S. virgatum	IS 18808	92.2	-	89.0
	S. bicolor	CSH 11	96.7	-	-
		S 18551	30.6	70.2	50.8
LSD (P 0.05)			5.8	19.6	18.9

Venkateswaran (2003).

Development of insect-resistant transgenic sorghums. Given the wide host range of some of the insect pests, and low levels of resistance in the cultivated germplasm against stem borers, head bugs, and armyworms, it would be highly desirable to combine conventional plant resistance with novel genes from *Bacillus thuringiensis,* protease inhibitors or plant lectins. For plant resistance to be successful in integrated insect pest management, they have to substitute completely or partially for the use of insecticides and/or other methods of pest management, and result in improved economic returns and reduced environmental impact. In addition to the reduction in losses due to insect pests, the deployment of transgenic plants with insecticidal genes will also lead to: i) reduction in insecticide sprays, ii) reduced exposure of farm labour to insecticides, iii) reduction in harmful effects of insecticides on nontarget beneficial organisms, iv) increased activity of natural enemies, v) reduced amounts of pesticide residues in food and food products, and vi) a safer environment to live in. The first transgenic plants were developed in the mid-1980s (Vaeck et al. 1987). Since then, there has been a tremendous progress in development and deployment of transgenic plants for insect resistance (Sharma et al. 2002, James 2003). Toxins from *Bacillus thuringiensis* var *morrisoni* have shown biological activity against the sorghum shoot fly, *A. soccata.* The *B. thuringiensis* toxins Cry 1 Ac and Cry2A are moderately effective against spotted stem borer, *C. partellus,* while Cry 1 Ac is effective against *H. armigera* (Sharma et al. 2004a). Sorghum plants having *cry1Ac* gene have been developed at ICR1SAT, and are presently being tested for resistance to spotted stem borer, C. *partellus* (Girijashankar et al. 2005). Combining transgenic resistance to insects with conventional plant resistance will render plant resistance an effective component for pest management in sorghum.

Genetic engineering of metabolic pathways. Many secondary plant metabolites such as flavonoids have been implicated in host plant resistance to insects in sorghum. Many compounds of the flavonoid biosynthetic pathway accumulate in response to biotic and abiotic stresses (Heller and Forkman 1993). Genetic engineering offers the opportunity to change the metabolic pathways to increase the amounts of various flavonoids that play an important role in host plant resistance to insect pests. Biotechnology also offers the opportunity to increase the production of secondary metabolites in plants to increase the levels of resistance to insect pests or inhibit the production of toxic metabolites such as HCN in forage sorghum.

Inducible resistance to insect pests - gene switches. A wide range of inducible genes have been identified in plants based on endogenous chemical signals such as phytohormones, response to insect attack, or wounding. Chemically induced expression systems or "gene switches" enable the temporal, spatial, and quantitative control of genes introduced into crop plants, or those that are already present in the plants. The best-studied system

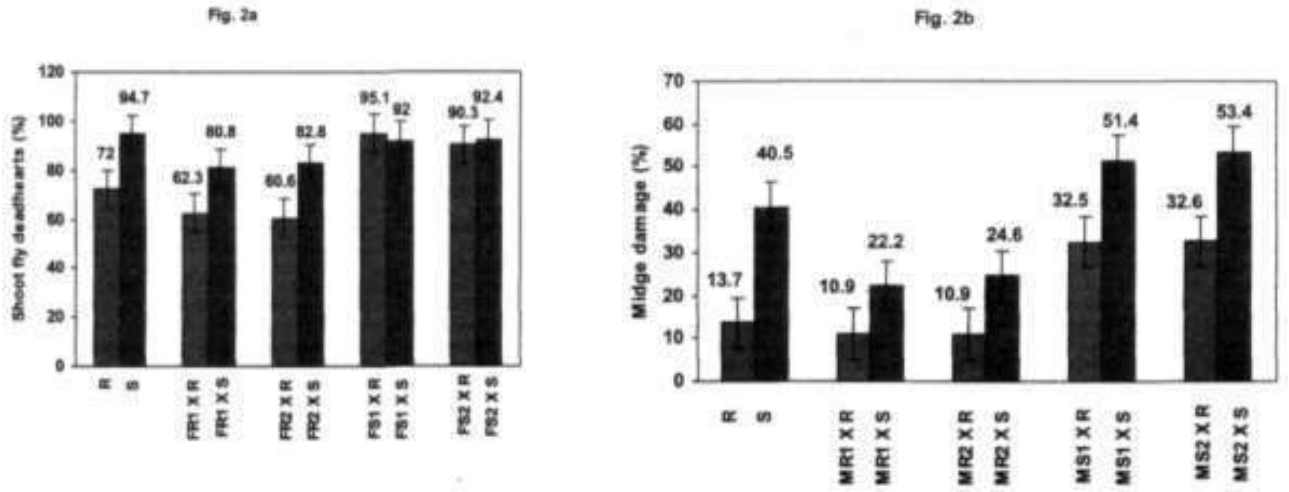

Figure 2. Insect damage in sorghum hybrids based on resistant (R) and susceptible (S) male-sterile and restorer lines, a: shoot fly, Atherigona soccata (FRI = SPSFR 94006, FR2 = SPSFR 94007, FS1 = ATx623, and FS2 = CK 60A). b: sorghum midge, Stenodiplosis sorghicola (MRI = ICSA 88019, MR2 = ICSA 88020, MS1 = 296A, and MS2 - ICSA 42). FR = Resistant female. FS = Susceptible female. MR = Resistant male. MS = Susceptible male. R = Resistant. S = Susceptible.
Sharma et al. (2004b).

utilizes pathogenesis-related protein-la (*PR 1-a)* gene expression in tobacco (Uknes et al. 1993). *PR 1-a* mRNA levels can also be induced by exogenous application of salicylic acid (Ward et al. 1991). Peptide hormones also induce production of proteinase inhibitors. Systemically induced responses are modified through synthesis and action of jasmonic acid via its lipid precursor, e.g., linoleic acid in tomato. Application of exogenous jasmonate induces the production of proteinase inhibitors. Enhanced resistance in transgenic rice plants by application of methyl jasmonate and abscisic acid has been reported (Xu et al. 1993).

Dominant repressible lethal genetic system. Traditionally, the sterile insect technique has been employed to control several insect pests. However, this system depends on

Table 5. Relative susceptibility of wild relatives of sorghum to spotted stem borer, *Chilo partellus.*

Section	Species	Accession	Deadhearts (%) Field conditions	Deadhearts (%) Greenhouse conditions	Larvae recovered (%)
Chaetosorghum	*Sorghum macrospermum*	TRC 24112	72.9	-	-
Heterosorghum	*S. laxiflorum*	IS 18958	0.0	82.5	6.0
		TRC 243492	0.0	15.3	0.0
Parasorghum	*S. australiense*	IS 18955	0.0	10.5	0.0
	S. matarankense	TRC 243576	0.0	5.2	0.0
	S. nitidum	TRC 243514	0.0	0.0	0.0
	S. purpureosericeum	RN 285	0.0	11.1	0.0
	S. timorense	TRC 243498	0.0	0.0	0.0
	S. versicolor	IS 14262	0.0	0.0	0.0
Stiposorghum	*S. angustum*	TRC 243499	0.0	0.0	6.0
	S. ecarinatum	TRC 243574	0.0	0.0	0.0
	S. extans	TRC 243601	0.0	0.0	0.0
	S. intrans	TRC 243571	0.0	0.0	0.0
	S. interjectum	TRC 243461	0.0	0.0	0.0
	S. stipoideum	TRC 243399	0.0	0.0	0.0
Sorghum	*S. aethiopicum*	IS 27584	86.7	-	-
	S. virgatum	IS 18808	94.5	98.2	55.0
	S. biculor	CSH 11	95.5	98.4	90.0
		S 18551	58.0	96.8	40.0
LSD (P 0.05)			10.5	4.4	-

Venkateswaran (2003).

Table 6. Molecular makers identified to be associated with resistance to insect pests in sorghum.

Linkage group (LG)	Primers	Linked traits/mechanisms
Sorghum shoot fly. *Atherigona soccata*		
LG F	*Xtxp 258* (bp 190/230) *Xtxp 289* (bp 270/294)	Trichome density
LG G	*Xgap 1* (bp 180/254) *Xtxp 141* (bp 154/169)	Deadhearts, leaf glossiness, and trichome density
LG I	*IS 328* (bp 144/166) *IS 264* (bp 153/207)	Leaf glossiness
LG J	*IS 258* (bp 170/193) *Xtxp 65* (bp 125/134)	Deadhearts and leaf glossiness
Sorghum midge. *Stenodiplosis sorghicola*		
LG A	*RZ* 543 *ST* 698	Antixenosis mechanism of resistance
LG G	*ST* 1017 *SG* 14	Antixenosis mechanism of resistance
LG J	7X5 1931 *SG* 37	Antibiosis mechanism of resistance
Green bug, *Schizaphis graminum*		
LG 3	*Xtxp 12 Xcup 20 Sbl* 10	Tolerance mechanism of resistance
LG 5	*Xt xp* 43 *Xtxp* 85 *Xtxp* 335 *Xtxp* 204	Tolerance mechanism of resistance

Folkertsma et al. (2003), Tao et al. (2003), and Nagaraj et al. (2005).

large-scale production of the target insect, and use of irradiation or chemical sterilization. Release of insects carrying a dominant lethal (RIDL) gene has been proposed as an alternative to the conventional techniques used for insect sterilization (Alphey and Andreasen 2001). This is based on the use of a dominant, repressible, female-specific gene for insect control. A sex-specific promoter or enhancer gene is used to drive the expression of a repressible transcription factor, which in turn controls the production of a toxic gene product. A non-sex specific expression of the repressible transcription factor can also be used to regulate a selectively lethal gene product. Insects produced through genetic transformation using this approach do not require sterilization through irradiation, and could be released in the ecosystem to mate with the wild population to produce sterile insects that will be self-perpetuating.

Need for future research

- Improvement in precision of screening and selection criteria for resistance to insect pests.
- Gene pyramiding and development of cultivars with multiple resistance to insect pests and diseases.
- Transfer of insect resistance genes into CMS, maintainer, and restorer lines, and exploitation of alternate CMS systems that are less susceptible to insect pests.
- Identification of toxin genes for shoot fly, stem borer, and head bugs, and development of transgenic plants with resistance to insect pests.
- Identification of molecular markers associated with resistance to shoot fly, midge, stem borer, aphids, and head bugs for use in MAS.

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Registration of ICSV 88032: A High Yielding Line Resistant to Sorghum Midge, *Stenodiplosis sorghicola*

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Sorghum [*Sorghum bicolor* (L.) Moench] midge [*Stenodiplosis sorghicola* (Coquilett)] is one of the most important pests of grain sorghum in Asia, Africa, Australia, and the Americas (Harris 1976, Sharma 1993). The larvae of sorghum midge feed on the developing ovary resulting in the production of chaffy spikelets. The damaged panicles present a blasted appearance. Midge damaged spikelets have a pupal case attached to the glumes or have a small exit hole of the midge parasite on the upper glume. Females lay 30-100 eggs singly in the spikelets at flowering during the morning hours, and die by the afternoon. Eggs hatch in 1-4 days. The larvae suck the contents of developing ovaries and complete development in 7-12 days. Larvae pupate inside the glumes, and the pupal period lasts for 3-8 days. Adults live for 2-48 h. Maximum midge abundance occurs during September-October. A small proportion of the larvae enter diapause in the spikelets in each generation, which may last as long as 3 to 4 years.

It is difficult to control sorghum midge with conventional insecticides, as the larvae remain hidden inside the spikelets. Therefore, it is important to develop midge-resistant cultivars to minimize the extent of losses due to this pest (Sharma 1993). Substantial progress has been made in identification and utilization of resistance to sorghum midge (Sharma et al. 1993). The accessions IS 2579C, IS 12666C, TAM 2566, AF 28, DJ 6514, IS 10712, IS 7005, IS 8891, and IS 8721 are diverse sources of resist-ance to sorghum midge (Sharma 1993), and efforts have been made to transfer resistance into high yielding cultivars in Asia, Australia, and USA. As a result of intensive efforts to breed for resistance to this pest, several midge-resistant varieties with high grain yield have been developed for cultivation by farmers or for use in the national sorghum improvement programs (Johnson et al. 1973, Agrawal et al. 1987, Sharma et al. 1994).

Sorghum line ICSV 88032 is highly resistant to sorghum midge, early, and less susceptible to leaf diseases. It combines high levels of resistance to sorghum midge with yield potential comparable to commercial cultivars. ICSV 88032 (PM 15936-1) is derived by pedigree breeding from a cross between ICSV 197 (midge-resistant line) and ICSV 1 (high-yielding sorghum variety). Its selection number is (ICSV 197 x ICSV 1) -22-1-1-1. The

segregating and the advanced lines were screened and selected for resistance to sorghum midge using the infester row and no-choice headcage screening techniques (Sharma et al. 1988a, b, 1992). In the international sorghum variety and hybrid adaptation trial, ICSV 88032 yielded 4.8 to 5.2 t ha^{-1} during the 1990-91 season compared to 5.0 to 5.3 t ha^{-1} for ICSV 112, a released commercial cultivar at Bhavanisagar and Patancheru (Table 1). At Surat, its grain yield was 2.6 and 4.6 t ha^{-1} compared to 2.9 and 3.4 t ha^{-1} for ICSV 112 during 1990 and 1991, respectively. In the preliminary variety trials of AICSIP, it yielded 3.1 t ha^{-1} compared to 2.81 ha^{-1} for ICSV 112 in 1990/91 (Table 2). In the 1991 and 1992 advance variety trials of AICSIP, it yielded 4.0 and 3.6 t ha^{-1} compared to 4.2 and 3.1 t ha^{-1} for ICSV 112, respectively.

The plant height of ICSV 88032 is 185 to 201 cm, and plant color is tan. Leaves are drooping with yellow midrib, and the leaf sheath encloses the stem. The stem is thin and non-juicy. Panicles are long and loose with long rachis. Glumes are straw colored covering l/3rd of the grain. It flowers in 67 to 68 days, and matures in 103 to 111 days compared to 112 to 115 days for CSV 10 and CSH 5. Grain of ICSV 88032 is pearly white, shining, plump, without sub-coat, and with white endosperm. Grain weight is 25 g per 1000 grains. Because of its pearly white grain, grain and food quality characteristics are comparable to ICSV 112.

ICSV 88032 is highly resistant to sorghum midge. It suffered 12-14% midge damage compared to 18-20% in the resistant check, DJ 6514; and 90 to 94% in the susceptible check, CSH 1 (Table 3). Visual damage ratings varied from 2.1-3.4 compared to 1.3-1.8 in DJ 6514, and 8.4-9.0 in CSH 1. Its resistance to sorghum midge has been confirmed across locations in India (Table 4), Latin America, and West Africa. During 1995/96, ICSV 88032 suffered 16-31% midge damage compared to 9-26% in ICSV 197 (resistant check), and 38-83% in Swarna - the susceptible check. The loose panicle of ICSV 88032 makes it less susceptible to head bugs and earhead caterpillars. Its susceptibility to shoot fly and stem borer is similar to that of ICSV 112 and CSH 5.

ICSV 88032 can be used for cultivation in midge-endemic areas. It can be used as a donor for combining resistance to sorghum midge, leaf diseases, grain quality, and high yield. It can escape terminal drought because of

Table 1. Grain yield (t ha^{-1}) of ICSV 88032 in India in the 1990-1991 International Sorghum Variety and Hybrid Adaptation Trial.

Genotype	Bhavanisagar 1990	Patancheru 1990	Patancheru 1991	Surat 1990	Surat 1991
ICSV 88032	5.1	5.2	4.8	2.6	4.6
Controls					
ICSV 112	5.0	5.1	5.3	2.9	3.4
CSH 11	4.7	6.7	6.0	4.0	5.4
Trial mean	4.01	4.6	4.4	3.0	4.6
SE ±	0.79	0.32	0.23	0.19	0.29

Table 2. Performance[1] of ICSV 88032 across nine locations in India (All India Coordinated Sorghum Improvement Project Trial, 1990/91).

Genotype	Plant ht (cm)	Days to 50% flowering	Days to maturity	Fodder yield (t ha^{-1})	Grain yield (t ha^{-1})
ICSV 88032	198	68	111	8.5 (18)	3.06 (2)
Controls					
CSV 10	219	74	112	9.5 (10)	2.62 (10)
ICSV 112	193		115	9.3 (13)	2.84 (7)
Trial mean	217	76	114	9 3	2.45
LSD at 5%	26	3	5	1.51	0.64

[1] Mean across nine locations (Parbhani, Akola, and Karad (Maharashtra), Dharwad (Karnataka), Patancheru (Andhra Pradesh), Surat (Gujarat), Udaipur (Rajasthan), and Kanpur and Jhansi (Uttar Pradesh).
Figures in parentheses indicate the ranking in the trials.

Table 3. Relative susceptibility of ICSV 88032 to sorghum midge under no-choice headcage screening and natural infestation (ICRISAT, Patancheru, 1985-88).

Genotype	Damage rating[1]		Midge damage (%)	
	Natural infestation	Headcage screening	Natural infestation	Headcage screening
ICSV 88032	3.4 ± 0.76	2.1 ±0.13	14	12
Controls				
DJ 6514 (R)	1.3 ±0.14	1.8 ± 0.43	18	20
CSH 1 (S)	8.4 ± 0.28	9.0 ±0.16	90	94
SE±	-	-	6.7	7.5
LSD at 5%	-	-	18.4	21.0

[1]Damage rating (1=<10% midge damage, and 9 = > 80% midge damage).
R = Resistant check. S = Susceptible check.

Table 4. Resistance of ICSV 88032 to sorghum midge across locations in India (1986-88).

Genotype	Dharwad	Patancheru		Bhavanisagar	Warangal	Mean
		Rainy season	Postrainy season			
ICSV 88032	3.5[1]	2.0	2.0	3.0	3.0	3.5 ± 0.20
Controls						
DJ 6514 (R)	2.0	1.0	1.5	2.0	1.0	1.5 ± 0.20
CSH 1 (S)	9.0	8.0	9.0	8.0	8.0	8.4 ± 0.21
SE±	0.21	0.16	0.18	0.21	0.18	-
LSD at 5%	0.57	0.45	0.49	0.58	0.51	-

[1] Damage rating (1 =<10% midge damage, and 9 = > 80% midge damage). R = Resistant check. S = Susceptible check.

its early maturity. It has hard corneous grain, which is associated with good grain quality. The use of midge-resistant varieties in integrated pest management in sorghum is promising as the levels of resistance to sorghum midge are quite high. This new cultivar will provide greater flexibility in planting times to obtain maximum yields, and proper utilization of available rainfall without risking midge damage.

This line has been released as ICSV 88032 by the Plant Material Release Committee of ICRISAT, and the seed is available in the Genebank at ICRISAT.

Acknowledgments. We thank the staff of breeding and entomology for their help in developing these lines.

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Registration of Sorghum Varieties ICSV 735, ICSV 758, and ICSV 808 Resistant to Sorghum Midge, *Stenodiplosis sorghicola*

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Sorghum [*Sorghum bicolor* (L.) Moench] is one of the most important cereals in the semi arid tropics (SAT). It provides food, feed and forage, but grain yields on peasant farms are generally low, partly due to insect pest damage. Nearly 150 species of insects have been recorded as pests of sorghum, of which sorghum midge [*Stenodiplosis sorghicola* (Coquillett)] is the most important worldwide (Harris 1976). As a result of feeding by the sorghum midge larvae on the developing ovary, the damaged spikelets become chaffy. Midge damage is sometimes confused with poor seed setting due to unfavorable weather, genetic sterility, and damage by head bugs and other insects (Sharma 2001). The midge-damaged panicles have pupal cases attached to the tip of the damaged spikelets, and often have a pinhole in the glumes, through which midge parasites have emerged. Sorghum midge is widely distributed in Asia, Australia, Americas, Mediterranean Europe, and Africa (CIE 1990). It has spread as diapausing larvae in chaffy spikelets in sorghum seed to most of the countries where sorghum is grown. Annual losses due to sorghum midge have been estimated to be $ 292 million in the SAT (ICRISAT 1992).

Early planting, cultural practices, natural enemies, resistant varieties, and insecticides have been recommended for pest management in sorghum. However, it is difficult to plant at times when insect damage can be avoided. Insecticides are costly, and beyond the reach of resource-poor farmers in the SAT. Therefore, it is important to develop cultivars with resistance to sorghum midge which maintains high grain yield. Nearly 15,000 sorghum germplasm accessions have been screened for resistance to sorghum midge at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India, and 25 lines have been identified as resistant to sorghum midge across seasons and locations in India. The germplasm accessions IS 2579C, TAM 2566, AF 28, DJ 6514, IS 3461, IS 8918, IS 8891, IS 7005, IS 10712, IS 22881, and IS 27103 are stable and diverse sources of resist-ance to sorghum midge (Sharma et al. 1993, Henzell et al. 1997). Efforts to develop sorghum cultivars with resistance to sorghum midge were initiated in the USA under the sorghum conversion program (Johnson et al. 1973), at ICRISAT (Sharma et al. 1993), and in Australia (Henzell et al. 1997), and several lines with high levels of resistance to sorghum midge have been developed. The midge-resistant varieties ICSV 735, ICSV 758, and ICSV 804 developed at ICRISAT have been found to perform well across locations in Myanmar, and have been released.

The sorghum midge-resistant varieties ICSV 735, ICSV 758, and ICSV 804 have been released as Yezin 6, Yezin 7, and Yezin 5, respectively in Myanmar. These varieties combine resistance to sorghum midge with yield potential close to the commercial cultivars Yezin 1 and Yezin 3. ICSV 735 (PM 14355-2-6) is derived from (ICSV 197 x ICSV 1)-9-1-1-2-6, ICSV 758 (PM 14403-1-1)

Table 1. Grain and fodder yield of midge-resistant sorghum genotypes fertilized with farmyard manure across three locations (Yezin Elite Sorghum Variety Trial 1993-94, Myanmar).

	Grain yield (t ha^{-1})				Fodder yield (t ha^{-1})			
Variety	Myingyan	Mahlaing	Zaloke	Mean	Myingyan	Mahlaing	Zaloke	Mean
ICSV 735	1.417	2.421	0.628	1.489	8.7	8.4	0.4	5.8
ICSV 758	1.309	3.533	1.004	1.947	3.4	6.9	0.3	3.6
ICSV 804	1.130	3.371	0.663	1.721	4.5	8.3	0.4	4.4
Control								
Local variety	0.502	1.094	2.659	0.622	6.2	12.3	1.5	6.7
SE	±0.1797	±0.3293	±0.1612	±0.1726	±0.60	±0.60	±0.10	±0.40

Table 2. Morphological characteristics of sorghum midge-resistant genotypes ICSV 735, ICSV 758, and ICSV 804.

Plant character	ICSV 735	ICSV 758	ICSV 804
Plant color	Tan	Tan	Tan
Leaf mid-rib color	White	White	White
Inflorescence compactness	Compact and elliptical	Semi-compact and broad at the tip	Semi-compact and broad at the tip
Glume color	Straw	Straw	Straw
Glume covering	1/3rd	1/3rd	1/3rd
Awns	Awnless	Awnless	Awnless
Grain color	Pearly white	Pearly white	Pearly white
Grain shape	Globular	Hat	Round
Endosperm	White and corneous	White and corneous	White and corneous
Threshability	Easy	Easy	Easy
Boot leaf	Small and erect	Long and erect	Small and erect
Leaves	Broad and erect	Broad and semi-drooping	Narrow and erect
Leaf sheath	Covering half of the next node	Covering the internode	Covering the internode
1000 grain mass (g)	19.17	28.04	25.30

from (ICSV 197 x A 13108)-I-2-I-I-I, and ICSV 804 (PM 14350) from (ICSV 197 x ICSV 1)-3-1-1-1-1. These varieties have been developed through pedigree breeding, and the segregating material has been selected for resistance to sorghum midge under field and no-choice headcage screening (Sharma et al. 1992). The grain yield of ICSV 735, ICSV 758, and ICSV 804 was 1.489, 1.949, and 1.721 t ha^{-1}, respectively compared to 0.622 t ha^{-1} for the local check in 1993/94 rainy season (Table 1). Under fertilizer application, grain yields of ICSV 735, ICSV 758, and ICSV 804 was 2.878, 3.389, and 3.416 t ha^{-1} compared to 1.910 t ha^{-1} for the local check. At ICRISAT Center, these varieties yielded 4.65 to 7.65 t per ha during the 1997 rainy season. The plant height of ICSV 735, ICSV 758, and ICSV 804 is 196, 236, 271 cm, respectively (Table 2). Days to 50% flowering ranged from 79-84 days for ICSV 735, 79-82 days for ICSV 758, and 78-84 days for ICSV 804 (Table 3). These lines are relatively less susceptible to leaf diseases than ICSV 1.

These lines are comparable to the resistant checks, DJ 6514 and ICSV 197 in midge resistance (Table 4). These are also less susceptible to the aphids, but as susceptible to shoot fly, head bugs, and stem borer as the commercial cultivars, ICSV 1 or CSH 9. Grains of ICSV 735, ICSV 758, and ICSV 804 are creamy white, shining, and with corneous endosperm. Grain mass per 1000 grain is 19.2 g for ICSV 735, 28.0 for ICSV 758, and 25.3 g for ICSV 804. Grain and food quality of these lines is comparable to commercial cultivars (CSH 9 and ICSV 1). These lines can be grown in midge-endemic areas as dual-purpose varieties, and have been released in Myanmar for this purpose. They can also be used as a base material for sorghum midge and leaf disease resistance in sorghum improvement. These lines have been used in the breeding program in Myanmar. ICSV 735 has also been distributed widely to farmers in Andhra Pradesh as a dual-purpose variety through the Indo-Swiss livestock project.

Significant progress has been made in developing sorghum cultivars with resistance to sorghum midge. There is a need to transfer midge resistance into cultivars with adaptation to different agro-ecosystems. Sorghum midge-resistant varieties exercise a constant and cumulative effect on insect populations over time and space. Sorghum midge-resistance will form the backbone of pest management in sorghum for sustainable crop production and environment conservation.

These varieties have been released as ICSV 735, ICSV 758, and ICSV 804 by the Plant Material Release Committee of ICRISAT, and their seed is available in the Genebank at ICRISAT.

Acknowledgments. We thank the staff of breeding and entomology for their help in developing these lines.

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Table 3. Sorghum midge and stem borer damage, grain yield, and days to 50% flowering in six sorghum genotypes across locations in India (1995-97).

Genotype	Midge damage (%)			Peduncle damage by stem borer (%)	Grain yield per plant (g)	Days to 50% flowering		
	Rahuri 1997	Dharwad 1995	Mean	Akola 1995	Dharwad 1995	Parbhani 1996	Parbhani 1997	Mean
ICSV 735	13.0	20.4	17.0	69.8	37.3	79.0	84.0	84.0
ICSV 758	13.7	36.3	25.0	55.4	22.7	79.0	82.0	82.0
ICSV 804	13.8	17.5	15.6	53.1	18.3	78.0	84.0	84.0
Controls								
DJ 6514(R)	4.1	10.3	7.2	61.3	35.7	74.0	83.0	83.0
CSH 9 (S)	–	–	–	58.1	61.3	–	–	–
ICSV 112 (S)	26.6	53.0	39.8	–	61.7	78.0	80.0	80.0
LSD at 5%	4.1	5.5		16.8	7.4	3.0	4.0	-

R = Resistant. S = Susceptible.

Table 4. Sorghum midge damage and agronomic expression of six sorghum lines (ICRISAT Center, 1995 rainy season).

	Midge damage rating[1]			Agronomic score[2]		
Genotype	S 1	S2	Mean	S 1	S2	Mean
ICSV 758	2.5	4.0	3.3	2.0	2.5	2.3
ICSV 804	3.0	3.5	3.3	2.5	2.5	2.5
ICSV 735	2.5	2.5	2.5	2.5	2.5	2.5
Controls						
DJ 6514 (R)	3.5	2.5	3.0	4.0	4.0	4.0
ICSV 197 (R)	3.5	2.5	3.0	2.5	1.5	2.0
Swarna (S)	8.5	9.0	8.8	1.0	1.5	1.3
SE ±	0.7	0.7	0.5	0.4	0.3	0.3
CV %	28.7	34.1	22.3	23.2	23.3	17.0

1. Damage rating (1= <10% midge damage, and 9 = >80% midge damage).
2. Agronomic score (1 = Good, and 5 = Poor).
S 1 and S 2 = First and second sowing, respectively.
R = Resistant. S = Susceptible.

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Plant Defense Responses to Sorghum Spotted Stem Borer, *Chilo partellus* under Irrigated and Drought Conditions

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Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is one of the most important cereal crops in the semi-arid tropics (SAT), and insect pests are a major yield-reducing factor. Sorghum is attacked by nearly 150 insect species, causing an annual loss of over $1 billion in the SAT (ICRISAT 1992). A number of stem borer species have been reported as serious pests of sorghum, of which spotted stem borer, *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae) is an important pest in India (Jotwani and Young 1972) and South and eastern Africa (Ingram 1958). Responses to stem borer infestation are influenced by environmental factors apart from genetic factors and their interactions. Moisture and nutrient availability influence plant growth, which in turn will influence the extent of losses due to stem borer damage. Therefore, we studied the reaction of a diverse array of sorghum genotypes to stem borer damage under irrigated and drought conditions.

Table 1. Reaction of sorghum genotypes to spotted stem borer, *Chilo partellus* damage under irrigated and drought stressed conditions (Kenya, Kiboko 1990–1991).

	Deadhearts (%)		Larvae plants^{-1}		Leaf damage (%)		Peduncle damage score		Recovery score	
Genotype	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought	Irrigated	Drought
ICSH 871001	66.8	76.0	53.0	28.7	95.3	94.3	5.2	7.3	5.3	5.7
ICSH 88065	63.2	67.0	27.7	19.7	100.0	90.7	5.0	6.7	3.5	6.3
ICSH 89020	70.9	80.0	51.3	31.3	93.7	90.0	6.0	6.7	6.0	5.3
ICSH 89034	70.5	81.0	51.0	28.7	93.7	96.3	5.3	7.0	4.3	6.7
ICSH 89051	72.0	73.7	37.7	22.7	100.0	95.0	6.5	7.3	5.3	6.0
ICSH 89123	72.6	70.3	56.7	23.0	100.0	95.0	6.3	7.3	5.5	6.3
ICSH 90002	78.4	74.3	38.0	23.7	99.0	86.3	5.3	6.7	5.0	7.0
ICSV 88002	73.9	67.7	34.0	24.0	94.0	92.0	5.8	7.0	5.3	7.0
ICSV 88013	68.7	85.0	43.0	31.3	91.0	99.0	6.2	8.3	5.7	7.7
ICSV 88032	80.5	71.3	45.3	34.0	96.0	96.3	6.2	6.7	5.2	5.3
ICSV 89101	73.1	77.3	54.3	30.7	93.3	92.7	6.2	6.0	6.0	6.0
ICSV 89106	76.4	68.3	40.2	27.0	99.3	96.0	6.7	7.7	6.2	7.7
IS 8193	72.9	69.0	57.3	30.0	86.3	90.0	5.7	7.7	4.5	7.0
IS 9302	80.8	68.3	29.0	25.0	94.0	74.7	7.0	7.3	5.3	6.3
S DX 106	70.2	90.3	37.7	31.0	97.0	95.7	6.8	6.7	5.8	5.7
KAT 83368	74.7	85.0	40.0	25.7	90.7	97.7	6.0	7.0	5.3	7.0
IS 23496	71.9	83.7	40.3	28.7	97.0	95.0	4.8	7.0	5.0	6.7
IS 23509	52.2	90.3	39.3	30.3	93.0	98.7	5.2	6.0	6.5	6.0
ICSV 401	56.7	83.7	39.0	22.7	93.7	94.7	5.2	6.7	4.5	6.7
ICSV 111	76.9	66.0	33.7	29.3	95.0	87.3	6.2	7.3	5.5	6.7
ISIAP DORADO	81.5	82.0	36.7	22.7	98.0	91.3	6.3	8.0	6.2	7.0
ICSV-CM865132	78.3	77.7	35.7	25.0	98.0	92.7	6.8	6.7	6.5	5.7
SPV 468	69.4	73.7	35.7	25.0	99.3	89.0	5.8	7.3	5.2	5.3
SPV 669	68.3	63.0	41.3	28.0	96.0	86.7	4.2	8.0	5.3	7.3
ICSV 112	62.4	72.0	49.7	27.7	88.0	91.3	4.8	5.3	5.3	5.0
ICSH 110	58.2	63.7	38.0	29.7	93.3	85.7	5.5	7.0	4.8	5.3
Local check	59.0	58.0	44.3	31.0	81.0	76.0	5.3	7.0	5.5	6.3
Mean	70.4	74.8	41.8	27.3	94.7	91.5	5.8	7.0	5.4	6.3
For comparing	LSD	Fp	LSD	Fp	LSD	Fp	LSD	Fp	LSD	Fp
Genotypes (G)	11.39	0.009	7.58	<0.001	8.26	0.005	0.97	<0.001	1.02	0.015
Treatment (T)	3.10	0.006	2.06	<0.001	2.25	0.006	0.27	<0.001	0.28	<0.001
G x T	16.11	0.003	10.73	0.019	NS	0.319	1.38	0.053	1.44	0.003

Note = The F-test was nonsignificant for genotype x treatment x environment, and hence the values in the table are means across seasons. Fp = F-probability. LSD = Least significant difference.

Materials and Methods

The experiments were conducted at the Kenya Agricultural Research Station, Kiboko during the 1990 and 1991 cropping seasons. The test material (27 sorghum genotypes) was sown in four row plots of 2 m row length, and the rows were 75 cm apart. There were three replications in a randomized complete block design (RCBD). Seed were sown five cm below the soil surface. The crop growth was maintained under two moisture regimes i.e., irrigated and non-irrigated (water stressed). Both irrigation regimes received a post-sowing irrigation to maintain uniform plant establishment. Data were recorded on deadheart formation due to stem borer, leaf area (%) damaged, number of larvae per five plants, peduncle damage, and recovery resistance under natural infestation. The number of plants with stem borer deadhearts was recorded at 35 days after seedling emergence (DAE) and expressed as a percentage of the total number of plants. Leaf feeding was evaluated at 20 DAE. The number of larvae was recorded from five randomly selected plants per plot at maturity. The peduncle damage (1 = <10% plants with broken peduncles, and 9 = >90% plants with broken peduncles) and recovery resistance was assessed on a 1 to 9 scale at maturity (1 = most of the damaged plants with 2 to 3 uniform tillers with panicles similar to the main plant, and 9 = <10% plants with tillers and productive panicles). Data were subjected to analysis of variance, and the significance of differences between the genotypes was tested by F-test, while the treatment means were compared by least significant differences (LSD) at P = 0.05.

Results and Discussion

The analysis of variance indicated significant differences due to genotype, treatments (irrigated and non-irrigated), and genotype x treatment interaction in plants with deadhearts, number of larvae, leaf feeding, peduncle damage, and recovery resistance for genotypes, except in case of leaf area damage (Table 1). Deadheart incidence was slightly lower (70.4%) in irrigated plots as compared to drought stressed plots (74.6%). Deadheart incidence ranged from 52.2 to 81.5% under irrigated and 58.0 to 90.3% under non-irrigated conditions. Leaf feeding was greater (94.7%) under irrigated than in the drought stressed plots (91.5%) (except in the case of ICSV 88013, IS 8193, KAT 83368, IS 23509, and ICSV 112). The peduncle damage rating varied from 4.2 to 7.0 under irrigated and 5.3 to 8.3 under drought conditions. Peduncle damage was lower (5.8) under irrigated than under drought stressed (7.0) conditions. The recovery resistance rating varied from 3.5 to 6.5 and 5.3 to 7.7 under irrigated and drought stressed sorghum, respectively. The plant recovery in response to stem borer damage was greater under irrigated condition (5.4) than under drought stress (6.3) (except in the case of ICSH 89020, IS 23509, and ICSV-CM 865132), suggesting that sorghum plants produce more axial tillers following damage by the stem borer to the main plant.

Moisture availability in the soil increases plant growth, and pushes the growing point upwards at a relatively faster rate, and as a result the larvae are not able to cause deadheart formation. Also, optimum moisture results in better nutrient uptake, rendering the plants more healthy and immune to damage by stem borer. Based on significantly lower damage under increased soil moisture, irrigation has been recommended for controlling corn stalk borer, *Elasmopalpus lignosellus* Zeller (All and Gallaher 1977). In the present study, the numbers of stem borer larvae were greater (41.8 larvae per 5 plants) in irrigated than in the drought stressed (27.3 larvae per 5 plants) plots. The moisture content of 10-day-old sorghum seedlings and the central whorl leaf at 20 DAE have been reported to be positively associated with leaf feeding and larval survival (Sharma et al. 1997). Greater plant biomass and more humidity favored the survival and development of stem borer larvae in irrigated plots. Karaman et al. (1998) reported that reduced water availability affected *Chilo agamemnon* Blesz. activity in sugarcane due to lower relative humidity. However, Reynolds et al. (1959) reported that timely irrigation decimated populations of *E. lignosellus* on sorghums in southern California. Irrigation reduces the deadheart incidence, peduncle damage, and recovery resistance in sorghum due to stem borer, and thus irrigation could be recommended as a component for the management of *C. partellus* in sorghum.

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Agronomic Characteristics of Different Cytoplasmic Male-Sterility Systems and their Reaction to Sorghum Shoot Fly, *Atherigona soccata*

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Introduction

The discovery of cytoplasmic male-sterility *(milo* cytoplasm) led to commercial exploitation of hybrid vigor in sorghum (Stephens and Holland 1954). Several CMS systems have been identified in sorghum for diversifying hybrid production. However, only the A_1 CMS system has been deployed for producing sorghum hybrids worldwide, with the exception of A_2 CMS-based hybrids in China (Shan et al. 2000). The use of a single source of male-sterility (A_1 cytoplasm) has narrowed the genetic base of sorghum hybrids. As a result, there is considerable risk of insect pest and disease outbreaks in cultivars based on a single source of male-sterility (Sharma et al. 2004).

Sorghum is damaged by over 150 species of insect pests, of which shoot fly *Atherigona soccata* (Rondani) is important in Asia, Africa, and Mediterranean Europe. Plant resistance is an important component for the management of this pest, and efforts are being made at ICRISAT to transfer resistance genes into male-sterile lines. Since there is considerable risk of single MS system-based hybrids becoming vulnerable to this major pest, it is important to determine the agronomic desirability and the reaction of different CMS systems to sorghum shoot fly, A *soccata.*

Materials and Methods

Plant material. The experimental material consisted of six isonuclear lines in six cytoplasmic backgrounds (A_1 A_2 A_3 A_4G_1 A_4M, and A_4V_7M), and six maintainer (B) lines, the test material was evaluated during the 2002 and 2003 rainy, and 2003 postrainy seasons. Each entry was planted in 4 row plots of 2 m row length, and the rows were 75 cm apart. There were three replications in a randomized complete block design. One week after seedling emergence, thinning was done to maintain a spacing of 10 cm between plants. Normal agronomic practices were followed for raising the crop. At the milk stage, the panicles were covered with nylon bags to avoid damage from birds.

Observations. Data were recorded on numbers of plants with shoot fly deadhearts in the central two rows at 14 days after seedling emergence, and expressed as percentage of plants with deadhearts. Data were also recorded on days to 50% flowering, plant height, and agronomic desirability. Plant height was recorded at maturity. Agronomic desirability was evaluated at crop maturity on a scale of 1 to 5 (1 = good productive potential and ability to withstand insect damage, 5 = poor productive potential and prone to insect damage). The data was analyzed using factorial analysis. The significance of differences between the treatment means was tested using least significant differences (LSD) at P 0.05.

Results and Discussion

There were significant differences among the CMS lines for all the traits under study (Tables 1 to 4). The mean squares due to genotype x CMS systems for plant height, agronomic desirability and shoot fly infestation were nonsignificant (Tables 2, 3, and 4). The isonuclear lines in A_1, A_2, and A_3 cytoplasmic backgrounds flowered 1-2 days earlier than in other CMS backgrounds. Similar results have earlier been reported by Quinby (1970). The A_4G_1 and A_4VzM cytoplasms flowered one-day later than the B-lines. These results are in conformity with those of Nagur and Menon (1974). The isonuclear lines in A_2 cytoplasmic background (except in case of ICSA 26 and ICSA 38) were shorter than in other cytoplasmic backgrounds, but the differences among the CMS systems were nonsignificant (Table 2). Similar observations have been reported by Williams-Alanis and Rodriguez-Herrera (1994). Pederson and Toy (1997) observed similar pattern for plant height in A_1, A_2, and A_3 cytoplasms. The

differences in agronomic score of different CMS systems were nonsignificant (Table 3). Ross and Kofoid (1979) reported comparable agronomic performance and grain yield in different CMS systems. However, Gangakishan and Borikar (1989) and Wang et al. (1990) observed that the hybrids based on *Maldandi* (A_4M) cytoplasm are bold and yield better than those on *milo* cytoplasm. Shoot fly deadhearts in different CMS systems varied from 69.9 to 88.7% (Table 4). The male sterile lines showed more deadhearts [77.1 (A_4M) to 81.0% (A_4G_1)] compared to the maintainer lines (74.4%) (Table 4). Among the cytoplasms tested, A_4M suffered lower deadheart incidence than the other CMS systems. Therefore, it can be exploited for producing shoot fly-resistant hybrids in future (Dhillon et al. 2005).

Conclusion

Isogenic lines in A_1, A_2, and A_3 cytoplasmic backgrounds flowered two days earlier than the other CMS and maintainer lines. The male-sterile lines in A_4G_1 and A_4VzM CMS backgrounds flowered one day later than the maintainer lines. The A_1, A_2, A_3, and A_4VzM CMS lines were comparable in height, but shorter than A_4M and A_4G_1 CMS and B-lines. The differences in agronomic

Table 1. Days to 50% flowering of different cytoplasmic male-sterile (A) and maintainer (B) lines of sorghum (1CR1SAT, Patancheru, India).

	Days to 50% flowering							
Genotypes	A,	A_2	A,	A_4G_1	A_4M	A_4VzM	B	Mean
ICSA 11	71.3	67.0	69.1	72.3	75.1	75.7	74.6	73.2
ICSA 17	71.3	73.0	73.3	71.7	69.7	73.6	70.7	71.4
ICSA 26	75.2	79.5	77.6	79.2	77.7	78.5	75.3	76.6
ICSA 38	72.7	77.2	68.6	76.6	75.6	76.6	80.6	77.6
ICSA 88001	74.7	76.0	73.6	77.7	79.6	76.7	76.9	76.6
ICSA 88004	78.7	75.7	77.6	80.6	78.1	79.0	76.0	77.1
Mean	74.0	74.7	73.3	76.3	75.9	76.7	75.7	
For comparing	SE±		LSD				F-test	
Cytoplasm (C)	0.63		0.83				0.002	
Genotypes (G)	0.58		0.89				<0.001	
CxG	1.54		2.18				0.004	

Genotypes (P = 0.05; df = 5); Cytoplasms (P = 0.05; df = 6); Cytoplasms x genotypes (P = 0.05; df = 30); Error (P = 0.05; df = 82).

Table 2. Plant height at maturity in different cytoplasmic male-sterile (A) and maintainer (B) lines of sorghum (ICRISAT, Patancheru, India).

	Plant height (cm)							
Genotypes	A_1	A_2	A_3	A_4G_1	A_4M	A_4VzM	B	Mean
ICSA 11	101.1	98.6	99.2	105.8	102.8	98.9	100.5	100.8
ICSA 17	88.3	80.0	88.6	94.4	91.1	93.1	86.9	88.1
ICSA 26	102.2	111.7	108.1	99.4	103.1	103.9	110.0	107.4
ICSA 38	104.7	103.1	103.3	101.7	103.1	101.7	109.2	106.1
ICSA 88001	125.0	122.5	122.5	123.3	129.2	126.9	123.3	124.1
ICSA 88004	110.0	108.3	109.2	119.7	112.8	109.2	113.9	112.7
Mean	105.2	104.0	105.2	107.4	107.0	105.6	107.3	
For comparing	SE±			LSD			F-test	
Cytoplasm (C)	1.68			NS			0.737	
Genotypes (G)	1.56			4.34			<0.001	
C x G	4.12			NS			0.748	

Genotypes (P = 0.05; df = 5); Cytoplasms (P = 0.05; df = 6); Cytoplasms x genotypes (P = 0.05; df = 30); Error (P = 0.05; df = 205). NS = Nonsignificant.

Table 3. Agronomic desirability of different cytoplasmic male-sterile (A) and maintainer (B) lines of sorghum (ICRISAT, Patancheru, India).

	Agronomic score[a]							
Genotypes	A_1	A_2	A_3	A_4G_1	A_4M	A_4VzM	B	Mean
ICSA 11	3.2	3.2	3.3	3.5	3.2	2.8	3.4	3.3
ICSA 17	3.3	3.5	3.3	3.2	3.5	3.2	3.5	3.4
ICSA 26	2.8	2.7	2.8	3.0	2.8	2.8	2.8	2.8
ICSA 38	3.2	3.5	3.5	2.8	3.0	2.8	3.2	3.2
ICSA 88001	3.0	3.3	3.2	2.8	3.2	3.0	3.2	3.1
ICSA 88004	2.8	3.2	3.0	2.8	2.8	2.7	2.8	2.9
Mean	3.1	3.2	3.2	3.0	3.1	2.9	3.2	
For comparing	SE±		LSD			F-test		
Cytoplasm (C)	0.10		NS			0.253		
Genotypes (G)	0.10		0.26			<0.001		
C x G	0.24		NS			0.995		

Genotypes (P = 0.05; df = 5); Cytoplasms (P = 0.05; df = 6); Cytoplasms x genotypes (P = 0.05; df = 30); Error (P = 0.05; df = 205). [a] = Agronomic score (1 = good, and 5 = poor). NS = Nonsignificant.

Table 4. Evaluation of different CMS systems of sorghum for susceptibility to shoot fly, *Atherigona soccata* (ICRISAT, Patancheru, India).

	Deadhearts (%) 14 DAE							
Genotypes	A_1	A_2	A_3	A_4G_1	A_4M	A_4VzM	B-line	Mean
ICSA 11	81.1	88.7	83.0	85.0	78.7	77.8	82.3	82.4
ICSA 17	84.0	73.9	74.0	81.0	77.1	78.3	80.7	79.4
ICSA 26	78.7	74.1	81.7	82.0	72.9	80.2	69.9	74.1
ICSA 38	78.8	84.7	81.2	81.1	81.7	81.2	71.9	76.7
ICSA 88001	78.2	78.1	79.1	76.4	75.4	81.2	70.6	74.4
ICSA 88004	77.1	74.0	77.4	80.7	76.9	78.1	71.1	74.2
Mean	79.6	78.9	79.4	81.0	77.1	79.5	74.4	
For comparing	SE±			LSD			F-test	
Cytoplasm (C)	1.33			3.71			0.016	
Genotypes (G)	1.23			3.43			0.005	
C x G	3.26			NS			0.314	

DAE = Days after seedling emergence. Genotypes (P = 0.05; df = 5); Cytoplasms (P = 0.05; df = 6); Cytoplasms x genotypes (P = 0.05; df = 30); Error (P = 0.05; df = 328). NS = Nonsignificant.

desirability of different CMS systems were nonsignificant. The A_4M (*Maldandi*) cytoplasm was less susceptible to sorghum shoot fly, *A. soccata,* and can be exploited for producing sorghum hybrids with less susceptibility to sorghum shoot fly.

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Morphology of Sorghum Grain in Relation to Resistance to Maize Weevil

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Introduction

The maize weevil *(Sitophilus zeamais)* is one of the most destructive insect pests of stored grain, including sorghum *[Sorghum bicolor* (L.) Moench] (Teetes et al. 1981, Teetes and Pendleton 2000). This weevil is abundant in warm, humid regions of the world. Maize weevils infest developing kernels in the field and storage. A female chews a cavity to deposit an egg in a kernel. The larva develops inside and damages the kernel.

Use of sorghum cultivars that resist damage in the field and in storage is an alternative to the use of insecticide. Chitio (2004) evaluated resistance to maize weevils in grain of 20 genotypes of sorghum. The goal of this research was to relate morphology of the sorghums to resistance to maize weevil.

Materials and Methods

Chitio (2004) measured grain weight, size, hardness, and protein content and evaluated resistance to maize weevils of 20 genotypes of sorghum (ATx623, ATx631, ATx635, B1, CE151, Kuyuma, Macia, Malisor84-7-167, Malisor84-7-476, RTx430-5362, RTx430-5451, Segaolane, SC630-11E11, Sima, SRN39, Sureno, Tegemeo, Tx2737, Tx2882, and Tx2911). One gram of grain of each genotype was weighed and the number of grains per gram counted to detemine the weight of an individual grain. This was repeated five times for each genotype. A Vernier caliper was used to measure the length, width, and height in millimeters of each of five grains of each genotype.

The density method was used to determine hardness of four 25-g samples of grain of each genotype. The grain was weighed and dried for 24 hours at 89°C in an oven. Each sample of grain was weighed again and put with 70 ml of water into a 100-ml glass graduated cylinder. The amount of water displaced by the weight of the grain was used as the volume of the grain. The dry weight of the grain was divided by the volume of the grain to determine the density of the grain in g ml^{-1}. The nitrogen content of grain of each genotype was determined by using a LECO model CN-2000 Carbon/Protein/Nitrogen Elemental Analyzer and converted to the amount of protein.

Five grams of sorghum grain were infested with three female and two male newly emerged maize weevils per each of 10 vials of the 20 genotypes of sorghum. Vials of each sorghum genotype were evaluated every 3 weeks for 105 days. Each day, each grain in the 10 vials of one kind of sorghum was evaluated for damage, numbers of live and dead weevil adults were counted, and the grain in each vial was weighed. A scale of 1-5 was used to score damage, where 1 = no evidence of damage; 2 = some feeding on the surface, involving 1-25% or one shallow hole in a kernel; 3 = two tunnels, causing 26-50% damage to a kernel; 4 = 51-75% damage or more than two holes in a kernel; 5 = 76-100% damage and many tunnels in a kernel.

For microscopic observation, grains of the sorghums were split, exposed to osmium vapor, and coated with gold-palladium. The cross-section of seed coat was observed by using a JEOL JSM 6400 at 15 KeV, 12-mm working distance, and 500-2000x magnifications. Pieces of seed coat were dried, fixed, and embedded in epoxy resin and sectioned for observation by using a Zeiss Axiophot compound light microscope at 100-600x magnifications.

Table 1. Mean weight, size, hardness, and protein content of individual grains of Sureno and SC630-11E11 sorghums; numbers of live, dead, and total maize weevil adults per gram; damage; and weight (± SEM) at 105 days after infestation of stored grain with maize weevils.

	Sureno	SC630-11E11
Grain weight (g)	0.021 ± 0.0003	0.026 ± 0.0005
Grain length (mm)	4.0 ± 0.07	4.2 ±0.12
Grain width (mm)	3.5 ± 0.05	4.0 ±0.11
Grain height (mm)	2.3 ± 0.04	2.8 ±0.13
Hardness (g cm^3)	1.23 ±0.017	1.15 ±0.013
Protein (g 100 g^{-1})	12.5 ± 0.07	8.8 ± 0.06
Live weevils g^{-1} of grain 105 days after infestation	0.4 ± 0.20	12.6 ±0.91
Cumulative dead weevils g^{-1} of grain 105 days after infestation	3.7 ± 0.46	0.5 ±0.17
Cumulative total weevils g^{-1} of grain 105 days after infestation	3.1 ±0.50	12.1 ± 1.01
Damage score (1-5 scale) 105 days after infestation	1.5 ±0.10	3.9 ± 0.21
Weight (g)/vial 105 days after infestation	5.0 ± 0.07	2.7 ± 0.02
% weight loss 105 days after infestation	0.8 ± 0.07	46.8 ± 0.21

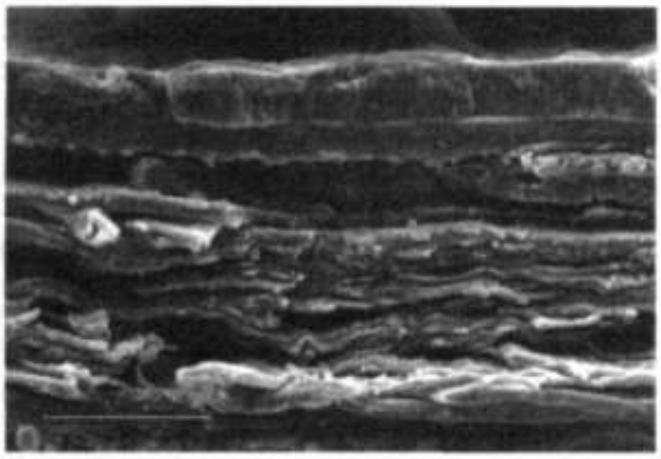

Figure 1. Scanning electron microscope photo of susceptible SC630-11E11. Scale bar = 10 µm.

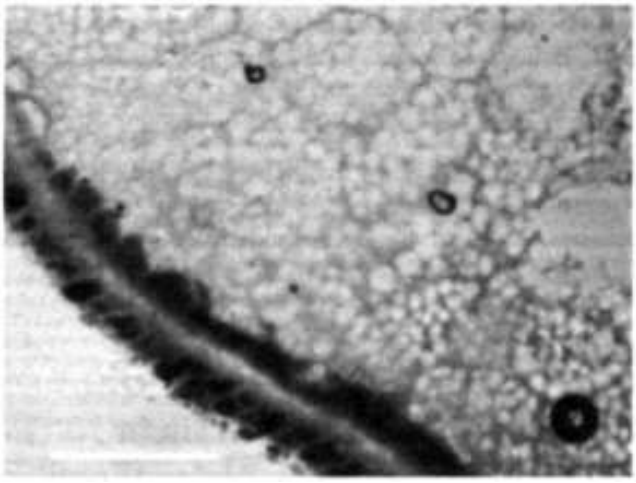

Figure 2. Light microscope photo of susceptible SC630-11E11. Scale bar = 60 µm.

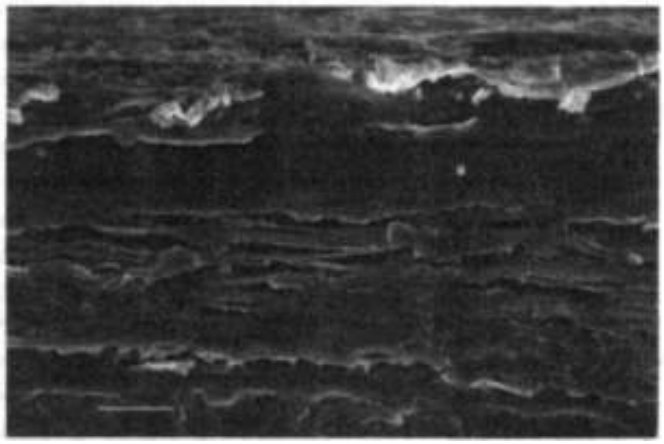

Figure 3. Scanning electron microscope photo of Sureno. Scale bar = 15 µm.

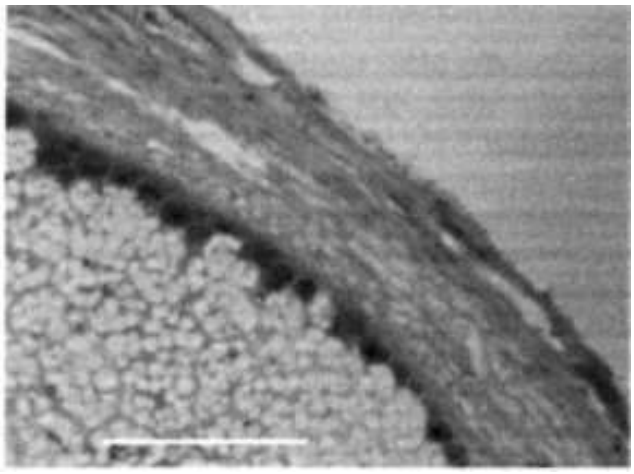

Figure 4. Light microscope photo of Sureno. Scale bar = 75 µm

Results and Discussion

Of the 20 genotypes of sorghum evaluated by Chitio (2004), Sureno and SC630-11E11 were most and least resistant, respectively. Features of Sureno and SC630-11 E11 are listed in Table 1. Individual grains of SC630-11E11 weighed 1.2 times more, were 1.1 times longer, 1.1 times wider, and 1.2 times taller than grains of Sureno. Grains of Sureno were 1.1 times harder than grains of SC630-11E11. Sureno grains contained 1.4 times more protein (12.5%) than did SC630-11E11 (8.8% protein).

At 105 days after infestation, 31.5 times more live weevils (12.6 versus 0.4) and 7.4 times fewer dead weevils (0.5 versus 3.7) were found in vials of SC630-11 E11 grain as in Sureno (Table 1). A total of 3.9 times more maize weevils (12.1 versus 3.1) was produced per g of SC630-11E11 grain as per g of Sureno at 105 days after infestation with maize weevils. The damage score for SC630-11 E11 (3.9) was 2.6 times greater than that for Sureno (1.5). Of the original 5.0 g of grain per vial, grain of Sureno weighed 5.0 g at 105 days after infestation with maize weevils, whereas grain of SC630-11E11 weighed only 2.7 g. Weight loss of grain of Sureno was 0.8%, while that of SC630-11E11 was 46.8%.

Scanning electron and light microscopies were used to determine that the thickness of the seed coats of the different genotypes of sorghum differed. The thickness of the sorghum seed coat was related to resistance to maize weevils. The thickness of the seed coat of the resistant Sureno was twice that of susceptible SC630-11E11 (Figures 1-4).

Acknowledgment. This research was supported in part by the International Sorghum and Millet Collaborative Research Support Program (INTSORMIL CRSP) sponsored by the U.S. Agency for International Development, under the terms of Grant No. LAG-G-00-96-90009-00.

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Identification of Sorghum Genotypes Resistant to Sorghum Midge in Niger

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Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is the most important cereal crop grown in savanna areas of West Africa, most production being in Nigeria (5 million tons in 1991), Burkina Faso (1 million tons), Mali, and Niger (FAO 1992). In Niger, insects feeding at different developmental stages of sorghum reduce yield and grain quality. Sorghum midge *(Stenodiplosis sorghicola),* a fly of the order Diptera, is the major insect pest of sorghum worldwide. ICRISAT (1992) reported unusual widespread infestation and severe damage by sorghum midge in the drier parts of northern Nigeria, Burkina Faso, Cameroon, and Niger in 1991. In 1992, interviews with farmers in Maradi and Konni revealed that symptoms of attack by sorghum midge were not properly identified or understood (Kadi Kadi 1993). The farmers thought a "night wind" caused empty glumes and reduced production of sorghum. To entomologists, this "night wind" is caused by sorghum midge depositing eggs in the early morning between the glumes of flowering spikelets of sorghum and larvae preventing kernel development.

Maïga (1988) found that sorghum midge caused more damage to local sorghums (Tanout local, Bagoba, and E1 Dele) because of their lengthy phenology (late flowering and maturity). Kadi Kadi (1994) reported TAM 2566, TX 2755, TX 2782, and TX 2890 sorghum lines introduced from Texas as resistant to sorghum midge in Niger. The research objective of this paper was to determine sorghum genotypes with stable resistance against sorghum midge in the field.

Materials and Methods

The head-cage technique developed and standardized by ICRISAT (Sharma et al. 1992) was used to evaluate sorghums for resistance to sorghum midge in the field at Maradi in East-Central Niger (15°26' North and 8°33' East). The mean annual rainfall there is 400-500 mm. Thirty-eight single seed descent sorghum lines and/or improved varieties were evaluated. The sorghums were grown in sandy soil.

Sorghum panicles at 25-50% anthesis were selected. A cage made of a cylindrical wire frame of 1.5-mm-diameter

Table 1. Scores of damage caused by sorghum midge and percentage of weight loss of sorghums, Maradi, Niger, 2001 and 2002.

	2001		2002	
Genotype	Damage score	% grain loss	Damage score	% grain loss
ICSV 745	1.5ab	8.9cd	1.5ab	16.9bc
ICSV 88032	1.0c	13.5cd	2.0ab	15.8bc
99 SSD F9 -34	1.0c	6.4cd	1.0c	28.9c
99 SSD F9 -28	2.5ab	18.2c	3.5b	18.4bc
99 SSD F9-14	1.0c	17.4c	1.0c	22.2c
99 SSD F9 -16	1.0c	21.3c	1.0c	19.5bc
99 SSD F9 -36	1.0c	2.1cd	1.0c	38.9ab
99 SSD F9 -35	1.0c	35.9abc	2.0ab	8.2bc
99 SSD F9 -37	1.0c	34.8abc	1.0c	13.4bc
99 SSD F9 -24	1.0c	13.5cd	4.0b	36.6ab
99 SSD F9 -2	1.0c	24.5c	1.0c	28.6c
99 SSD F9 -29	1.0c	35.1abc	1.0c	18.4bc
99 SSD F9-17	1.0c	31.5abc	3.0ab	24.3c
99 SSD F9 -33	1.0c	28.9c	1.0c	27.4c
99 SSD F9 -13	1.0c	21.9c	1.0c	37.2ab
99 SSD F9 -8	1.0c	31.4abc	7.0a	28.6c
99 SSD F9 -1	1.0c	30.0abc	1.0c	32.2ab
99 SSD F9-21	2,0ab	28.0c	2.0ab	37.2ab
99 SSD F9 -26	1.0c	52.5a	1.0c	18.6bc
99 SSD F9 -23	1.0c	33.5abc	1.0c	38.0ab
99 SSD F9 -20	1.0c	35.6abc	1.0c	38.5ab
99 SSD F9 -19	1.0c	33.5abc	1.0c	41.0ab
99 SSD F9 -11	1.0c	36.3abc	1.0c	38.6ab
99 SSD F9-18	1.0c	40.9ab	1.0c	34.5ab
99 SSD F9 -32	2.0ab	27.3c	2.5ab	48.2b
99 SSD F9 -7	1.5ab	46.6ab	1.0c	37.5ab
99 SSD F9 -3	2.0ab	23.6c	3.0b	65.4a
99 SSD F9 -27	1.0c	16.7cd	4.0b	72.5a
99 SSD F9 -30	1.0c	51.0a	1.0c	38.7ab
99 SSD F9 -5	1.0c	42.6ab	1.0c	49.2b
99 SSD F9 -4	1.0c	45.8ab	1.0c	48.7b
99 SSD F9 -6	1.0c	47.4ab	2.5ab	48.8b
99 SSD F9-31	1.0c	45.1ab	1.0c	62.4a
IRAT 204	4.0b	45.5ab	4.5b	65.3a
MR 732	3.5b	55.6a	3.5b	55.8a
Mota Maradi	4.5b	67.3a	6.5a	45.6b
99 SSD F9 -10	6.0a	51.6a	1.0c	61.8a
TX 623 A	3.0b	73.3a	4.5b	74.5a
LSD	1.6	39.3	1.8	37.3
CV	0.346	4.78	0.334	4.87
P	0.5074	0.1231	0.0208	0.0132

Means (± SE) followed by the same letter in a column are not significantly different (Student t-test, $P < 0.05$).

galvanized iron wire was placed around the sorghum panicle and covered with a mosquito net bag (20 cm wide by 40 cm long). The net bag had an extension 5 cm in diameter by 10 cm long at the top. A 200-ml aspirator was used between 0600 and 0900 hours to collect adult female sorghum midges from flowering sorghum panicles. Twenty sorghum midges were released into each cage and the inlet closed. The procedure was repeated the following day. Five panicles of each genotype of sorghum were infested. The panicles were examined 5-7 days after infestation, and other insects such as mirid bugs, panicle-feeding caterpillars, or predatory spiders were removed. Cages were removed 15 days after infestation.

Damage by sorghum midge was evaluated visually using the ICRISAT scale of 1-9. All panicles from the middle row(s) or hills were harvested at maturity, and panicle and grain weights were recorded. Data for scores of damage by sorghum midge and percentages of grain loss were analyzed by using SAS. The Student t-test was used to compare means and determine sorghum genotypes resistant to sorghum midge.

Results and Discussion

In 2001, damage scores for the single seed descent parents were 4.5 and 1.0 for Mota Maradi (susceptible local parent) and ICSV 88032 (introduced resistant parent) (Table 1). Grain loss was 67.3% for Mota Maradi but only 8.9% for ICSV 88032. The greatest damage score of 6.0 was estimated for 99 SSD F9-10, with 51.6% grain loss. The damage score was 4.0 and yield loss 45.5% for IRAT 204. Least grain losses in 2001 were 2.1, 6.4, 8.9, and 13.5% for 99 SSD F9-36, 99 SSD F9-34, ICSV 745, and 99 SSD F9-24, respectively. Damage was scored 1.0 for all but ICSV 745, which scored 1.5.

Mean damage scores and percentages of loss were greater in 2002 than 2001. Damage scores were 6.5 and 2.0 for Mota Maradi (susceptible local parent) and ICSV 88032 (introduced resistant parent), while grain losses were 45.6% for Mota Maradi but only 15.8% for ICSV 88032 in 2002 (Table 1). Least grain losses were 8.2, 13.4, 16.9, 18.4, 18.4, 18.6, and 19.5% for 99 SSD F9-35, 99 SSD F9-37, ICSV 745, 99 SSD F9-28, 99 SSD F9-29, 99 SSD F9-26, and 99 SSD F9-16, respectively. Damage scores varied from 1.0 for 99 SSD F9-16, 99 SSD F9-26, 99 SSD F9-29, and 99 SSD F9-37, to 3.5 for 99 SSD F9-28. In 2002, 99 SSD F9-8 had the greatest damage score of 7.0, but only 28.6% yield loss. Greatest yield losses were 72.5, 65.4, 65.3, 62.4, and 61.8% for 99 SSD F9-27, 99 SSD F9-3, IRAT 204, 99 SSD F9-31, and 99 SSD F9-10, respectively, with damage scores of 1.0-4.5. Damage scores were 3.0 and 4.0 for 99 F9-17 and 99 F9-24, with yield losses of 24.3 and 36.6%. Damage scores were 4.0 and 4.5 for 99 SSD F9-27 and TX 623 A, with yield losses of 72.5 and 74.5%, respectively.

Three years (2000-2002) of evaluation led us to conclude that ICSV 745, 99 SSD F9-21, 99 SSD F9-33, and 99 SSD F9-35 were resistant to sorghum midge. However, in 2001, 99 SSD F9-24, 99 SSD F9-27, 99 SSD F9-34, and 99 SSD F9-36 were also resistant to sorghum midge. Sorghum lines 99 SSD F9-16, 99 SSD F9-26, 99 SSD F9-29, and 99 SSD F9-37 were also resistant in 2002.

Acknowledgment This research was supported by the Institut de la Recherche Agronomique du Niger (INRAN) and the West Africa Regional Program of the International Sorghum and Millet Collaborative Research Support Program (INTSORMIL CRSP).

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Use of Local Plants to Control Sorghum Insect Pests in the Field

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Introduction

Insects can attack sorghum [*Sorghum bicolor* (L.) Moench] from seedling through maturity stages (Teetes and Pendleton 2000). Insect pests of sorghum in Africa include aphids, especially the sugarcane aphid *[Melanaphis sacchari* (Zehntner)]; sorghum midge *[Stenodiplosis sorghicola* (Coquillet)]; and panicle-feeding bugs such as *Eurystylus immaculatus* (Autrique and Perreaux 1989). Sugarcane aphid nymphs and adults suck sap from all stages of sorghum. Chlorotic spots appear on the leaves and plant growth may be stunted. Aphids also excrete honeydew on which molds develop. A sorghum midge lays an egg between the glumes of a flowering spikelet of sorghum, and the larva that develops inside destroys the kernel (Diarisso 1997). Yield losses of 100% can occur to panicles in a field. Bugs such as *E. immaculatus* puncture developing kernels during feeding and oviposition, causing the kernels to shrivel and increasing the plant's vulnerability to pathogens (Doumbia 1992). Pathogens, especially grain mold, decrease grain quality and yield. Panicle bugs can cause 60% yield loss.

Materials and Methods

Sorghum variety 'S34' susceptible to panicle-infesting bugs was grown in a field at Samanko, Mali. The plots were five rows 5 m long 75 cm apart, and plots 2 m apart. Juice from leaves of local giant milkweed *(Calotropis procera,* Asclepiadaceaea) and neem seed jelly (*Azadirachta indica* Meliaceae) was filtered and sprayed on sorghum at the seedling, end of flowering, and hard-dough stages. The experimental design was a Fisher block with three replications of six treatments: 200 or 250 g L^{-1} of neem seed jelly, 25 kg of fresh leaves of *Calotropis procera* with 30 L of water and 100 g of local soap of Koulikoro filtered after 12 h and applied at a rate of 10 L of C. *procera* juice per hectare, 80 ml L^{-1} of Dursban (chlorpyrifos), sorghum at the boot stage covered by a mesh bag, or nontreated check. Insects were counted a day before and a week after treatment from 10 panicles in each plot. At maturity, damage by insects and grain mold on 10 panicles was rated on a scale of 1-5, where 1 = <10% grain mold, to 5 = >75% grain mold. The grain from 20 panicles in the middle rows of each plot was weighed when the sorghum was harvested.

Results and Discussion

The number of aphids on seedling sorghum decreased after treatment (Figure 1). The greatest control was observed in plots treated with Dursban or either dose of neem seed jelly. A few aphids were present in sorghum treated with juice from *C. procera* leaves. The number of aphids on nontreated sorghum was twice that in plots treated with C. *procera.* Neem seed jelly at 200 or 250 g L^{-1} was more effective in controlling insects than 80 or 160 g L^{-1} of neem used in previous years.

Except on nontreated check plants, the number of panicle bugs was less after treatment (Figure 2). No bugs were found before or after treatment of panicles protected with mesh bags. Fewest bugs were in plots sprayed with Dursban (5 bugs), 250 g L^{-1} neem seed jelly (7), or 200 g L^{-1} neem seed jelly (10).

Table 1. Damage by sorghum midge, panicle bugs, and grain mold, and weight of S34 sorghum treated with local plants and Dursban at Samanko, Mali, in 2004.

Treatment	Damage score by sorghum midge on 10 panicles	Damage score by bugs on 10 panicles	Rate of grain mold on 10 panicles	Weight (g) of grain from center rows
Nontreated check	3.1 a	4.0 a	2.2 ab	433.3 a
Neem seed jelly (200 g L^{-1})	3.1 ab	3.2 b	2.2 ab	566.3 c
Neem seed jelly (250 gL^{-1})	2.2 bc	3.0 b	2.1 ab	600.3 c
Calotropis procera juice (10 1 ha^{-1})	2.4 abc	4.1 a	2.5 a	466.7 b
Dursban (5.3 ml L^{-1})	1.5 cd	2.0 c	1.9 c	666.7 cd
Panicle protected by bag	1.0d	1 4 4	1.0 c	700.0 d
CV	0.3673	0.1416	0.0677	
Probability	0.048	0.00	0.00	
Significance	S	HS	HS	HS

Means followed by the same letter in a column are not significantly different (Duncan's range test, $P < 0.05$).

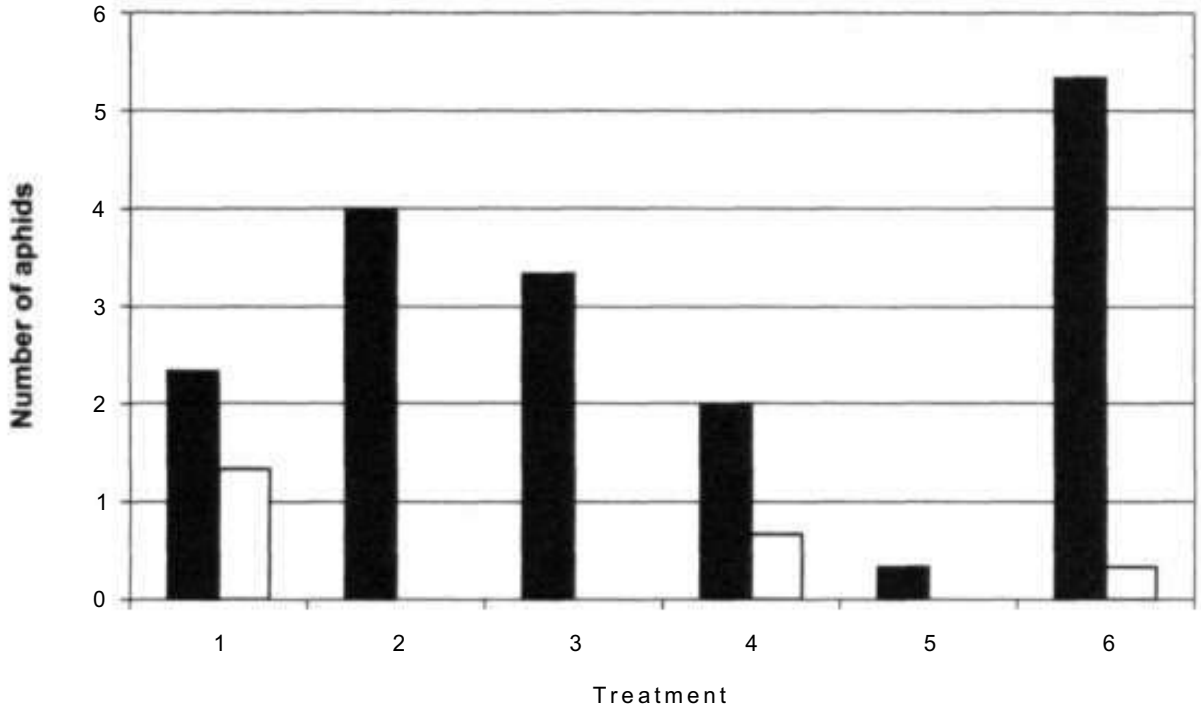

Figure 1. Number of aphids on seedling sorghum before (black bars) and after (white bars) treatment at Samanko, Mali, in 2004; 1 = nontreated, 2 = 200 g L^{-1} neem, 3 = 250 g L^{-1} neem, 4 = 10 L ha^{-1} *Calotropis procera,* 5 = Dursban, and 6 = protected panicle.

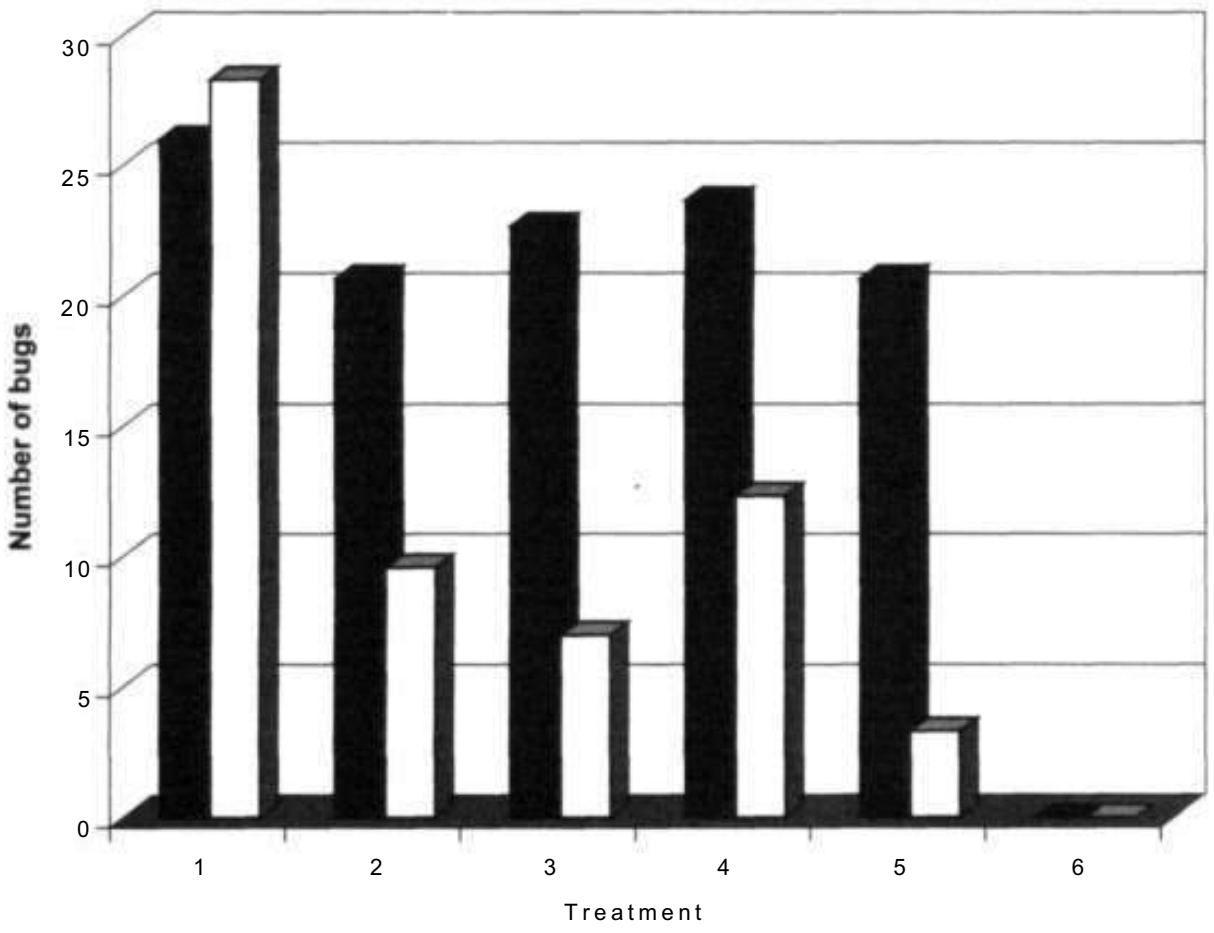

Figure 2. Number of panicle bugs before (black bars) and after (white bars) treatment of sorghum at Samanko, Mali, in 2004 ; 1 = nontreated, 2 = 200 g L^{-1} neem, 3 = 250 g L^{-1} neem, 4 = 10 L ha^{-1} *Calotropis procera,* 5 = Dursban, and 6 = protected panicle.

Grain weight, and scores of damage by sorghum midge, panicle bugs, and grain mold differed significantly among the treatments. Panicles protected by mesh bags were not infested by sorghum midge, bugs, or grain mold (Table 1). Damage to panicles protected by mesh bags or Dursban did not differ from each other but did differ significantly from damage to panicles sprayed with extracts from local plants. There was no significant difference in damage by insects to sorghum sprayed with either dose of neem, but kernels on panicles sprayed with the greater dose of neem seed jelly were less molded than panicles sprayed with the lesser dose. Scores of damage to panicles sprayed with Dursban were 1.5, 2.0, and 1.9 for sorghum midge, panicle bugs, and grain mold, respectively. Scores of damage by sorghum midge, panicle bugs, and grain mold on nontreated sorghum were 3.1,4.0, and 2.2, respectively. The weight of protected grain was greater than the weight of nontreated sorghum or panicles treated with juice from leaves of *C. procera,* but weight of grain from panicles protected by mesh bags did not differ from that of panicles sprayed with Dursban. Overall, mesh bags or Dursban better protected panicles from damage by insects and grain mold than did treatment with extracts from local plants, which were in turn better than the nontreated check.

Acknowledgment. This research was supported by IDA/ PSAOP3783 MLI and the International Sorghum and Millet Collaborative Research Support Program (INTSORMIL CRSP) sponsored by USAID, under the terms of Grant No. LAG-G-00-96-90009-00.

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Effectiveness of Plant Powder in Controlling Lesser Grain Borer in Stored Sorghum Grain

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Introduction

Insect pests can cause major damage to stored grain, especially in warm, humid regions of the world (Ratnadass et al. 1994). Lesser grain borer, *Rhyzopertha dominica* (Fabricius), is one of the smallest grain-infesting beetles but one of the most damaging. Lesser grain borer originated in the tropics, but is now cosmopolitan in distribution (Teetes et al. 1983). Eggs laid on grains hatch into larvae that bore into and feed inside kernels or on flour produced by the feeding adults. The larva pushes odoriferous dust composed of feces and flour out of the entry hole (Wilbur and Mills 1985). Feeding by larvae and adults may leave only the bran covering. The life cycle can be completed in 1 month. Three to four generations are produced per year. This study examines the effectivenss of powdered leaves of local plants *Calotropis procera* and *Cassia nigricans* in controlling lesser grain borer in stored sorghum.

Materials and Methods

Leaves of local *Calotropis procera* or *Cassia nigricans* plants were ground into powder and used to treat sorghum [*Sorghum bicolor* (L.) Moench] 'Malisor 92-1' at Sotuba, Mali. One kilogram of grain was treated with 3 or 6 g of *C. procera* powder, 3 or 6 g of C. *nigricans* powder, or not treated (check). The grain was placed in cotton-cloth bags. Four replications of each treatment were used. The bags of grain were placed in a storage room infested with lesser grain borer so the grain could be infested naturally. Damaged and nondamaged grains per treatment and replication were counted and weighed each month for 6 months to determine percentage of grain loss due to lesser grain borer.

Results and Discussion

Damage began the third month after treatment and increased over time (Table 1). Nontreated grain was

Table 1. Sorghum grain loss to lesser grain borer after treatment with powder of *Calotropis procera* or *Cassia nigricans* plants at Sotuba, Mali, in 2004.

Grain treatment	Loss (%) at months after treatment			
	3 months	4 months	5 months	6 months
Calotropis procera powder (6 g kg^{-1} grain)	0.3 c	1.3 c	2.3 c	4.4 b
Calotropis procera powder (3 g kg^{-1} grain)	2.2 b	3.2 b	4.4 b	5.0 b
Cassia nigricans powder (6 g kg^{-1} grain)	0.5 c	1.7 c	2.7 c	4.2 b
Cassia nigricans powder (3 g kg^{-1} grain)	2.4 b	3.3 b	4.5 b	5.1 b
Nontreated check	3.4 a	4.7 a	5.7 a	7.1 a
CV	0.2619	0.1706	0.1274	0.1392
PPDS	0.7641	0.7595	0.7641	1.137
Significance	HS	HS	HS	HS

Means followed by the same letter in a column are not significantly different (Duncan's range test, P <0.05).

significantly damaged. Least damaged was grain treated with 6 g of *Calotropis procera* or *Cassia nigricans* powder per kg of grain. Six grams of *Calotropis procera* and *Cassia nigricans* powder resulted in 0.3 and 0.5% loss 3 months after treatment and 4.4 and 4.2% loss 6 months after treatment. Losses to nontreated grain were 3.4 and 7.1% at 3 and 6 months, respectively, after treatment. The greater dose (6 g kg^{-1} grain) was more effective than the lesser dose (3 g kg^{-1} grain) of plant powder. Efficacy of the plant powders decreased and number of insects increased over time.

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Iridomyrmex sp. (Hymenoptera: Formicidae) and *Helicoverpa armigera* (Lepidoptera: Noctuidae) can be Insect Vectors of Sorghum Ergot

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Introduction

Ergot of grain sorghum [*Sorghum bicolour* (L.) Moench], caused by *Claviceps africana* Frederickson, Mantle, & de Milliano, remains an important pathogen of sorghum in Australia. This is due to the production of alkaloids that are toxic to a range of livestock (with similar toxic effects to rye ergot alkaloids) (Blaney et al. 2001) and increased costs of grain and hybrid seed production.

C. africana very rapidly produces large numbers of airborne secondary conidia. Secondary conidiation does not occur in all ergot species, or even on all species infecting grain sorghum. Secondary conidiation depends upon certain environmental conditions, including honeydew age, osmotic potential, and environmental parameters, particularly temperature and relative humidity (Ryley et al. 2003).

Insect vectors have been considered of little relevance to sorghum ergot, because of the production of abundant airborne secondary conidia. *Helicoverpa zea* has been reported as a vector of C. *africana* in the USA (Prom et al. 2003). While this species is not present in Australia, five other species are, of which *H. armigera* is the major

and single pest species of sorghum (Adam Hardy personal communication).

Ants have not been reported as ergot vectors. Large numbers of ants *(Iridomyrmex* sp.) were observed feeding on sorghum ergot honeydew during the dry 2002/2003 summer in south-east Queensland (a time with no secondary conidia production). *Iridomyrmex* spp. are one of the largest and most frequently encountered groups of ants in Australia. They are general scavengers and opportunistic nectar feeders (Shattuck 1999). Observations of insects feeding on ergot honeydew prompted the question of whether they were able to vector the disease. Experiments were conducted with *Iridomyrmex* sp., and the corn ear caterpillar, *H. armigera.*

Materials and Methods

Sorghum inoculation with *Iridomyrmex* sp: A number of preliminary experiments were conducted to determine a robust method of working with the ant *Iridomyrmex* sp. in glasshouse experiments. To capture the ants, a suction tube was used, which avoided handling and damaging the ants, and enabled the use of naturally ergot inoculated ants. In order to maintain the ant presence on the sorghum panicle for over 24 hours, the panicle was enclosed in a plastic bag. To encourage the ants to roam the panicle (rather than the plastic bag), they were released directly onto the panicle. To minimize confounding of the results through pollination, a male-sterile sorghum genotype was used (AQL41).

These methods were followed in the glasshouse to conduct an ant inoculation experiment. Ten flowering sorghum panicles were used, with five bagged without ants, and the other five bagged with ten ants per panicle. All panicles were left bagged for 38 hours, and rated for percentage ergot infection three weeks after bag removal.

***Helicoverpa armigera* Cage Experiments:** Two large cages were utilised (1.5 m length by width by height). Three flowering male-sterile plants were placed into each cage, along with ergot infected panicles as a honeydew source. No obvious secondary conidiation was visible.

Six laboratory-reared moths (two male and four mated female) were released into one cage, and the other cage left as a control. Using well-fed laboratory moths can mean that they will not feed in the first 24 hours (Renee Herde, personal communication), so moths were left caged for 48 hours to avoid this potential problem.

Moths were removed after 48 hours, and flowering panicles bagged for the following 24 hours. Panicles were rated three weeks after bag removal for the percentage of spikelets infected with ergot.

The experiment was repeated, with slight modification. Four flowering male sterile plants were used in each cage. Six laboratory reared moths were again released into one cage, but with an even ratio of the sexes (three male and three mated female), and the other cage left as a control.

Moths were removed after 48 hours, and all panicles bagged for 24 hours. Panicles were rated three weeks after bag removal for the percentage of spikelets infected with ergot.

Results and Discussion

Sorghum inoculation with *Iridomyrmex* sp: A significant difference in ergot infection was found between the control (0% ergot) and panicles where ants were introduced (13.1% ergot) (Table 1).

Vectors such as ants will contribute to localised infections. Ants will only be a serious vector in cropping systems without frequent tillage, or when sorghum is grown as a ratoon crop, enabling ant colonies to become established. In the case of widely dispersed weed hosts in non-cultivated land, such as *Sorghum halepense* and 5. *album,* ants may play a role in dispersing ergot over time, rather than over wide areas.

***Helicoverpa armigera* Cage Experiments:** The first cage test with H. armigera moths did not show a significant difference between the treatments. The control had a trace of ergot, with a mean of 0.5% ergot infection, while the H. armigera treatment had 14.5% ergot infection (Table 1). The variability of moth-related infection, combined with a low number of panicles available for

Table 1. Summary results of three glasshouse experiments on testing the ability of insects as vectors for sorghum ergot.

Experiment	Control (% ergot infection)	Insect Vector (% ergot infection)	*P* Value[1]
Iridomyrmex sp. inoculation	0.0	13.1	0.05
Helicoverpa armigera cage test 1	0.5	14.5	0.19
Helicoverpa armigera cage test 2	0.0	16.6	0.01

1. Values d™ 0.05 indicate the insect vector has caused a significantly higher level of ergot disease compared to the control.

testing would have contributed to the non-significance of the statistical test. The trace amount of infection in the control plants was unexpected due to the apparent absence of secondary conidiation. A low level of secondary conidia must have been present on the ergot source.

The second experiment showed a significant difference between the treatments. The control had a mean of 0% ergot infection, while the *H. armigera* treatment had a mean of 16.6% ergot infection (Table 1). Overnight viewing of the caged moths revealed a number of them feeding on the ergot honeydew, suggesting moth contact with ergot honeydew is not merely random.

The levels of ergot infection due to moth transmission (14.5% and 16.6%) are reasonably high, although only significant for the second experiment because of the fluctuations in ergot severity between panicles. The ergot infection induced by *H. zea* in cage experiments was higher than this (means from 12% up to 53%) (Prom et al. 2003), but the number of moths used was also much higher (15 or 20 moths per cage), suggesting that using a comparable number of moths would also produce higher ergot infection.

Epidemiologically, insect vectors will still have a negligible role when conditions are conducive to secondary conidia formation. However, under environmental conditions where secondary conidia are not being produced, insect vectors will have a role to play. Insect vectors such as *H. armigera* may contribute to disease spread over a large area. Adult moths feeding on honeydew will then carry conidia to flowering panicles when seeking ovipositing sites, effectively inoculating the visited panicles. This would result in point sources of infection occurring over a wide area to act as disease foci when conditions become suited for production of airborne conidia.

In summary, insect vectors are important factors in the lifecycle of many ergot species. They have been considered of little relevance to C. *africana,* due to the presence of aerially dispersed conidia. However, under certain conditions, insect vectors may play a role in sorghum ergot epidemiology. *Iridomyrmex* sp. and *Helicoverpa armigera* were both found to feed on ergot honeydew. Both were found to act as vectors of sorghum ergot, causing significant ergot infection.

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Development of Technique for Obtaining Transgenic Sorghum Plants by Agrobacterium-Mediated Transformation *In Planta*

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Introduction

Development of simple and efficient methods for obtaining genetically engineered plants is one of the main goals of plant biotechnology. During the past years a few reports on obtaining transgenic sorghum plants have been published (Casas et al. 1993, 1997). These experiments used the "microbombardment" technique, which needs expensive equipment and laborious steps for obtaining embryogenic callus, and for selection and regeneration of transformed plants. Moreover reported frequencies of transgenic plants were very low. Another method of genetic transformation based on cocultivation of *A.tumefaciens* cells with callus or explants tissues is unsuitable for sorghum because of frequent necrosis of sorghum cells after cocultivation (Carvalho 1998; our unpublished results). Development of a simple and reliable method for obtaining transgenic sorghum plants was the main purpose of our investigations.

Materials and Methods

A. tumefaciens strains containing the plasmids *pGV3101* and *pAS47* with kanamycin-resistance *(npt)* and p-glucuronidase *(gusA)* marker genes were used. *pAS47,* which was generously supplied by Dr. R.G.F.Visser, in addition bears anti-sense sequence of the maize granule-bound starch-synthase gene (GBSS; EC 2.4.1.21) under the control of the 35S-promotor.

Plants of the lines with cytoplasmic male sterility (CMS) A_2KVV-181 and A_4Milo-10 were bagged before anthesis and were pollinated by their fertile analogs. After a certain time cell suspension of *A. tumefaciens* grown on the acetosyringone-containing medium was put on the surface of the stigmas. To select transgenic plants the seeds developed on the treated panicles were sterilized, pre-soaked in water (selection method #1) or kanamycin solution (selection method #2, 300 or 600 mg L^{-1} for A_2KVV-181 or A_4Milo-10, respectively) for 18-24 h and were grown *in vitro* on kanamycin-containing medium (200 mg L^{-1}, for both selection methods). Green kanamycin-resistant seedlings were transferred to soil. Histochemical examination of GUS activity in young leaves and nodes of shoots and seedlings was done (Jefferson et al. 1987).

Results and Discussion

Taking into account a high level of kanamycin resistance of sorghum cells we used the medium with high kanamycin concentration and tested different selection methods. In Method #1 kanamycin action (albinism) manifested beginning from the 2nd leaf in 100% of A_2KVV-181 and approx. 90% of A_4Milo-10 control seedlings. In Method #2 albinism manifested beginning from the 1 st leaf and at the two-leaf stage all control seedlings had either bleached or curled leaves and were unviable in both genotypes.

Unlike these, seedlings developed from seeds obtained from the panicles treated with *A. tumefaciens* expressed much higher levels of kanamycin resistance, remaining green at two or three-leaf stages. Altogether, out of 404 seedlings of the line A_4Milo-10, which were obtained from 5 panicles treated with *A. tumefaciens* bearing *pGV3101* and selected by Method #1, 119 (29.0%) seedlings maintained green phenotype at the two-leaf stage. The proportion of green seedlings among those selected by Method #2 was 40.9% (335 out of 818). The analysis of the GUS activity in the leaves and first nodes of these seedlings showed that the majority (8 out of 10 studied) contained reporter gene *gusA.* Endogenic GUS activity was absent in control plants.

From 8 panicles of the line A_2KVV-181 treated with the *A. tumefaciens,* 104 (19.6%) out of 530 seedlings selected by Method #1, and 55 (40.1%) out of 137 selected by Method #2 maintained green phenotype at the two-leaf stage. However, on the next stages the majority of selected seedlings also developed albino leaves, possibly as a result of the toxic effect of extremely high levels of kanamycin used for selection. Nevertheless, four plants survived and three of them expressed GUS activity in the shoot tillers. These plants maintained CMS phenotype and set seed under pollination by their fertile analogs.

It should be noted that in the experiment with *A. tumefaciens* bearing *pAS47* plasmid, which was performed in a closed growth chamber in winter, in the line A_2KVV-181 we have obtained one kernel (out of 9 formed on the treated panicle) with semi-waxy endosperm, which could possibly have developed as a result of inhibiting GBSS activity and reduction of amylose synthesis by

anti-sense sequence of the transgene, as it occurred in transgenic potato with anti-sense GBSS gene (Visser et al. 1991). Such type of endosperm was never observed in this line.

Thus, we have developed a simple and effective method for obtaining transgenic sorghum plants by agrobacterium-mediated transformation *in planta,* probably via the pollen tube pathway. This method does not need the tissue culture stage, which significantly restricts the amount of transformants and induces genetic variation. Moreover, transformation via pollen tube pathway excludes chimerity of transgenic plants that is possible using multicellular explants.

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Isolation of Fertility-Restoring Revertant Obtained from Tissue Culture of Cytoplasmic Male-Sterile Sorghum

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Introduction

One of the most effective approaches for isolation of genes involved in genetic control of cytoplasmic male sterility (CMS) is the induction of fertile revertants from male-sterile lines. This approach allowed the identification of mitochondrial CMS-inducing genes in maize with *T*- and S-types of CMS, and in other plant species (Hanson and Bentolila 2004). Along with cytoplasmic revertants, the nuclear male-fertile revertants are of a special interest for studying mechanisms of cytoplasmic male sterility and nuclear-cytoplasmic interactions. The aim of this research was induction of reversion to male fertility in cytoplasmic male-sterile sorghum plants with the A, cytoplasm using genetic variation in tissue culture combined with the mutagenic effect of streptomycin.

Materials and Methods

Male-sterile plant donors of morphogenic callus cultures were isolated from the F_2 hybrid population derived from the cross A1 Efremovskoye-2 ' KVV-28. Callus cultures were induced from immature panicles from the secondary tiller according to previously elaborated methods (Elkonin et al. 1986). After two passages on the medium (MS + 2,4-D, 1.0 mg L^{-1} + 6-BAP, 0.5 mg L^{-1}) embryogenic callus cultures were treated with streptomycin solution (500 mg L^{-1}) for 15 h, for subculturing and then transferred to regeneration medium (MS + IAA 1.0 mg L^{-1}). The few green plants that appeared among numerous albino regenerants were transferred on to soil and were grown in the greenhouse.

The progeny of semi-sterile panicle that developed on the secondary tiller of one regenerated plant as well as its test-cross hybrids were grown in an experimental field located at the Agricultural Research Institute for South-East Region (Saratov, Russia). Fertility level was determined by the percentage of seed set on panicles bagged before anthesis. Depending on the percent seed set, the panicles were classified as sterile (0 seed setting or 1-2 seed), partially sterile (1-40%; usually no more than basal 1/3rd part of the panicle), partially fertile (40-75%; usually 2/3rd part of the panicle) and fertile (> 75%). The x^2-test was

used to determine the fit of observed segregation ratios of sterile and fertile plants to the expected segregation ratio of restored vs. non-restored plants fit well to a 9:7 ratio by grouping fertile, partially fertile and partially sterile plants in one class. This segregation suggested interaction of two dominant genes in restoration of male fertility. Investigation of allelic relationships of induced and standard fertility-restoring genes for the A1 cytoplasm is in progress now.

Results and Discussion

Among seven green plants regenerated from streptomycin-treated callus cultures (R_0 generation), one plant produced a partially sterile panicle that developed on its secondary tiller. The R_1 progeny of this plant ranged from sterile to a few partially sterile plants (Fig. 1). In the next generation (R_2), one partially sterile R_1 plant yielded only sterile progeny, while another produced partially fertile and partially sterile plants alongside with numerous sterile plants. In the R_3 generation, after self-pollination of the partially fertile plant from R,, we could obtain the progeny with more stable expression of male fertility. In the R_4 and R_5 generations, it did not segregate male-sterile plants; however, partially sterile plants did occur in the R_5 and R_6 generations. In addition, in the R_6 generation, segregation of completely sterile plants was also observed. This instability did not correlate with changes of environmental factors (temperature, amount of precipitation) either before or during plant flowering and seemed to be the result of genetic instability of the revertant line.

To study the genetic nature of reversion to male fertility, completely fertile plants from the R_4 generation were crossed to the CMS line A1 Saratovskoye-3 (Table 1). All F_1 plants from these crosses were fertile which proved nuclear location of mutation(s) to male fertility. In the F2 generation, segregation of numerous sterile plants was observed which indicated a sporophytic mechanism of action of fertility-restoring gene(s). The

Thus, using tissue culture-induced variation combined with the mutagenic effect of streptomycin and subsequent selection for seed setting, we could obtain the nuclear male-fertile revertant line of sorghum. Remarkably, in a

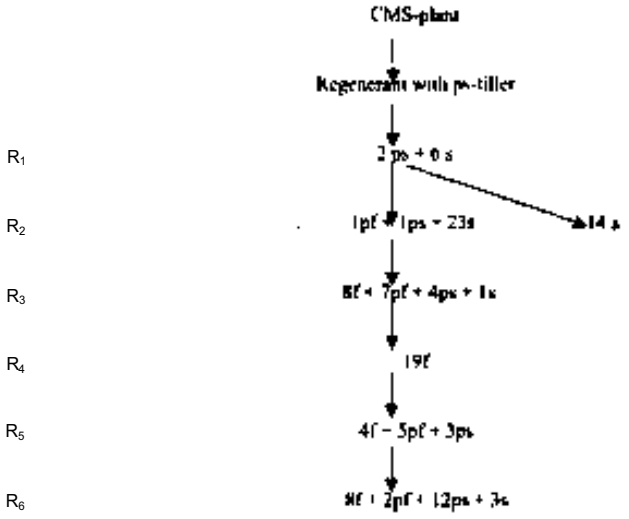

Figure 1. Schematic outlining of origin of male-fertile revertant. f - fertile, pf - partially fertile, ps - partially sterile, s - sterile plants.

Table 1. Inheritance of male fertility in test-crosses of induced male-fertile revertant of sorghum (line 39/02).

Hybrid combination	Generation/Year	Number of plants[1]				Ratio-	r	P
		f	Pf	ps	s			
Line 39/02	R_4/2002	19	-	-	-			
A1 Saratovskoye-3 x 39/02. cross 1	F_1/2003	28	-	-	-			
A1 Saratovskoye-3 x 39/02. cross 2	F_1/2003	29	-	-	-			
A1 Saratovskoye-3 x 39/02, cross 3	F_2/2003	32	-	-	-			
Line 39/02[3]	R_5./2003	4	5	4	-			
A1 Saratovskoye-3 x 39/02, cross 1	F_2/2004	11	7	7	21	9:7	0.068	0.75-0.90
Line 39/02	R_6/2004	8	2	12	3			

1. f - fertile, pf - partially fertile, ps - partially sterile, s - sterile plants;
2. ratio of (f+pf+ps) to s;
3. the progeny of paternal plant from cross 1.

similar work with CMS-*T* maize, Umbeck and Gengenbach (1983) obtained cytoplasmic male-fertile revertants. Assuming that previously we have reported on obtaining another nuclear male-fertility revertant from tissue culture of a CMS-sorghum plant with an A, cytoplasm, the line AS-1 (Elkonin et al. 1995), the CMS-inducing cytoplasmic genes of the A, sorghum cytoplasm seems to be rather stable in tissue culture conditions. The reasons of such stability are unclear.

At the same time, the data reported herein are of special interest because they illustrate the process of formation of dominant fertility-restoring genes. Taking into account a weak expression of male fertility in the R_1 and R_2 generations, it is evident that an initial partially sterile plant from $R_{()}$ did not bear dominant fertility-restoring genes. Therefore, one could assume that these dominant fertility-restoring genes occurred as a result of recombination and/or mutation processes in the genomes of partially sterile plants during their self-pollination. According to modern data, fertility-restoring loci in different plant species have a complex structure being composed of tandem repeats of a number of identical sequences (copies of pentatricopeptide repeat motif) (Hanson and Bentolila 2004). It was supposed that these loci might arise by repeated gene duplication through unequal crossing over, which takes place in response to expression of CMS-inducing genes (Touset and Budar 2004). In this connection, our data may represent an example of evolution of dominant fertility-restoring genes in sorghum.

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Development of a New Genetic Transformation System for Sorghum using *Agrobacterium* and Immature Inflorescences

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Introduction

Genetic improvement of sorghum [*Sorghum bicolor* (L.) Moench] has depended on conventional plant breeding methods. As a result, sorghum insect management has mainly relied on the development of pest resistant varieties through traditional breeding and improved cultural management practices. In recent years, there has been increased pressure to sustain, and even enhance, crop productivity with less use of chemicals. Conventional breeding has made a great contribution to sorghum production in the past and will continue to be an important component of future sorghum improvement programs, but traditional breeding has some inherent limitations due to natural barriers (i.e. sexual incompatibility) and the narrow genetic variability (limited gene pool) that is available (Able et al. 2001).

Plant biotechnology is a promising tool for changing agriculture, potentially providing new solutions to age-old agricultural problems. In particular, significant advances in gene identification and gene transfer techniques have allowed the incorporation of beneficial genes for specific agronomic traits into diverse crop plants. Today these new tools enable plant breeders to design new varieties by inserting desired foreign genes, including insect resistance genes, into existing commercial lines in an extremely short period of time. During the last decade, many of the world's most important crops (including wheat, maize, rice, soybean and cotton) have already been engineered with increased resistance to insects and diseases (Sahrawat et al. 2003). Although similar research has been attempted in sorghum, it has lagged behind that of other cereal crops. The limited progress in sorghum transformation is partly due to difficulties associated with its tissue culture and partly due to lack of efficient protocols for transformation. There have been only a few reports on sorghum transformation, and the majority of these transformation experiments were based on microprojectile bombardment devices for delivering foreign genes into the plant cells. As for the starter tissues, these transformation protocols have relied on use of mature or immature embryos. However, it has been suggested that

Agrobacterium may be a better system for DNA delivery in higher plants, including graminaceous monocots (Ishida et al. 1996, Schlappi and Hohn 1992). Thus, our current research has been directed towards the development of the *Agrobacterium-mcdidied* transformation system for sorghum. This paper reports the progress of the project on sorghum transformation using *Agrobacterium* and immature inflorescences.

Materials and Methods

Plant Materials. Two commercial varieties of sorghum, the genotypes RTx430 and RTx2737 were used in these studies because of their high regeneration capacity in preliminary experiments (Kaeppler and Pedersen 1997). Donor plants were grown in greenhouse soil beds where the growth conditions were maintained at 25-35°C during the day and 18-25°C at night, and 14 hours of light. Plants were watered on alternate days and fertilized every two weeks.

Bacterial strain and vector. The *Agrobacterium tumefaciens* strain LBA4404 harboring one of several binary vectors was used for all experiments. The vector PBI121 contains a kanamycin resistance gene as the selectable marker and a GUS gene as the screenable marker. The *Agrobacterium* was prepared for inoculation by streaking one loop of the bacterial stock onto yeast-extract-peptone (YEP) agar plate and grown in an incubator al 28°C. When bacterial colonies appeared, they were used to inoculate the YEP liquid medium with appropriate antibiotics and continued to grow for 6-12 h at the same conditions. Freshly grown bacteria were collected by centrifugation at 5,000 rpm for 5 min and the resultant bacterial pellets were resuspended in MS liquid medium supplemented with 10 g L^{-1} glucose and 200 µM of acetosyringone and adjusted to a concentration of 0.5 OD_{600}.

Transformation and plant regeneration. Explants used in this study included sorghum immature inflorescences and shoot apical meristems from the plants described above. The sorghum immature inflorescences (approx. 0.5-1.0 cm in length) were obtained from the donor plants and sterilized while wrapped in the inner leal' sheaths. Sorghum spikelets (floral primordia) were isolated aseptically and immediately transferred into 6 cm Petri dishes containing *Agrobacterium* inoculum, ensuring that all explants were completely submerged. Inoculation was carried out in the dark at 26°C from 15 min to 2 h. The inoculum was then pipetted out and infected explants were blotted on sterile Whatman filter paper (grade 1), then plated onto callus induction medium containing 10 g l^{-1} glucose and acetosyringone at 200 uM. Co-cultivation was carried out in the dark at 26°C for 2-4 days. Following co-cultivation, the explants were transferred to the regeneration medium with or without selection, where the resulting embryogenic callus was induced to form shoots and roots using the methods described by Lusardi and Lupotto (1990). Alternatively, shoot apical meristems were isolated from sorghum seeds that were germinated on the water-agar medium. After surface-sterilization, the explants were incubated in a freshly growing *Agrobacterium* cell suspension for 15 minutes, plated on co-cultivation medium, and incubated in the dark for 3 days. The meristems were then transferred onto callus-induction medium (C1M) containing carbenicillin (100 mg L^{-1}) and incubated under the light. Finally, transformed plants were regenerated via in vitro organogenesis.

Results and Discussion

Our current research aimed to identify a tissue culture system with a high capacity for producing regenerable cells which may be amenable to *Agrobacterium* infection, thus integrating the two systems into an efficient protocol to recover transgenic plants in sorghum. Immature

Table 1. Transformation frequencies of two explant types infected by *Agrobacterium tumefaciens.* This table contains data obtained from several experiments.

Genotype	Explant type[1]	Number of explants	Frequency of GUS spots (mean spot number)[2]	Stable kanamycin resistant cell lines[3]
RTx430	S.A.M.	76	4/24 (16.7%)	1/24 (4.2%)
RTx430	I.I.	120	9/28(32.1%)	4/28 (14.3%)
RTx2737	S.A.M.	61	3/18 (16.7%)	0
RTx2737	I.I.	68	5/22 (22.7%)	1/22 (4.5%)

Notes: 1. Explants S.A.M., shoot apical meristem and I.I., immature inflorescence; 2. number showing GUS activity / number of explants tested; [3] number showing kanamycin resistance lines / total number of explants.

inflorescences of sorghum were a good source of starter explants for in vitro culture and regeneration of whole plants in earlier experiments (Lusardi and Lupotto 1990). Furthermore, one of the simplest available plant transformation systems involves the infiltration of *Agrobacterium* cells into Arabidopsis plants before flowering and the direct selection for transformants in the resultant seedling population. Given these special considerations, we believed that immature inflorescences would be an ideal system to be integrated into the *Agrobacterium-medialed* transformation system. Indeed, the results from our current studies demonstrated that sorghum immature inflorescences were responsive to DNA delivery by *Agrobacterium.* After being infected by *Agrobacterium* under the conditions described above, many treated immature inflorescences continued to proliferate *in vitro* and consequently developed into embryogenic cells. Molecular analysis was able to detect that some cells accepted the alien genes delivered by the gene vector.

As shown in Table 1, immature inflorescences resulted in more transgenic cells when compared to the apical meristems, which were subjected to the same transformation treatments. Although the overall transformation frequency is still very low compared to those obtained from the well-established transformation systems in other cereal crops like wheat and rice, immature inflorescences show a promise for a practical source of young, competent tissues for genetic transformation and plant regeneration. Apparently immature inflorescences as target tissues for genetic transformation have several clear advantages over other types of explants. A large amount of inflorescent tissues exist in a single plant, providing plenty of material for transformation treatment. Another advantage is the ease of manipulation including tissue preparation and sterilization. Further, unlike immature embryos, sorghum immature inflorescences have a better ability to withstand physical damage as well as the cellular toxins associated with *Agrobacterium* (Rasco-Gaunt and Barcelo 1999).

The common process for cereal crop transformation has depended upon the use of biolistic gun, which involves "shooting" the genes carrying desired traits into the plant cells. The transgenic plants generated through this process sometimes can be problematic, since the process often introduces a high number of copies of the inserted genes or delivers DNA fragments of differing lengths with various rearrangements, which could cause significant disruption to the plant, as well as potentially causing the desired traits to stop expressing (i.e. gene silencing). However, *Agrobacterium* has proved to be a better DNA transfer system, although many graminaceous monocots are less responsive to *Agrobacterium* infection. It possesses several obvious advantages over other transformation systems, including simple technology with lower cost, the ability to insert larger segments of DNA with minimal rearrangement, and the precise insertion of transgenes at a lower number of copies of inserted genes, resulting in higher transformation precision and less disruption. Now, new biotechnology approaches are being developed for sorghum, which could have a major impact on sorghum genetic improvement.

A critical step in the development of *Agrobacterium*-mediated transformation system is the establishment of optimal conditions for T-DNA delivery into the target tissues from which whole plants can be regenerated. Future research efforts will entail the optimization of the protocol, especially with respect to the age of explants, the concentration of *Agrobacterium* inoculum, and the selection strategy of transformed cells for subsequent regeneration.

Developing and optimizing the *Agrobacterium*-mediated transformation system is an important first step to manipulate defense genes in sorghum cells and produce genetically modified plants for enhanced insect resistance to protect this crop from insect pests. Plant cell and tissue culture is fundamental to most aspects of plant biotechnology and in vitro plant regeneration is the bottleneck of successful production of transgenic plants (Able et al. 2001). Tissue culture is an enabling technology, providing powerful resources to both basic and applied biological research. These tools can be used by plant breeders to increase the speed and efficiency of the breeding process and to create new varieties for crop improvement, by plant biologists to deliver and subsequently test novel genes (genes with unknown function) in a particular plant species or certain tissues in order to define their function, by biochemists to analyze metabolic pathways and achieve metabolic engineering, and by entomologists and plant pathologists to study interactions between plants/plant tissues and insects or pathogens. Finally, using the transformation and in vitro regeneration systems, the desired genes conferring host plant resistance to insect pests, or any newly isolated genes awaiting confirmation of function can be transferred into the sorghum genome.

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Indications of Bee Pollination in Sorghum and its Implications in Transgenic Biosafety

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Introduction

The family Graminae is a large, diverse group that includes some of the world's most important crops such as maize *[Zea mays],* rice [Oryza *sativa* L.], wheat [*Triticum aestivum* L.] and sorghum [*Sorghum bicolor* (L.) Moench]. These crops are generally considered to be wind pollinated, and transgenic risk assessment studies on gene flow studies have focused on wind-mediated pollen only (Arriola 1995; Arriola and Ellstrand 1996; Song et al. 2003; Song et al. 2004). On some occasions, however, bees have been reported to visit Graminae crops and their wild relatives. On some indigenous grasses in South Africa, honey bees were recorded collecting pollen (Anderson et al. 1983). Solitary bees from the genus *Lipotriches* (family Halictidae) are known to collect pollen from 21 different grass species including *Sorghum bicolor* ssp. *arundinaceum,* the wild progenitor of cultivated sorghum (Immelman and Eardley 2000). Honey bees and bees of the genus *Nomia* (family Halictidae) (The genus *Nomia* was later divided into several genera, one of which is *Lipotriches)* were observed on indigenous grasses in Kenya (Bogdan 1962).

During a field trial (See "Crop-to-crop gene flow in sorghum and its implications for transgenic biosafety by the same authors in this volume) at the research farm at Roodeplaat near Pretoria, South Africa, investigating crop to crop pollen flow in sorghum, a number of honey bees, wild bees or solitary bees and one beetle species were observed on sorghum flowers. An additional investigation on the flower-visiting insects was therefore conducted. The field trial was not specifically designed to investigate insect pollination, so that the insect role cannot be conclusively demonstrated. Nonetheless, the observations made and samples collected provide strong evidence for bee pollination in sorghum.

Materials and Methods

The sorghum field trial was conducted on the 4000 ha Agricultural Research Council (ARC) research farm at Roodeplaat, approximately 20 km northeast of Pretoria, South Africa (25° 31' S and 28°21 'E, altitude approximately 1160 m). The trial took place in a non-sorghum growing area, at least 5 km from any other sorghum fields and at least 2 km from wild or weedy sorghum plants. As the pollen source, a central block, measuring roughly 30 X 30 m, was planted with the B-line Redlan Pannar Ps 1051 B/168(015), containing 35 rows approximately 90 cm apart with 30 cm within-row plant spacing. The surrounding vegetation was dominated by the local veld type (grassland) and some male sterile plants for the original field trial. The sorghum was grown using standard agronomic practices. Trial plants were planted on 28 December 2002. The plants in the central field started to flower in early March, approximately 70 days after planting, and maximum flowering was reached about 75 days after planting. Bees and beetles were observed and photographed on 12, 15 and 17 March and collected on 15 and 17 March 2003 during morning hours (9:00 - 11:15). Insects visiting sorghum flowers were collected with simple plastic boxes and then stored in the refrigerator before preparation for electron microscopy. In addition, fresh sorghum anthers from the central field were collected on 15 March and also prepared for electron microscopy, using standard preparation techniques. Bees and beetle specimens were identified in May 2003 by the Biosystematics Division of the Agricultural Research Council, Plant Protection Research Institute, Pretoria, South Africa. Halictidae bees were identified by C. Eardley, the Apidae variety by A. Lubbe and the beetle (family Melyridae) by E. Grobbelaar.

Results and Discussion

Specimens from 7 genera were recorded visiting sorghum flowers: 6 bee genera and 1 beetle species. The bees were observed to collect pollen from sorghum. They not only visited one flower, but several flowers consecutively . From the 6 bee genera, 5 were from the family Halictidae and one was a local honey bee variety (Table 1). Figure 1 shows *Apis mellifera* and a solitary bee collecting pollen on sorghum flowers. *Apis mellifera, Astylus astromaculatus* and two *Lipotriches* species were scanned for pollen grains under the electron microscope. Pollen grains were found on all the investigated insects; the grains were identical to pollen obtained directly from sorghum anthers. Pollen morphology was additionally compared with reports in the literature, where a good characterisation of sorghum pollen was available. Pollen characteristics matched in terms of pollen size, pore diameter, annulus diameter and exine ornamentation (Chaturvedi et al. 1994). The pollen size of sorghum was 40 µm (37-45 µm), the pore diameter was approximately 3 µm, the annulus diameter 9 µm and the exine ornamentation can

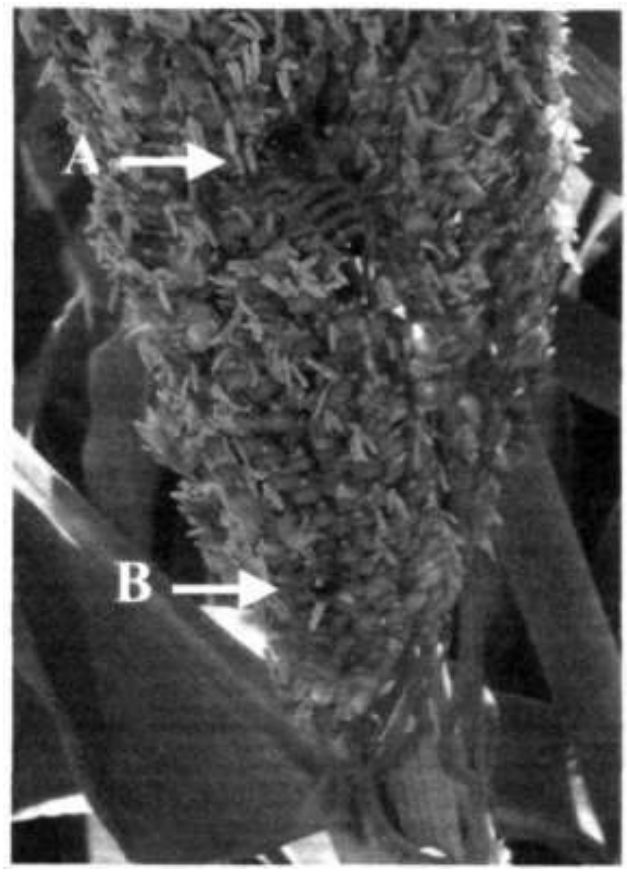

Figure 1. *Apis mellifera* (A) and a solitary bee (B) collecting pollen side by side. Note the size difference.

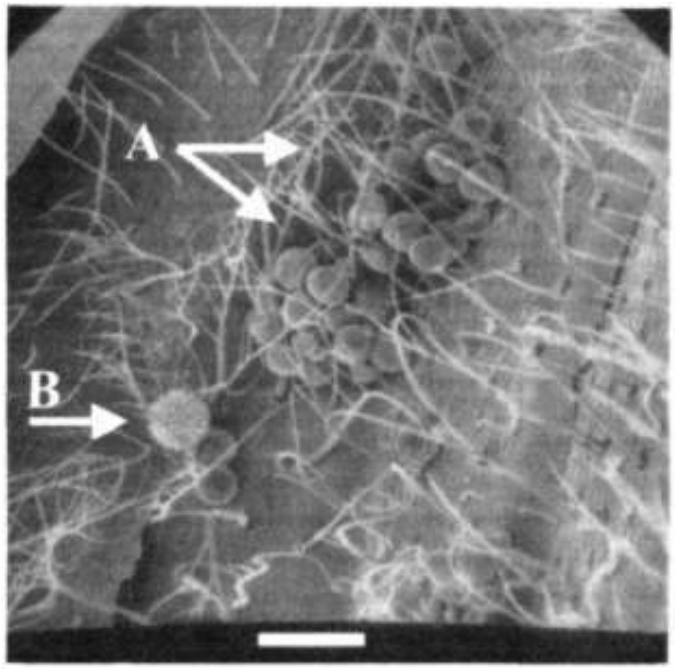

Figure 2. Pollen grains found on the body surface *of Lipotriches* sp.1. (A) Pollen grains from *Sorghum tricolor.* (B) Pollen grain from a different species. Scale bar = 100 µm.

Table 1: Taxonomy of insects collected from sorghum flowers.

Pollinator	Family	Species (Genus)
Wild bee	Halictidae	*Spatunomia* sp.
	Halictidae	*Patellapis* (Zonalictus) sp.
	Halictidae	*Lipotriches* sp.
	Halictidae	*Nomia (Acunomia)* sp.
	Haliclidae	*Lasioglossum (Evylaeus)* sp.
Honey bee	Apidae	*Apis mellifera* prob. *scutellata* Lepeletier
Beetle	Melyridae (Melarinae)	*Astyhis atromaculatus* Blanchard

be described as "insular type" (resembling a number of small pieces fitted together). Figure 2 depicts pollen found on *Lipotriches* sp. 1.

The pollen found on the investigated species originated without a doubt from the sorghum in the central field of the field trial. In terms of quantity, *A. mellifera* specimens carried the greatest amount of pollen, followed by the medium-sized Halictidae and the small-sized Halictidae. The beetle *A. atromaculatus* carried the least amount of pollen. (In the honey bees, the pollen was glued to the hind leg but also loosely attached to body hairs, as in Halictidae species.)

Previous studies on the risk of gene flow of transgenic sorghum and outcrossing have focused on wind pollination only (Arriola 1995 and Arriola and Ellstrand 1996). The findings presented in this study are a strong indication for bee pollination in *S. bicolor.* The results cannot be considered conclusive because the field trial was not specifically designed to distinguish between wind and insect pollination. However, the fact that several bees visited several sorghum flowers and collected pollen is strong evidence for this pollination mechanism. The contribution of bee pollination to total pollination in sorghum is difficult to estimate, as wind pollination is still believed to be mainly responsible for sorghum outcrossing. Bee pollination, however, could have effects beyond distances where wind pollination normally plays a major role (up to several hundred meters). Even though sorghum pollen morphology is clearly classified as anemophily (single pollen, no sticky exine, smooth surface), it may be foraged and used as a food source for bees, especially when other nearby pollen sources are unavailable during the sorghum flowering period. Another factor favoring bee visits in crop sorghum could have been the design of the field trial, namely the close vicinity of crop plants to local undisturbed grassland.

The sorghum gene flow field trial was not designed to confirm the occurrence of, nor quantify the extent of, bee pollination in sorghum. No differences between wind and bee pollination could be detected, and, since the receptor plants in the surrounding areas were male sterile, the bees were not rewarded for visiting these flowers. For future studies investigating the role of bee in sorghum outcrossing, wind pollination must be excluded (e.g. by carrying out the study in a glasshouse and providing beehives for pollen active time period) and the receptor plants should be male fertile. The relative frequencies of self pollination and outcrossing will have to be investigated using either molecular or unambiguous dominant phenotypic markers. The observations presented here not only show the flexibility of ecological interactions and the complexity of biological systems but also demonstrate the challenges of comprehensive risk assessment in transgenic sorghum (as well as in other Graminae crops). The inclusion of bee pollination in the risk assessment of transgenic sorghum adds more uncertainty to the prediction of gene flow, which was previously thought to be driven by wind alone. These considerations should be kept in mind when assessing the risk of gene flow, as they affect three different biosafety aspects:

1. When determining adequate buffering distances between transgenic sorghum fields and other sorghum fields (or wild relatives), the role of bee-transmitted pollen has to be considered. In contrast to wind pollination - which is believed to occur mainly within a distance of a few hundred meters - bee pollination and foraging may extend up to 5 km.

2. The impact on beekeepers active in the vicinity of transgenic sorghum fields and their honey production will have to be investigated within this new perspective.

3. The potential effects of transgenic pollen on honey bees and solitary bees will have to be investigated, especially when new transgenic characteristics deal with insect resistance. The effect of "built-in" insecticides on nontarget organisms such as bees will have to be considered in future studies of formerly-considered "wind pollinated" crops.

One possibility to impede gene flow (whether by wind or bees) has been proposed by Pedersen et al. (2003), namely the use of cytoplasmatic male sterility in transgenic sorghum together with male fertile conventional lines. This seed production system could prevent gene flow in transgenic sorghum as no pollen is dispersed by wind and no bees are attracted to the sterile lines.

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Utilization

Performance of Layers on Sorghum-Based Poultry Feed Rations

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Introduction

Sorghum *[Sorghum bicolor* (L.) Moench] in India is grown in the rainy as well as postrainy seasons, generally by resource-poor small farmers in the semi-arid regions. The rainy season sorghum is often vulnerable to grain deterioration due to grain mold attack, making it unfit for food use. However, normal and molded grain has enormous demand for industrial uses such as preparation of animal/poultry feed and alcoholic beverages. A lack of assured supply of the sorghum grain produced in rainy season limits its use to only about 10% of the potential industrial demand. By 2010, the demand for rainy season sorghum for industrial use is estimated to increase by 10-30%; with the major demand expected to be from the poultry industry, which is growing at a rate of 15-20% per annum (Kleih et al. 2000). But, apprehension about energy levels of sorghum-based feed rations among feed manufacturers and poultry producers is one of the major limiting factors for its use in the poultry industry.

Considering the expected increase in demand and to assess the feasibility of the use of sorghum grain based rations in poultry industry, ICRISAT along with Acharya NG Ranga Agricultural University (ANGRAU) conceptualized and implemented a project (funded by Department for Internationa] Development, UK) in collaboration with the non-governmental organizations. Federation of Farmers Associations (FFA), Andhra Pradesh Poultry Federation (APPF) and Janaki Feeds (a private sector partner). One of the project's goals is aimed at enhancing the use of rainy season sorghum in poultry feed rations in layer production as a potential alternative to maize and to create sustainable marketing linkages between sorghum growers and the poultry industry through innovative institutional systems. Performance of layers on rainy season sorghum grain-based

feed rations was studied and the results are reported in this article.

Materials and Methods

Improved sorghum cultivars such as CSV 15, PSV 16, CSH 16 and S 35 were supplied in the rainy season of 2003 to 74 selected farmers in four villages of Mahabubnagar and Ranga Reddy districts of Andhra Pradesh, India. In each village, meetings were held with farmers where the objectives and methodology of the experiment were explained and participation was solicited on a voluntary basis. Efforts were also made to encourage female farmers to participate. Farmers were selected randomly with the help of FFA. Farmers selected to participate in these trials broadly possessed the following characteristics, as guided by Ray et al. (1989).

- They were willing to accept the innovations (such as high-yielding moderately mold resistant sorghum cultivars)
- They were traditional sorghum farmers using normal agronomic practices
- They were willing to be guided by research staff and to carry out operations as prescribed.
- They all agreed to cooperate without any financial incentives other than subsidized seed.

The project staff monitored the crop frequently and the farmers were advised appropriately on following recommended production practices. After harvest, the surplus grain was bulked at village level and supplied to Janaki Feeds (poultry feed manufacturer) and poultry feed rations were prepared using the sorghum in different proportions. The Poultry Feed Trials (PFTs) were conducted at Poultry Experimental Station, ANGRAU, Rajendranagar, Hyderabad. Maize-based diets were used as control in the PFTs.

Experimental diets. Sorghum was included part-by-part replacing maize at 0%, 50%, 100% and 100%, + *3% Stylo* of control diet. Each diet was prepared both in mash and pellet forms, making for a total of 8 treatments. *Stylosanthes* leaf meal was included in place of deoiled rice bran as a source of dietary yellow pigment for egg yolk color. Feed and water was offered ad libitum during the experimental period.

Birds and housing. Five hundred, four day-old commercial chicks of egg type (White Leghorn) were classified into 42 groups based on body weight. All the groups were randomly allotted to six treatments i.e., 0%, 50% and 100% sorghum inclusion diets, each in both mash and pellet forms. Each of the dietary treatments was allotted to seven replicate groups during the growth period (day old to 18 weeks age) and four replicate groups during laying period (24-44 weeks age). Birds were housed in electrical battery brooders up to the age of 8 weeks, and then all the birds were shifted to grower cum layer cages up to 18 weeks age (At this stage, the experiment was disturbed due to mortality across all the treatments in experimental station. During this period, all the birds were given control diet. After six weeks the trials were continued). At the age of 24 weeks, 256 birds were allotted to 32 groups (8 birds/group) based on their egg production and housed individually. All the groups were randomly allotted to 8 treatments i.e., 0%, 50%, 100% and 100% + 3% *stylo* sorghum inclusion diets, each in both mash and pellet forms. A common layer vaccination schedule was followed.

Body weight and feed intake of birds were recorded at 2-week intervals during the growing period. Feed conversion ratio (FCR) was calculated as feed/body weights at 8 and 18 weeks of age. Mortality if any during growing period was also recorded. Layer trial data were collected for a total of 5 periods from 24 weeks to 44 weeks of age. Each period comprised of 4 weeks (28 days) duration. Data on egg production and mortality if any were recorded daily. Data on body weight and feed intake were recorded. To study egg quality traits, two eggs were taken on 3 consecutive days from each replicate at the end of each period. Eggs were weighed individually and internal egg quality parameters such as albumin index, yolk index, Haugh unit score, shell thickness and yolk color scores were recorded period-wise. Yolk color score was recorded by comparing the standard Roche Fan color scale. FCR was calculated at the end of each period and expressed as feed (kg)/ 12 eggs and feed (kg) /kg egg mass. Feed cost up to 8, 18 week age period and also feed cost per egg during each period of the laying stage was estimated.

Results and Discussion

There were no significant differences between the control and sorghum grain-based diets with respect to body weight, feed intake and feed conversion ratio (FCR) up to 8 weeks of age (Table 1). These results are in line with those of Madacsi et.al. (1988). However, at 18 weeks of age, there were significant differences in body weight and feed intake among the treatment groups. It was interesting to note that the body weight and feed intake of birds fed on control diet in mash form were high compared to that of other dietary treatments despite the nonsignificant differences in FCR among the treatments. All the birds achieved standard body weight of 1.2 kg by the end of 18 weeks irrespective of their diet treatment.

Table 1. Performance of egg type commercial chickens on different sorghum grain-based diets.

Treatment	Feed form	8th week age: Feed intake (g)	8th week age: Body weight (g)	8th week age: FCR (feed/gain)	8th week age: Feed cost up to 8th week (Rs)	18th week age: Feed intake (g)*	18th week age: Body weight (g)*	18th week age: FCR (feed/gain)	18th week age: Feed cost up to 18th week (Rs)
Sorghum @ 50%	Mash	1933	637	3.038	14.44	6100[a]		5.083	42.15
	Pellet	1911	652	2.935	14.75	6073[a]	1206[a]	5.037	43.48
Sorghum @100%	Mash	1897	637	2.978	13.88	6136[a]	1208[a]	5.083	41.54
	Pellet	1896	645	2.938	14.35	6176[ab]	1202[a]	5.138	43.35
Control	Mash	1901	621	3.061	14.47	6324[b]	1233[b]	5.130	44.58
	Pellet	1884	658	2.865	14.80	6129[a]	1202[a]	5.102	44.74
SEm±		41.3	16	0.091		76.9	9.4	0.071	

*Values followed by the same letter in the column are not significantly different at 5% level.

Table 2. Performance of commercial layer birds on sorghum grain-based diets.

Treatment	Feed form	Egg production Hen-day%	Feed intake (g)*	FCR/ 12 eggs (g)	FCR/kg egg mass (g)	Egg weight (g)*	Feed cost/egg (Rs)
Sorghum @ 50%	Mash	84.9	112.3[ab]	1.603	2.426	55.1[ab]	1.10
	Pellet	86.6	115.3[bcd]	1.598	2.396	55.5[bc]	1.04
Sorghum @ 100%	Mash	87.4	115.3[bcd]	1.586	2.434	54.2[a]	1.07
	Pellet	87.9	112.6[ab]	1.543	2.341	54.9[ab]	1.01
Sorghum @ 100% + *Stylo* 3%	Mash	85.3	111.1[a]	1.570	2.401	54.4[ab]	1.01
	Pellet	86.0	113.7[abc]	1.588	2.343	56.4[c]	0.99
Control	Mash	87.1	117.1[d]	1.614	2.481	54.1[a]	
	Pellet	86.7	116.5[cd]	1.615	2.377	56.6[c]	1.07
SEm±		0.92	1.61	0.040	0.068	0.57	1.04

*Values followed by the same letter in the column are not significantly different at 5% level.

Table 3. Egg quality parameters of birds fed on sorghum grain-based diets.

Treatment	Feed form	Haugh unit score	Albumen Index	Yolk index	Shell thickness (mm)	Yolk color score
Sorghum @50%	Mash	74	0.070	0.377	0.376	++
	Pellet	72	0.068	0.376	0.371	++
Sorghum @ 100%	Mash	73	0.073	0.379	0.369	+
	Pellet	74	0.070	0.368	0.355	+
Sorghum@ 100%+Stylo 3%	Mash	72	0.067	0.370	0.371	++
	Pellet	72	0.066	0.394	0.355	++
Control	Mash	75	0.072	0.383	0.361	++++
	Pellet	75	0.073	0.388	0.376	++++
SEm±		1.6	0.003	0.011	0.006	

Note: Cost of maize and sorghum was considered as Rs.6 kg^{-1} and Rs. 5.40 kg^{-1}, respectively.

Highest reduction in cost of feed was observed in diets on 100% replacement of maize with sorghum in both mash and pellet forms as well up to 8 weeks of age. Similar cost reduction was observed on sorghum-based diets up to 18 weeks of age.

The egg production performance of commercial layers (24-44 weeks) did not appear to be influenced by their diet treatments (Table 2). The results reported by Ambula et al. (2003) lend sufficient support to the present findings. Inclusion of *Stylosanthes* leaf meal at 3% in the diet comprising 100% sorghum in place of maize resulted in lower feed consumption by commercial layers compared to control diet. However, diet form did not have any influence on feed consumption. FCR was similar in all the experimental diets. Significant differences in egg weight among the treatments were observed with pellet diets resulting in higher egg weight compared to mash diets. The results are in congruence with the findings of Madacsi et.al. (1988). Feed cost per production of an egg was low in sorghum diets compared to control. Inclusion of 3% *Stylosanthes* leaf meal in sorghum diets further lowered the feed cost.

There is no reason to believe that inclusion of sorghum at any level in feed ration affects internal egg quality parameters (Table 3). However, the egg yolk color assessed by visual score method indicated a proportionate reduction due to inclusion of sorghum at different levels in diets. Though egg yolk color improved with the inclusion *of Stylosanthes* leaf meal at 3% level in 100% sorghum diet, the improvement was only 50%. Mortality throughout the experiment was within normal limits.

Conclusions

The experiments showed that maize can be replaced by sorghum in poultry feed rations without affecting body weight and egg production performance of layer birds. Considerable cost reduction was also achieved with sorghum-based diets, particularly in mash form. Quality traits of eggs produced by layer birds fed with sorghum-based diets were also comparable to that of maize-based diets except yolk colour. However, yolk colour was partially improved with addition of 3% *Stylosanthes* leaf meal at 100% inclusion level of sorghum with considerable positive cost effectiveness. These results provide sufficient evidence to dispel the apprehensions among poultry feed manufacturers and poultry producers about the use of sorghum grain as an alternative to maize in poultry feed rations. The encouraging results have been already disseminated to poultry feed manufacturers and poultry producers through stakeholder workshops.

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Sweet Sorghum - A Potential Alternate Raw Material for Bio-ethanol and Bio-energy

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Introduction

Sweet sorghum [*Sorghum bicolor* (L.) Moench] is a special purpose sorghum with a sugar-rich stalk, almost like sugarcane. Besides having rapid growth, high sugar accumulation, and biomass production potential, sweet sorghum has wider adaptability (Reddy and Sanjana 2003). Given that water availability is poised to become a major constraint to agricultural production in coming years (Ryan and Spencer 2001), cultivation of sugarcane becomes difficult. Sweet sorghum would be a logical crop option in lieu of sugarcane in such situations. Sweet sorghum can be grown with less irrigation and rainfall and purchased inputs compared to sugarcane. The sugar content in the juice extracted from sweet sorghum varies from 16-23% Brix. It has a great potential for jaggery, syrup and most importantly fuel alcohol production (Ratnavathi et al. 2004a). The stillage after extraction of juice from sweet sorghum can be used for co-generation of power.

Need for alternate raw material

The Supreme Court of India informed the Government of India (GOI) to use Compressed Natural Gas (CNG) as an alternative to petrol and diesel for fuelling automobiles to reduce environmental pollution. However, considering the reduced output by the Oil and Natural Gas Corporation (ONGC), and thereby likely shortage of CNG in future (Anonymous 2001), the GOI has made it mandatory to blend petrol and diesel with ethanol (to reduce carbon monoxide emission in automobiles) initially up to 5% and gradually hiking it to 10% in the second phase. There are two objectives in this strategy, reducing both the environmental pollution and the fuel-import bill for the country. According to the Federation of Indian Chambers of Commerce and Industry (FICC1), India could save nearly 80 million L of petrol annually if petrol is blended with alcohol by 10%. Burning quality of alcohol-blended petrol is more eco-friendly than that of CNG (Arbatti 2001). These environment and cost considerations have triggered a debate on the availability of adequate raw material to meet the possible increased demand for ethanol production. Molasses (a by-product of sugarcane after the extraction of sugar), the traditional source of raw material for ethanol production, is unlikely to meet the actual demand in the long run (Ratnavathi et al. 2004b). The requirement of ethanol in India to blend with petrol (10%) is about 1000 million L, and for blending with diesel (5%) another 3000 million L per annum. Total ethanol requirement including other purposes is 5000 million L per annum. The possible ethanol production from available sugarcane molasses (8.2 million t) and other sources is 2000 million L per annum. This leaves a deficit of 3000 million L of ethanol per annum. Further, the molasses-based ethanol distilleries operate only for 180 days (during sugarcane crushing season) because of the limited availability of the molasses to run the distillery throughout the year as well as the problems associated with the spent wash to comply with pollution control standards [Personal communication from Patil, Vasanthadada Sugar Institute (VS1), Pune, India]. The existing distilleries therefore, operate at 50% efficiency and needs alternate raw material(s) to operate at their full efficiency (Anonymous 2004). The underutilization of the existing molasses-based ethanol distilleries and the deficit in ethanol requirement can be made good if sweet sorghum cultivation is promoted for ethanol production.

Comparative advantages of sweet sorghum

In recent years, there has been increased interest in the utilization of sweet sorghum for ethanol production in India as its growing period (about 4 months) and water requirement (8000 m^3 over two crops) (Soltani and Almodares 1994) are 4 times lower than those of sugarcane (12-16 months and 36,000 m^{-3} $crop^{-1}$ respectively). The cost of cultivation of sweet sorghum is three times lower than that of sugarcane (Dayakar Rao et al. 2004) (Table 1). Further, sweet sorghum is best suited for ethanol production because of its higher total reducing sugar content and poor sugar content compared to sugarcane juice (Huligol et al. 2004). The presence of reducing

sugars in sweet sorghum prevents crystallization and sweet sorghum cultivars have 90% fermentation efficiency (Ratnavathi et al. 2004a). These along with its suitability for mechanized crop production, seed propagation and higher ethanol production capacity of sweet sorghum (2800 L ha^{-1} yr^{-1} over two crops; 70 t ha^{-1} of millable stalk per two crops @ 40 L t^{-1}) *vis-a-vis* sugarcane molasses (850 L ha"^{1} yr^{1}; 3.4 t ha^{-1} @ 250 L t^{-1}) (as per the pilot study by VSI, Pune, India) (Table 1) makes sweet sorghum the best alternative source of raw material that can be used as a supplementary raw material rather than as a substitute to ethanol production in India.

Comparative economics of ethanol production by sweet sorghum and sugarcane

A techno-economic feasibility study undertaken by the National Research Center for Sorghum (NRCS), Hyderabad, Andhra Pradesh, with active collaboration with M/s Renuka Sugars Ltd, Belgaum, Karnataka, indicated that the per liter cost of production of ethanol from sweet sorghum (Rs. 13.11) is slightly lower than that from sugarcane molasses (Rs. 14.98) (Table 2). In addition to sweet-stalk, grain yield of 2 to 6.0 t ha^{-1} (which can be used as food or feed) could be harvested from sweet sorghum. Further, the stillage from sweet sorghum after the extraction ofjuice has a higher biological value than the bagasse from sugarcane when used as forage for animals, as it is rich in micronutrients and minerals (Seetharama et al. 2002). It could also be processed as a feed for ruminant animals (Sumantri and Edi Purnomo 1997). The stillage contains similar levels of cellulose as sugarcane bagasse, and therefore has a good prospect as a raw material for pulp product. According to a pilot study by Shree Renuka Sugars Ltd., Karnataka, India, blending sweet sorghum juice up to 10% in sugarcane juice does not affect crystallization; hence it is compatible with the sugarcane industry (Huligol et al. 2004). Apart from these, the pollution level in sweet sorghum-based ethanol production has 1/4th of the biological oxygen demand (BOD, 19,500 mg L^{-1}) and lower chemical oxygen demand (COD, 38,640 mg L^{-1}) compared to molasses-based ethanol production (Personal communication from Patil, VSI, Pune, India). Further, ethanol is a "clean burning fuel" with a high octane rating because of its low sulphates and aldehydes and existing automobile engines can be operated with Gasohol (petrol blended with

Table 1. Comparative advantages of sweet sorghum vs. sugarcane/sugarcane molasses for ethanol production.

Crop	Cost of cultivation (Rs. ha^{-1})[1]	Crop duration[2]	Water requirement[2]	Ethanol productivity (L ha^{-1})[3]
Sweet sorghum	17820	4 months	8000 m^3 over two crops	2800 year-1 over two crops[(4)]
Sugarcane	49250	12-16 months	36,000 m^3 $crop^{-1}$	6500 $crop^{-1}$ [(5)]
Sugarcane- molasses				850 $year^{-1}$[(6)]

1. Source: Dayakar Rao et al. (2004);
2. Source: Soltani and Almodares (1994)
3. Personal communication from Patil, Vasanthadada Sugar Institute, Pune, India
4. 70 t ha^{-1} millable stalk ovcrtwo crops @ 40 L t^{-1};
5. 85-90 t ha^{-1} millable cane $crop^{-1}$ @ 75 L t^{-1};
6. 3.4 t ha^{-1} @ 250 L t^{-1}.

Table 2. Comparative per liter cost of ethanol production from sweet sorghum and sugarcane molasses.

Particulars	Sweet sorghum[1] (Rs. L^{-1})	Sugarcane molasses[2] (Rs. L^{-1})
Manpower	0.50	0.25
Steam	1.00	1.00
Electricity	1.00	1.00
Yeast	0.10	0.10
Management/Administration	0.10	0.25
Pollution control	Nil	0.25
Raw material	10.41	12.13
Total	13.11	14.98

1. Sweet sorghum stalk @ Rs. 500 t^{-1};
2. Sugarcane molasses @ Rs. 2000 t^{-1}

Source: Dayakar Rao et al. 2004

ethanol) without any need for engine modification (Ratnavathi et al. 2004a). Thus, from both economics and environment protection point of view, sweet sorghum offers good prospects for ethanol production as an additional feed stock to existing distilleries.

Prospects of enhancing genetic potential of sweet sorghum

The wide range of variability for Brix reading (from 13 to 24%), sucrose% (from 7.2 to 15.5), stalk yield (from 24 to 120 t ha^{-1}) and biomass yield (from 36 to 140 t ha^{-1}) in sweet sorghum (Almodares et al. 1997) under long growing seasons in Iran; and Brix readings (from 14.1 to 19.2%) and millable stalk yield (27.1 to 47.6 t ha^{-1}) under Indian conditions (AICSIP 2004-2005) indicates the high potential for genetic improvement to produce high sweet-stalk yield coupled with high sucrose% sweet sorghum lines. Genotypic differences for extractable juice, total sugar content, and fermentation efficiency and alcohol production have also been reported (Ratnavathi et al. 2003). The predominant role of non-additive gene action for plant height, stem girth, total soluble solids, millable sweet-stalk yield and extractable juice yield (Sankarapandian et al. 1994) indicates the importance of breeding for heterosis for improving these traits. The substantial magnitude of standard heterosis for all the traits related to ethanol production (plant height: up to 46.9%, stem girth: up to 5.3%, total soluble solids (%): up to 7.4%, millable stalk yield: up to 1.5% and extractable juice yield: up to 122.6%) (Sankarapandian et al. 1994) further supports breeding for heterosis for genetic enhancement of sweet sorghum.

Sweet sorghum research at ICRISAT

Hybrid parents. Sweet sorghum research at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) was initiated in 1980 to identify lines with high stalk-sugar content in part of the sorghum world germplasm collection maintained at ICRISAT's gene bank initially by chewing the stalks at maturity. Seventy accessions that tasted sweet were evaluated during the rainy season of 1980 and nine accessions with high sugar content were planted in the 1981 rainy season. Two cultivars, IS 6872 and IS 6896, were selected. The mean stalk-sugar content of the nine accessions grown in 1980 and 1981 varied by roughly 3% between the two seasons, indicating that differences between growing seasons had little influence on the stalk-sugar content. The density of juice did not show appreciable variation among the accessions (Subramanian et al. 1987). Apart from this, several sweet sorghum lines with high Brix values were identified among Nigerian and Zimbabwe lines and within advanced breeding progenies at ICRISAT-Patancheru.

Due to changed focus driven by donor perceptions and the needs of national agricultural research systems (NARS), sweet sorghum research at ICRISAT was discontinued in the late 1990s. However. ICRISAT renewed its sweet sorghum research in 2002 to meet the possible increased demand created for ethanol following the Indian Government's policy to blend petrol and diesel with ethanol. As a short-term strategy for immediate utilization for hybrid cultivar development, a set of 92 hybrid parents (among the existing diverse set of grain sorghum hybrid parents) with high Brix values were identified based on field evaluations during the 2002 rainy, 2002-03 postrainy and 2004 rainy seasons. Promising among these are ICSB 264, ICSB 401, ICSB 405, ICSB 472, ICSB 474 among the seed parents (B-lines), and ICSR 93034, S 35, ICSV 700, ICSV 93046, Entry# 64 DTN among the varieties/R-lines (Table 3). Four of these lines, S 35, ICSV 700, ICSR 93034 and Entry# 64 DTN, were tested in the All India Coordinated Sorghum Improvement Project (AICSIP) during 2004 rainy season and two of these, ICSV 700 and ICSR 93034, have been promoted for advanced testing.

Table 3. Promising sweet sorghum hybrid parents identified at ICRISAT, Patancheru, India.

Year / season of evaluation	Promising lines	Stalk Brix (%)	Sweet-stalk yield (t ha^{-1})
Hybrid seed parents			
2002 rainy and 2002-03 postrainy seasons	ICSB 472, ICSB 401, ICSB 405, ICSB 731	15.9 to 17.9	17 to 35
2004 Rainy season	ICSB 264, ICSB 293. ICSB 213, ICSB 654, ICSB 474	13.3 to 16.8	14 to 24
Varietal/Restorer lines			
2002 rainy and 2002-03 postrainy seasons	GD 65003, Entry#64 DTN. GD 65080, ICSV 96143, ICSV 93046	13 to 20	26 to 46
2004 Rainy season	ICSR 93034, ICSR 91005, S 35. ICSV 574. ICSR 93026-2, ICSV 700	16.8 to 20.3	30 to 45

Further, five other varieties, ICSV 574 (SPV 422), SPV 1411, ICSV 93046, Seredo and NTJ 2, were sent to the NRCS, Hyderabad, India, for preliminary testing under A1CSIP during the 2005 *kharif* season.

A set of 48 varieties/restorers selected based on high Brix reading and sweet-stalk yield from a set of 92 entries were evaluated during 2003 rainy season at ICRISAT-Patancheru, Andhra Pradesh. The sugar% in the entries ranged from 11.5 to 20.6 (trial mean 17.3) and sweet-stalk yield from 14.6 to 62.5 t ha^{-1} (Trial mean 36.2; SE ± 4.3). Two varieties (NTJ 2 and Seredo) and one restorer line (ICSR 93034) were on par with SSV 74 and SSV 84 for sugar% and sweet-stalk yield. They showed 10% superiority for sugar% and sweet-stalk yield over S 35. In another trial consisting of 28 sweet stalk B-lines, the sugar% in the B-lines ranged from 10.9 to 21.8 (trial mean 16.4) and fresh fodder yield ranged from 9.6 to 53.9 t ha^{-1} (trial mean 26.6) with sugar% being on par with sweet stalk check varieties, SSV 53, SSV 74 and SSV 84. Among these, ICSB 293 and SP 20656 B produced 46% higher grain yield than 296 B (widely used grain sorghum seed parent).

Hybrids. Research experience at ICRISAT and elsewhere, shows that hybrids are known to be relatively more tolerant and produce higher biomass, besides being early and photo- insensitive than the pure-line varieties. The requirement of photoperiod- and thermo-insensitiveness is essential to facilitate plantings at different dates to ensure a year-round supply of sweet sorghum stalks for ethanol production. Therefore, at ICRISAT hybrid parents' research is being given strategic importance for enhancing genetic potential of sweet sorghum. As mentioned in the previous section, several promising sweet-stalk hybrid parents have been identified at ICRISAT. To strengthen the hybrid parents' research and to delineate method(s) of developing sugar-rich high stalk yielding hybrids, 144 hybrids synthesized by crossing nine male sterile lines (five with low Brix and four with high Brix values) with 16 restorer lines (eight with low Brix and eight with high Brix values) were evaluated under protective irrigation at ICRISAT, Patancheru, India, Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri, and at the University of Agricultural Sciences (UAS), Dharwad, under the Indian Council of Agricultural Research (ICAR)-ICRISAT partnership project and as a part of the research work of a PhD scholar from the UAS, Dharwad, during the 2004 *kharif* season. Results indicated that the hybrids were 15 days earlier in maturity than their parents. Some of the hybrids produced higher milleable cane yield (t ha^{-1}), juice yield (kL ha^{-1}), sugar yield (t ha^{-1}) on juice yield basis than the check variety SSV 84 (Table 4). Further, it was found that the probability of producing high-yielding sugar-rich hybrids is significantly higher if both the hybrid parents or at least the male parent are sugar-rich. A Special/Sweet Sorghum Hybrid, CSH 23 (NSSH-104) developed by the NRCS from ICSA 38, an ICRISAT-bred male-sterile (seed) parent and SSV 84 (the sweet-stalk sorghum variety bred and released for general cultivation by the national program in India through extensive testing in AICSIP in 1992/93) is being recommended for release for commercial cultivation. Several new hybrids were developed during 2004 *kharif* season and promising hybrids were identified. A total of 15 promising hybrids (ICSA 56 x SPV 1411, ICSA 293 x SPV 1411, ICSA 474 x SPV 1411, ICSA 52 x SSV 84, ICSA 293 x SSV 84, ICSA 686 x SSV 84, ICSA 264 x SSV 74, ICSA 293 x SSV 74, ICSA 560 x NTJ 2, ICSA 675 x NTJ 2, ICSA 293 x ICSV 700, ICSA 474 x ICSV 700, ICSA 502 x ICSV 574, ICSA 561 x ICSV 574 and ICSA 502 x Seredo) were sent to NRCS, Hyderabad, India, for

Table 4. Performance of selected sweet-stalk hybrids at ICRISAT-Patancheru, India- 2004 *kharif* season.

Hybrid	Days to 50% flowering	Brixreading at maturity	Millable cane yield (t ha^{-1})	Juice yield (k1 ha^{-1})	Sugar yield[1] (t ha^{-1})	Grain Yield (t ha^{-1})
ICSA 264 x ICSV 93046	70	16.0	43.6	18.5	2.6	6.5
ICSA 213 x S 35	72	17.0	48.6	17.6	2.6	5.3
ICSA 474 x ICSV 574	75	19.0	52.2	17.2	2.9	3.8
ICSA 474 x SSV 74	74	17.7	50.7	17.1	2.7	4.4
ICSA 657 x SSV 84	74	17.7	48.8	16.8	2.6	4.7
ICSA 474 x SSV 84	74	19.7	50.0	16.7	2.9	3.9
SSV 84 (control)	88	22.7	41.0	12.1	2.4	0.2
Mean	69	15.7	32.9	10.8	1.5	4.6
CV (%)	1.7	8.1	15.0	21.6	24.3	22.0
LSD (5%)	1.9	2.1	7.9	3.8	0.6	1.6

1. Sugar% based on formula (Brix at maturity x 0.8746) + 0.1516.
2. Sugar yicld= (sugar%/100) x juice yield (k1 ha^{-1}) (Source: Corleto et al. 1986).

preliminary evaluation under AICSIP during the 2005 kharif season.

Sweet sorghum research in Indian national programs

Sweet sorghum research in India is carried out at NRCS, Hyderabad, and at six AICSIP centers, Rahuri, Parbhani, Akola, Surat, Coimbatore and Dharwad. The NRCS has been engaged in sweet sorghum research since 1989. The sweet sorghum varieties and hybrids bred at NRCS, Hyderabad, have the ability to produce extremely high stalk yields of up to 50 t ha^{-1}, with juice Brix reading between 18% and 22% and 1.5 to 2.5 t ha^{-1} grain (Table 5).

Concerted research efforts at AICSIP centers have resulted in the identification of several promising sweet sorghum varieties such as SSV 96, GSSV 148, SR 350-3, SSV 74, HES 13, HES 4, SSV 119 and SSV 12611 for TSS% and juice yield (AICSIP 1991-92), GSSV 148 for cane sugar (AICSIP 1993-94), NSS 104 and HES 4 for green cane yield, juice yield, juice extraction and total sugar content (AICSIP 1999-2000), and RSSV 48 for better alcohol yield during 2001-02 (AICSIP 2001-2002). An evaluation of 12 sweet sorghum varieties bred at different AICSIP centers in Advanced Sweet Sorghum Varietal Trial at four locations in India-Parbhani, Akola, Rahuri, S-wadi and NRCS, Hyderabad, indicated the significant superiority of one hybrid, NSSH 104, and two varieties, RSSV 56 and PAC 52093 for millable cane yield and juice yield by 31% and 18%, 17% and 4% and 17% and 18%, respectively, over the check, SSV 84 (AICSIP 2004-2005). The Brix reading of the test hybrid, NSSH 104 (20%), was comparable to the check, SSV 84 (19.2%).

A National Agricultural Technology Project (NATP) on sweet sorghum for bio-energy (RNPS 24) ran from 2000-2005 at NRCS, Hyderabad. A strong network with AICSIP program is currently underway to identify high biomass and bio-energy sweet sorghums from multi-environment trials.

Opportunities for ICRISAT-public and private sector collaboration

Genetic enhancement

ICRISAT-public sector. Recognizing that NRCS, Hyderabad, is the leader in sweet sorghum research in India, ICRISAT desires active collaboration with the NRCS in the following areas of genetic enhancement of sweet sorghum:

- Study the relationship between stem sugar% and agronomic traits
- Study the inheritance of sugar% and related traits
- Identify/develop sorghum hybrid parents with high sugar% and traits related to high sugar% and their further improvement

Table 5. Promising sweet sorghum varieties identified for ethanol production in Indian national programs.

Sl. No.	Variety	Stalk yield (t ha^{-1})	Grain yield (t ha^{-1})	Juice extract-ability (%)	Brix (%)	TSS (%)	RSS (%)	Sucrose (%)
1	RSSV 59	48.4	2.2	50.1	17.7	15.1	1.5	13.6
2	RSSV 46	47.6	2.8	42.5	16.2	13.1	1.7	11.3
3	RSSV 24	45,7	1.5	46.7	16.1	13.1	1.4	11.2
4	RSSV 45	45.5	2.0	39.1	16.8	14.1	1.8	11.9
5	RSSV 57	44.3	2.5	45.7	16.6	13.7	1.3	12.0
6	RSSV 44	44.5	2.6	43.1	15.9	13.2	1.3	12.3
7	RSSV 58	42.4	2.2	46.1	17.2	13.3	1.4	11.9
8	NSS 219	40.5	2.1	42.3	16.6	14.1	2.0	12.0
9	NSS 216	39.2	2.7	41.3	16.2	13.8	1.9	11.7
10	NSS 218	38.6	2.2	39.4	16.9	13.0	2.2	10.6
11	NSS 209	38.1	1.8	46.3	16.9	13.6	1.5	11.6
12	NARISS 41	34.5	1.9	48.8	14.2	12.9	1.3	9.5
13	AKSS 01-03	28.7	2.9	44.8	14.9	11.6	1.9	9.6
14	NARISS 83	27.9	2.3	41.1	16.2	14.4	1.9	12.3
15	SSV 84 (C)	43.6	1.8	47.1	16.5	14.1	2.1	11.8

TSS: Total soluble solids; RSS: Reducing sugar

Source: Ratnavathi et al. 2003

- Develop and identify sorghum hybrids suitable for use in bio-ethanol and bio-energy production

ICRISAT-private sector. Identification of several promising hybrid parents (Table 3) within a short span of two years since ICRISAT renewed sweet sorghum research during 2002 stands testimony to ICRISAT's strength in sweet sorghum research. It is expected that private seed companies in India would complement the efforts of the national programs in the development and marketing of location-specific, high yielding, sweet-stalk hybrids (using hybrid parents developed at ICRISAT and the national programs) in a business approach through the well-established ICRISAT-Private Sector Sorghum Hybrid Parents Research Consortium.

Technology Collaborations

ICRISAT-Industry. Research on sweet sorghum for ethanol production involves two distinct but simultaneous phases: (1) genetic enhancement of sweet sorghum for quantity and quality of juice for ethanol production and (2) development of cost-effective economy-scale ethanol production technology. While the former requires crop improvement research expertise, the latter requires industrial expertise. Therefore, "ICRISAT's strategy is two-pronged." ICRISAT, besides developing sweet sorghum hybrid parents and varieties suitable for ethanol production, facilitates private sector involvement in ethanol production technology from sweet sorghum. Ever since the establishment of Agri-Business Incubator (ABI)-ICRISAT, there have been perspective proposals for sweet sorghum-based ethanol production from major industries. These industries could be classified into two types: (1) start-up and stand-alone industries and (2) sugarcane-based industries.

Start-up and stand-alone industries. These encompass proposals from start-up entrepreneurs and stand-alone units mainly based on sweet sorghum as raw material for ethanol production. These firms require ABI-ICRISAT and NARS program (NRCS and AICSIP) support for providing sweet sorghum cultivars suitable to their command areas and agricultural extension consultancy. Some of them even need backstopping in terms of project consultancy and business facilitation support. The ABI at ICRISAT signed a memorandum of agreement (MoA) with Rusni Distilleries Pvt. Ltd., a private sector industry based in Hyderabad, which obtained licence to establish 40 KLPD distillery at Sanga Reddy Mandal in Andhra Pradesh for supply chain management, seed development, supply and procurement of raw materials. ABI-ICRISAT offers technical back-stopping on suitable sweet sorghum cultivars and sweet sorghum production technology besides laboratory and land facilities. Several other private firms such as M/s XL Telecom, M/s Minerva Industries, M/s Matrix Power Pvt. Ltd., M/s Shriba Agro and M/s Morita Biotech Pvt. Ltd., all based at Hyderabad have shown interest in incubating ethanol production technology at ABI-ICRISAT.

Sugarcane-based industries. These encompass proposals from major sugar industries. Sugar industries are looking to complement their existing molasses-based ethanol production capacities with alternative raw material to fill-in the lean sugarcane crushing periods for year-round operation. They are interested in research and development support on sweet sorghum seed-based and production technologies from ABI-ICRISAT. An MoA was signed by ICRISAT and VS1, Pune, Maharashtra, for identification/development of improved sweet sorghum varieties and characterization of juice and ethanol quality and quantity. VSI has well-equipped alcohol production and quality testing laboratories and also has connections with the distilleries in the state of Maharashtra. Several other industries such as Bannari Amman Sugars India Ltd, EID Parry India Ltd and Thiruarooran Sugars India Ltd, have expressed interest in incubating ethanol production technology at ABI-ICRISAT.

Public sector industry. Shree Renuka Sugars Ltd, located at Manoli village in Belgaum district, Karnataka, India commissioned an ethanol plant recently in 2003 and initiated a pilot project with the need for finding new substrates for producing ethanol for the National Fuel Ethanol program in collaboration with the United States Agency for International Development (USAID) and NRCS, Hyderabad (Huligol et al. 2004). Three varieties - SSV 74 from the University of Agricultural Sciences, Dharwad; cv. Madhura from Nimbakar Agricultural Research Institute, Maharashtra; and cv. SSV 84 from NRCS, Hyderabad; were supplied to farmers for evaluation. These varieties gave an average sweet-stalk yield of 10-12 t acre^{-1} and grain yield of 0.8-1.0 t acre^{-1} under normal conditions in farmers' fields. For grain yield, Madhura and for stalk yield, SSV 84, were found better. The laboratory analysis indicated that sweet sorghum juice is very rich in total reducing sugars (TRS) and comparatively poor in sugar content and hence suitable for making alcohol. From a trial al the distillery, it was reported that 112 t of sorghum stalk (25 t ha^{-1}) has 23.47% juice with 8.5% TRS, Brix of 12 and pH 5. Alcohol yield was about 16.38 L t^{-1} of stalk. The study also indicated that sweet sorghum could be cultivated in the lean period of sugarcane (as crushing period of sugarcane varies from state to state), thus extending the crushing period before and after sugarcane crushing (Ratnavathi et al. 2003).

The NRCS is also interacting with industries like Praj Industries at Pune, Maharashtra, GMR sugar industries at Sankili, Andhra Pradesh, and Mohan breweries and distilleries at Chennai, Tamil Nadu and Sagar Sugars and Allied Products, Chittor district, Andhra Pradesh, India. These industries have expressed an interest in conducting large-scale trials and big mill tests on sweet sorghum as an alternative source of raw material for ethanol production to supplement sugarcane molasses. The NRCS intends continued collaboration with these industries in the coming seasons to popularize sweet sorghum for ethanol production.

Future outlook

The promising sweet-stalk sorghum varieties identified at ICRISAT and NARS (NRCS and A1CSIP) programs need to be re-evaluated in a more systematic manner. Also, basic research aspects such as inheritance of sweet-stalk yield and juice quality-evident traits and inter-relationships among juice quality-evident traits and with ethanol quality and yield and strategic research aspects such as method(s) of developing shoot fly, stem borer and shoot bug resistant high yielding sweet sorghum varieties and hybrids and the effects of stalk-sugar content on stalk yield, juice quantity and quality and reaction to insect pests and diseases will be delineated in partnerships with NRCS and AICSIP programs. Formation of an ICRISAT-led consortia (similar to the existing and highly successful ICRISAT-Private sector Hybrid parents Research Consortia and NRCS-led NRCS-AICSIP-Alcohol industry collaborative project) comprising of NRCS, private seed companies, alcohol distilleries and sugar industries for comprehensive research and development of sweet sorghum hybrid parents and hybrids, integrated sweet sorghum production packages, and ethanol production technology would help enhance the demand for sorghum, which ultimately benefit the poor farmers of the semi-arid tropics. The existing collaboration with the private sector for developing cost-effective economy-scale ethanol production technology will be strengthened further. The partnership technologies developed, improved breeding products and ethanol production processes will be scaled-up and transferred to farmers and entrepreneurs by involving private seed companies and distilleries.

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Socioeconomics

Evaluation of Farmer-Grown Improved Sorghum Cultivars for Stover Quality Traits

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Introduction

Sorghum [*Sorghum bicolor* (L.) Moenchl is an important staple food crop in semi-arid tropical tracts of India where mixed crop and livestock farming systems are mostly prevalent. Sorghum not only provides grains for human consumption, but also provides fodder for livestock. For farmers, both grain and stover (crop residue) are of equal importance and they consider harvested stover as almost equal in market value to harvested grain. However, the feeding/nutritive value of sorghum crop residue is generally poor and it provides sub-maintenance levels of nutrients. The limited adoption of improved sorghum varieties has been mainly attributed to the lower nutritional value of their crop residue (Kelley and Rao 1994). The farmers feel that the stover yield and stover quality of improved cultivars in terms of nutrition and digestibility is lower than that of the local cultivars. This study attempts to examine the validity of farmers' perceptions about stover yield and stover quality traits of improved cultivars *vis-a-vis* local cultivars. The study was conducted as one of the activities of the Department For International Development (DFID)-funded project on *Exploring marketing opportunities through a research, industry and users coalition: sorghum poultry feed* aimed at enhancing the access to and availability of rainy-season sorghum for poultry feed rations.

Materials and Methods

Selection of villages and farmers. The Mahabubnagar and Ranga Reddy districts of Andhra Pradesh, India, where rainy season sorghum cultivation is predominant were selected for farmer participatory evaluation of improved and local cultivars for stover yield and stover

quality traits. After studying district profiles, two mandals were selected from each district. Four villages (one from each mandal) that are close to market yards, accessible in all seasons, and have existing farmers associations were selected for the study. During the 2003 rainy season, based on their willingness, 48 farmers were selected from these four villages for participation in the evaluation trials. The farmers were apprised of objectives and methodology of the study. The farmers' fields in which cultivars were grown could be classified into four soils type (Table 1), Black, *Barka, Chalka* and Red (*Chalka* - red colored with large pebbles and low fertility; *Barka* - light black in color with low fertility and low moisture retention capacity). All sorghum farmers in the selected villages traditionally cultivate a yellow sorghum variety, locally called *patcha jonna* intercropped with pigeonpea [*Cajanus cajan* (L.) Millsp.].

Cultivars and design of experiment. Seed of four improved high-yielding sorghum cultivars CSH 16, CSV 15, PSV 16 and S 35 were supplied to the 48 farmers from the selected villages for planting in a 4000 m^2 area. Each improved cultivar was planted by 12 farmers along with the traditional yellow sorghum cultivar as a check intercropped with a local pigeonpea cultivar in a row ratio of 5 sorghum: 1 pigeonpea. Leaflets containing information on production practices printed in the local language were supplied along with seed bags to the farmers. The project staff monitored the evaluation trials frequently for proper conduct of the trials.

Stover sampling for laboratory analysis. An entire field planted by a farmer to a particular cultivar was divided into four plots and from each plot, one sample was collected at random, using an 1 m^2 area sampler. While samples from improved cultivars were drawn from 48 farmers for stover quality analysis, those from local cultivars were drawn from only 5 randomly selected farmers bringing the total sample size to 48. During sampling, sufficient care was taken to draw samples from each of the predominant soil types of the region. The grain and stover yield from each 1 m^2 area were estimated. The stover samples were then ground to 1 mm particle size and analysed in the laboratory of the International Livestock Research Institute (ILRI) - South Asia Project located at ICRISAT, Patancheru, India for stover nitrogen content and stover in vitro digestibility using combinations of conventional laboratory analysis with near infra red spectroscopy (NIRS). For calibration and validation procedures for the development of NIRS equations, stover nitrogen content was determined by auto-analyser and stover in vitro digestibility as per the technique of Menke and Steingass (1988). Computed mean of data from four samples was treated as one replication, making each farmer field one replication.

Statistical Analysis. Mean values of stover nitrogen content and in vitro digestibility of each cultivar were used for statistical analysis considering cultivar and soil type as fixed factors. A Restricted Maximum Likelihood (REML) variance components analysis (fixed model) (Payne 2002) was carried out to assess variability due to cultivars, soil types and cultivar x soil type interaction for stover nitrogen and in vitro digestibility. The Wald statistic, which follows an approximate Chi-square distribution, was used to test the overall significance of differences among treatments (Thompson and Welham 1993).

Results and Discussion

The REML analysis indicated significant cultivar differences only for stover in vitro digestibility (Table 2). Differences due to either soil type or cultivar x soil type interaction were not significant for stover nitrogen content and in vitro digestibility. These findings suggested that cultivars rather than soil types and cultivar x soil type interaction contribute towards the variation in stover nitrogen content and in vitro digestibility and strongly supported options for genetic enhancement for stover quality traits.

Table 1. Distribution of soil types of selected farmers' fields across villages.

		Village Name			
Soil type	Manmarry	Udityal	Ganagpur	Kandawada	Total
Black	3	*	2	*	5
Barka	5	*	*	7	12
Red	*	4	6	6	16
Chalka	*	10	5	*	15
Total	5	14	13	13	N=48

*Data not available.

Stover nitrogen content in improved cultivars was slightly higher than in the local check (Table 3). However, these differences are unlikely to be of nutritional significance. In ruminant nutrition, a feed nitrogen content of about 1.2% is considered to be the minimum requirement for rumen microbes to degrade feed effectively (Van Soest 1994) and the nitrogen content of stover from both improved and local cultivars investigated in the present study were well below this minimum level. In contrast, stover in vitro digestibility was on an average about five units higher in the improved compared to the local cultivar (Table 3). Five units difference in in vitro digestibility is considered to be of practical nutritional significance, as livestock productivity will be higher on a stover with a digestibility of 45% than of 40% (Van Soest 1994). While the stover yield of improved cultivars was better or on par with that of local cultivar, the grain yield of improved cultivars was two to three times that of the local cultivar. The average stover yield of the improved cultivars in Mahabubnagar and Ranga Reddy districts was 2297 kg ha^{-1} and 1560 kg ha^{-1} compared to 1900 kg ha^{-1} and 1260 kg ha^{-1} for local cultivars and the cost benefit ratio of improved cultivars was estimated at 2.02 and 1.44 compared to 1.35 and 0.98 for local cultivars, respectively. The soil type did not appear to have significant bearing on stover nitrogen content and in vitro digestibility. The improved cultivars were found better or comparable to local cultivars for stover nitrogen content and in vitro digestibility.

Conclusions

It appears from me present study that the genetic component rather than soil type and genotype x soil type interaction components is important in total variation of the cultivars for stover nitrogen content and stover in vitro digestibility. The nonsignificant mean squares due to soil type and genotype x soil type interaction would support genetic improvement of stover nitrogen content and stover in vitro digestibility for wide adaptation. Stover nitrogen dry matter (NDM) content was highest in CSH 16 followed by S 35, CSV 15, and PSV16. The stover digestibility of improved cultivars was better than that of the local sorghums. While the improved cultivars were on par with the local cultivars for stover nitrogen content irrespective of soil type, they were significantly superior to local cultivars for stover digestibility in *Barka* and red soils. The study provides sufficient evidence to dispel farmers' perceptions that improved cultivars have poor stover nutritive value and digestibility when compared to local cultivars. Complementing the stover quality, the quantity obtained by the farmers with improved cultivars was better or comparable with local cultivars.

Table 2. REML components variance analysis for sorghum stover nitrogen and in vitro digestibility of five sorghum genotypes, across four soil types.

		Wald Statistic/df.	
Source	Degrees of freedom	Nitrogen	In vitro digestibility
Genotype	4	1.77	5.82**
Soil type	3	1.03	2.12
Genotype x Soil type	9	1.11	0.59

** Significant at 0.01 level of probability.

Table 3. Estimated mean values of nitrogen, crude protein content and in vitro digestibility of stover of improved and local sorghum cultivars (values based on dry matter).

Cultivar	Nitrogen Dry Matter (NDM) (%)	Crude Protein (CP) content* (%)	*In vitro* digestibility (%)	Grain yield (t ha^{-1})	Fodder yield (t ha^{-1})
CSH 16	0.43	2.7	43.0	0.9	1.9
CSV 15	0.36	2.2	45.9	0.9	2.3
PSV 16	0.36	*22*	46.0	0.8	*2.2*
S 35	0.40	2.5	*46.3*	0.6	*2.5*
Local	0.39	2.4	40.5	0.3	2.5

* Crude protein content = 6.25 x NDM

However, a word of caution is necessary here taking into consideration the variable number of farmers representing each soil type and that the sampling of fanners was not based on soil type. A more systematic evaluation of the cultivars involving higher number of farmers sampled from different soil types spread across larger areas would validate the present results.

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Farmers' Perception of Feeding Value of Sorghum Stover Obtained in Different Seasons

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Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is an important food and fodder crop of dryland agriculture in India. Its stover forms important roughage for feeding cattle in rainfed agricultural systems. Kelley et al (1991) reported that on an average, stover contributes 40% to the total value of sorghum crops. It is grown as a dual-purpose crop and farmers try to maximise grain and fodder yields within the constraints imposed by the environment and their production systems.

Sorghum is the staple food crop of Dharwad region of Karnataka. It is grown in the rainy (July-October), late rainy (August-December) and postrainy seasons. The varieties grown and main purpose of growing in each season vary. Rainy season hybrids are grown for fodder. The cultivar M-35-1 is grown in the postrainy season primarily for grain, but its stover forms an important source of fodder for livestock from March through October. Many studies have been conducted to evaluate farmers' perceptions on the fodder value of stover of hybrid vs. local sorghum cultivars, but similar reports on stover of rainy vs postrainy sorghum are rare. This paper reports on farmers' perceptions of feeding value of sorghum stover obtained from different seasons, and the scientists' responses.

Materials and Methods

Data drawn from the major project on participatory planning of fodder and forage resources in milkshed and non-milkshed areas were utilized for this study. The study was carried out in the Dharwad region of Karnataka, which represents the northern transitional zone. It covered ten villages. Data was generated by focused participatory rural appraisal (PRA) exercises using pair wise ranking, matrix ranking, transect, resource map and small group discussions. Sorghum stover formed the major part of the discussion during PRAs as it is utilized primarily as livestock feed. All the attributes of sorghum stover mentioned by the farmers were recorded. Attributes that were closely related were combined and were listed separately for stover of rainy

Tabie 1. Farmers' observations and scientists' responses.

Rainy sorghum stover	Postrainy sorghum stover	Test value for scientists' responses
More biomass, leafier, more and softer leaf sheath, thin stem, less wastage, more juicy	More pith, more sugary, less fibrous	r_c = 0.41 X^2 = 12.11*
More fibrous, less tasty	More wastage, less leafier, less biomass, thick stem	

* - Significant at 5% level.

and postrainy sorghum. Based on the attributes listed, a questionnaire was structured to elicit scientist's responses on farmers' observations. This questionnaire was mailed to 15 sorghum specialists working at the Agricultural College in Raichur, Bijapur, and Dharwad. Their responses were sought based on a three-point continuum scale: right, partially right, or incorrect. Percentages, contingency coefficient, and chi square values were calculated for analysing scientists' responses to farmers' observations.

Results and Discussion

Farmers observations. Dry stover of rainy season sorghum is valued for its higher leaf sheath, leaf portion and juiciness as it is harvested during a cool period (i.e, in October, temperature between 23-26°C) and its thin stem. Because of these factors animals consume the stover completely, resulting in less waste of the fodder. The total biomass obtained from rainy season sorghums was greater compared to postrainy sorghums. Though hybrids, which tend to be dwarf in stature, are cultivated in the rainy season, the higher biomass of this crop may be associated with closer plant spacing (10-13 cm) and narrower row spacing (38 cm). Balasubramaniyan and Palaniyappan (1991) reported that straw yield of rice showed an increase of 800 kg with increasing plant density in the Kharif season.

Maldandi (M-35-1) is more popular in the postrainy season. It is mainly valued for more pith, less fibrous stems and leaves, and greater palatability. Due to its thick stem, smaller leaf proportion, and moisture level, farmers reported a greater percent of waste from this sorghum compared to stover of rainy sorghums. Low moisture content facilitates storage for longer periods of time (as much as 24 months), which is not the case with rainy season sorghum stover. Farmers utilize leftover stover either for fuel or for composting. Lower biomass of the postrainy crop is said to be due to the spacing between plants, which is perceived to be good for grain formation and filling. Laki and Wolf (1989) reported that maize stover yield increased as plant density increased from 40-60 thousand ha^{-1} to 60-80 thousand ha^{-1}. However, net energy, crude protein (CP) content and CP digestibility of the stover decreased with increasing density and the lowest density gave the best combination of stover quantity and nutritive value. This finding suggests that quality aspects of kharif sorghum stover need to be explored from a farmer's viewpoint.

There existed a difference of opinion among farmers regarding their preference for stover of rainy or postrainy sorghum. About half of the farmers preferred rainy sorghum stover due to its higher leaf sheath and leaf portion with the assumption that leaf provides more nutrients to the livestock. Another group of farmers reported that postrainy sorghum provides more energy to livestock due to more pith, so would result in improvement in livestock health. Also as it is grown during dry weather, there is higher accumulation of sugar, which adds to the taste and energy. Work reported by Kelley and Parthasarathy Rao (1994), Badve et al. (1994), and Walli et al. (1994) support the latter argument.

Scientist's responses. The calculated contingency coefficient (r_c) indicated that scientists agree with approximately 41% of farmers' observations (Table 1). Chi square test showed association between farmers' observations and scientist's responses at a 5% level of significance. Scientists expressed that though postrainy sorghum yields less biomass, stover quality is excellent. They agreed that it has less leaf portion and less moisture content. However, farmers were able to get clean and healthy fodder from postrainy stover.

Conclusion

Farmers related their observations on spacing and season of harvest to judge the yield and quality of the stover. This indicates that their observations are based not only on the cultivar per se but also on agronomic practices followed. While farmers placed emphasis on waste of stover from postrainy sorghum, scientists viewed getting

clean, hygienic and healthy fodder free from mud and fungal infections as important.

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Genetic Enhancement and Breeding

Combining Ability and Heterosis for Grain Yield and its Component Traits in Finger Millet under Irrigated Conditions

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Introduction

Finger millet [*Eleusine coracana* (L.) *Gaertn]* is a widely cultivated crop of the tropical and subtropical regions of the world. India is the largest producer of finger millet in the world. Africa, Madagascar, Sri Lanka, Malaysia, China and Japan, are the other finger millet growing countries. In India, it is cultivated on 2 million ha with a production of 2.8 million t and productivity of 1.5 t ha^{-1} (Senthil et al. 2005). Finger millet is a major source of grain and fodder and is an indispensable crop in semi-arid tropical region of several states in India. Its grains have high nutritive value. Combining ability has been extensively used for choosing suitable parents in crossing programs to combine traits of interest in high-yielding background. Combining ability analysis also provides estimates of genotypic variance components, useful for genetic enhancement of crops. Considering that hybrids offer good opportunity to improve yield levels, as evident from several other self-pollinated crops such as rice and sorghum, evaluation of hybrid potential within finger millet was undertaken. The present study assessed the combining ability, nature of gene action and the extent of heterosis for yield and its components within select finger millets.

Material and Methods

Five parents (CO 9, CO 10, CO 11, CO 12 and CO 13) selected on the basis of desirable agronomic and morphological characters, were crossed with three pollen parents (TNAU 946, GPU 28 and DPI 2011) in line x tester mating design during 2002 *kharif* season. Hybridization was done by hot water treatment (Raj et al. 1964). Based on morphological characters such as plant pigmentation, earhead shape and seed coat color of the parents and the vigor of the plants, F_1 hybrids were identified. The resulting 15 hybrids along with eight parents were evaluated in a Randomized Complete Block Design (RBD) with three replications and a spacing of 22 x 10 cm. The experiment was conducted at the Department of Millets, Tamil Nadu Agricultural University (TNAU), Coimbatore, during 2002-2003 *rabi* season. Data on days to 50% flowering, days to maturity, plant height, number of tillers $plant^{-1}$, number of fingers $earhead^{-1}$, average finger length (cm), 1000 seed weight (g) and grain yield $plant^{-1}$ (g) were recorded from five randomly selected plants in each entry. The mean values of the data in each replication were subjected to statistical analysis. The general combining ability *(gca)* and specific combining ability *(sca)* effects and their variances (σ^2gca and σ^2sca, respectively) were estimated as per Kempthorne (1957). The mean superiority of the crosses over check (standard heterosis) was estimated as per standard formulae.

Table 1. Analysis of variance for combining ability in finger millet.

Source of variation/ variance component	df	Days to 50% flowering	Plant height (cm)	No.of tillers $plant^{-1}$	No.of fingers $earhead^{-1}$	Finger length (cm)	1000 seed weight (g)	Grain yield $plant^{-1}$ (g)
Females	4	34.38**	81.67	1.23	1.43	1.49	0.07	25.16*
Males	2	0.100**	56.28	0.96	3.36	0.03	0.16*	8.57
Females x Males	8	3.81*	80.75**	1.60**	1.32**	1.67	0.44**	6.09*
Crosses	14	12.87**	77.52**	1.40**	1.65**	1.39**	0.07**	11.89**
Males *vs* females	1	7.35*	32.34*	0.02	4.54**	3.15**	0.06**	40.88**
Error	14	1.32	5.07	0.21	0.24	0.10	0.002	1.43
σ^2 *gca*		0.48	-0.17	-0.01	0.02	-0.02	0.001	0.31
σ^2 *sca*		1.24	37.84	0.698	0.54	0.78	0.02	2.33
σ^2 *gca*/σ^2 *sca*		0.39	-0.005	-0.015	0.031	-0.02	0.06	0.13

* - Significant at P = 0.05; ** - Significant at P = 0.01

Table 2. Estimates of mean values and general combining ability effects of parents for grain yield and its component traits in finger millet.

Parents	Days to 50% flowering		Plant height (cm)		No.of tillers plant^{-1}		No.of fingers earhead^{-1}		Finger length (cm)		1000 seed weight (g)		Grain yield plant^{-1} (g)	
Females	Mean	Gca effects	Mean	Gca effects	Mean	Gca effects	Mean	Gca effects	Mean	Gca effects	Mean	Gca effects	Mean	Gca effects
CO 9	68.50	-3.27**	87.05	-3.96**	4.95	-0.23	8.80	0.21	6.35	-0.19	2.15	0.15**	19.93	-0.59
CO 10	66.50	-0.60	85.85	3.72**	3.50	0.00	8.05	-0.10	5.95	0.38*	2.63	0.00	18.27	-1.69**
CO 11	77.00	0.90	92.75	3.67**	3.90	0.53*	7.30	-0.74**	6.55	-0.72**	2.44	-0.13**	16.86	0.42
CO 12	78.50	3.57*	101.3	-0.06	5.55	-0.62**	7.60	0.03	7.35	0.54**	2.33	0.05*	22.27	-1.48**
CO 13	72.50	-0.60	84.70	-3.38**	5.30	0.32	10.0	0.60**	6.80	-0.01	2.15	-0.07**	16.78	3.34**
Males														
TNAU 956	73.00	0.10	92.50	-2.73**	5.10	0.30	9.35	-0.66**	7.40	0.06	2.56	-0.09**	17.54	-0.94*
GPU 28	76.50	-0.10	94.80	1.17	4.25	-0.32*	8.95	0.26	7.70	-0.01	2.38	0.14**	23.32	0.03
DPI 2011	72.50	0.00	92.50	1.56*	4.80	0.01	10.05	0.41*	7.45	-0.04	2.45	-0.05**	25.52	0.91*
SE ±(lines)		0.469		0.92		0.19		0.20		0.13		0.02		0.49
SE ± (Testers)		0.363		0.71		0.14		0.15		0.10		0.02		0.38

* – Significant at P = 0.05; ** – Significant at P = 0.01

Results and Discussion

Components of variation. The significant mean squares due to females x male interaction for all the traits differed significantly for only a few traits (Table 1), which suggests that females x male interaction resulted in increased genetic differentiation in the crosses for all the traits, which is supported by significant mean squares due to crosses. It therefore appeared that, non-additive gene action is the major cause for significant variation among the crosses for all the traits, which is further supported by higher magnitude of variances due to *sea* effects than those due to *gca* effects for all the traits. These findings are in agreement with those reported by Vigneswaran (1996) and thus justifying exploitation of heterosis in finger millet.

General combining ability effects. The estimates of combining ability effects (Table 2) indicated that CO 13 was the most desirable female parent with significant positive *gca* effects for traits such as grain yield plant^{-1} and number of fingers earhead^{-1}. CO 9 was a good general combiner for days to 50% flowering and 1000-grain weight. Among males, DPI 2011 was a good general combiner for the traits such as plant height, number of fingers earhead^{-1} and grain yield plant^{-1}. The *gca* effects are a reflection of additive and/or additive x additive epistastic gene effects, which represent the fixable genetic components of variance. Thus, the parents CO 13 and DPI 2011 appeared to be worth exploiting in breeding programs.

Parental mean performance and gca effects. The poor correlation between mean performance of parents and their *gca* effects for all the traits (Table 2) indicated that *per se* performance of the parents may not be good indicator of their *gca* effects. Thus the selection of the parents for hybrid development should be largely based on *gca* effects although mean performance, especially of seed parents cannot be ignored from a hybrid seed production point of view. Ravikumar (1988) also reported poor association between *per se* performance of parents and their *gca* effects for days to 50% flowering, number of tillers plant^{-1} and number of fingers earhead^{-1} in finger millet.

Specific combining ability effects. Five of the 15 hybrids, CO 9 x GPU 28, CO 11 x TNAU 956, CO 13 x TNAU 956, CO 13 x GPU 28 and CO 13 xDPI 2011, had significant and positive *sea* effects and were found to be good specific combiners for grain yield (Table 3). While the hybrids, CO 11 x DPI 2011, CO 12 x TNAU 956 and CO 13 x GPU 28, had significant negative *sea* effects, they were good specific combiners for earliness. Two

Table 3. Estimates specific combining ability effects and standard heterosis of crosses for grain yield and yield component traits in finger millet.

Cross	Days to 50% flowering		No.of fingers earhead^{-1}		Finger length (cm)		1000-seed weight (g)		Grain yield plant^{-1} (g)	
	sca effects	Standard heterosis	sca effects	Standard heterosis	sca effects	Standard heterosis	sca effects	Standard heterosis	sca effects	Standard heterosis
CO9 x TNAU956	-1.93*	-5.52**	0.40	-13.50**	-0.84**	-6.62	-0.06	0.23	-0.79	5.84
CO 9 x GPU 28	0.27	-2.26	-0.32	-11.50*	0.63*	13.97**	0.01	14.65**	2.75**	32.77**
CO 9 x DPI2011	1.67	-0.69	-0.07	-7.50	0.21	7.35	0.05	7.44	-1.96*	9.92
CO10 x TNAU956	1.40	2.76	-0.04	-21.0**	0.64*	23.53**	-0.03	-5.12	-0.28	6.37
CO10 x GPU 28	0.10	0.69	-0.06	-12.00*	0.81**	25.00**	0.1	12.09**	-14.45*	5.99
CO 10 x DPI2011	-1.50	-1.38	0.09	-9.0	-1.46**	-8.82	-0.07	-4.88	-10.78*	16.38*
CO11 x TNAU956	0.40	3.45*	0.75*	-19.50	-0.26	-5.88	0.06	-6.74	21.41**	24.43**
CO11x GPU 28	0.60	3.45*	-0.97*	-27.50**	-0.69**	-13.24**	-0.16**	-6.05	-10.54	7.09
CO 11x DPI2011	1.00	1.38	0.23	-14.00**	0.94	10.29*	0.10**	-3.02	6.95	35.03**
CO12 x TNAU956	-0.77	5.52**	0.23	-17.00**	-0.17	13.97**	-0.09**	-5.58	-10.50	6.14
CO12 xGPU 28	-0.07	6.21**	0.16	-8.50	-0.15	13.24**	0.21**	19.30**	-18.2**	11.08
CO 12 x DPI2011	0.83	7.59**	-0.39	-12.50**	0.33	19.85**	-0.13**	-5.58	-19.0**	15.25
CO13 x TNAU956	0.90	2.07	-1.34**	-27.00**	0.63*	17.65**	0.12**	-1.16	24.72**	27.52**
CO13 x GPU 28	-0.90	-0.69	1.19**	7.50	-0.60*	-1.47	-0.16**	-3.49	19.19**	42.39**
CO 13 X DPI2011	0.00	0.69	0.14	-1.50	-0.02	6.62	0.04	-3.26	18.12**	48.85
SE ±	0.81		0.34		0.23	0.29	0.03	0.08	0.84	1.30
CD (5%)	2.47		1.04		0.68		0.09		2.57	

* – Significant at P=0.05; ** – Significant at P=0.01

hybrids CO 11 *x* TNAU 956 and CO 13 x GPU 28 had significant positive *sea* effects for number of fingers earhead^{-1}; CO 13 x TNAU 956 had significant positive *sea* effects for finger length and 1000-grain weight; and CO 9 x GPU 28 had significant positive *sea* effects for finger length and they were all identified as good specific combiners. These crosses need to be evaluated further to confirm their superiority and could be used for generation advancement to select and derive elite genotypes.

Heterosis. The magnitude of standard heterosis for grain yield plant^{-1} ranged from 5.84 to 48.85% over the check CO 13 (Table 3); of the 15 crosses, only seven hybrids, CO 9 x GPU 28, CO 10 x DPI 2011. CO 11 x TNAU 956, CO 11 x DPI 2011, CO 13 x TNAU 956, CO 13 x GPU 28 and CO 13 x DPI 2011, showed significant positive standard heterosis over the check CO 13. Eight hybrids for finger length and three hybrids for 1000-grain weight expressed significant positive standard heterosis. Manifestation of substantial heterosis for grain yield and other *traits* was also reported earlier by Tamil Covane (1995) and Vigneswaran (1996).

In general, most of the hybrids involving at least one parent with good *gca* showed higher *sea* effects and standard heterosis for grain yield suggesting that parental diversity for combining ability is necessary for greater manifestation of heterosis.

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Combining Ability Analysis of Dual-Purpose Pearl Millet Genotypes

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Introduction

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is a staple diet for the vast majority of poor farmers and also forms an important fodder crop for livestock population in arid and semi-arid regions of India. Increased emphasis on development of dual purpose (grain and fodder) pearl millet is, therefore, necessary to ensure high grain yield as well as high dry fodder yield under rainfed cultivation (Dangaria and Atara 2004).

Combining ability and performance *per se* serve as useful tools in identifying superior genotypes for any trait. However, information available on combining ability of dual purpose lines is limited. Hence, this study was undertaken to obtain genetic information on dual purpose pearl millet for various yield and yield contributing traits.

Materials and Methods

The study included a set of 11 diverse pearl millet genotypes (which were selected based on the diversity study for grain and stover yield and its attributing traits). These genotypes were IP 20381 (P_1), PT 5665 (P,). GP 15071 (P_3), PT 5600 (P_4), PT 5651 (P_5), IP 20334 (P_6), PT 5136 (P_7), IP 19125 (P_8), IP 20389 (P_9). GP 16239 (P_{10}) and IP 20350 (P_{11}). They were crossed in a diallel mating system (excluding reciprocals) during the 2003 *kharif* season (rainy season). The 55 F_1s and 11 parents were grown in a randomized block design with three replications in *rabi* 2003. The experiment was conducted at the Department of Forage Crops, Center for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore. The entries were planted in two rows of 3-m length with 45-cm row spacing and 15 cm between plants. Recommended cultural practices were followed before and after sowing. Data were recorded on five randomly selected plants in each treatment and replication for grain and stover yield and their associating characters. Combining ability analysis was carried out by following model 1 method 2 of Griffing (1956).

Results and Discussion

The mean squares of parents and hybrids were significant for all characters studied. Parents vs hybrids interaction mean squares indicated existence of heterotic effects for all the traits except panicle width. The variances due to general combining ability (GCA) and specific combining ability (SCA) were highly significant. The GCA variances were higher in magnitude than the SCA variances for all the characters (except for leaf breadth), indicating the preponderance of additive gene action. For leaf breadth, both the variances were equal, indicating the prevalence of both additive and non-additive gene action.

The estimates of *gca* effects of parents (Table 1) showed that the germplasm accession, IP 20381, was a better combiner for plant height, number of tillers, number of productive tillers, number of leaves, leaf length, stem diameter, grain yield per plant and stover yield per plant. IP 19125 was also a good combiner for all the above traits, in addition to panicle length and panicle width. Another germplasm accession, IP 20334, was a good combiner for leaf breadth and 1000-grain weight. Likewise, GP 15071 for days to 50 percent flowering. Since each of these parents possessed one or more of the desirable characters, an intermating population involving all possible crosses among them may be synthesized in order to harness genetic variability. Selecting desirable combinations of traits in such a population could result in a population of lines combining superior grain and fodder yield.

The *per se* performance of the parents provided a fairly good indication of their combining ability in most cases, except for panicle width. When individual cases were examined, IP 20381 had high *per se* performance for plant height, number of tillers, number of productive tillers, number of leaves, leaf length, leaf breadth, stem diameter and grain yield, and also had high *gca.* However, this was not always true. For example, IP 20381 was superior as a general combiner for stover yield, but GP 15071 was the high *per se* performer. Similar results have been reported by Khangura et al. (1980). Thus, *gca* as well as the *per se* performance may be taken into account while selecting the parents for a hybrid breeding program.

An examination of the *per se* performance and the *sca* effects (Table 2) of the hybrids indicated that the hybrids having high *sca* effects might have high *per se* performance. For example, the combination IP 20381 x PT 5665 (P_1 x P_2) had the highest *sca* and *per se* value for plant height, number of tillers, number of productive tillers and grain yield. Similarly the cross IP 20381 x GP 15071 (P_1 x P_3) also had the highest *sca* and *per se* value for number of leaves and panicle width. But this relation was not good for trait leaf breadth. The cross IP 20381 x GP 15071 which involved two good combiners also exhibited high *sea* effect for two traits. However, the

Table 1. General combining ability (GCA) effects of parental lines for both grain cum stover yield and its contributing characters.

Parents	Days to 50 percent flowering	Plant height (cm)	No. of tillers plant	No. of productive tillers	No. of leaves	Leaf length (cm)	Leaf breadth (cm)	Panicle length (cm)	Panicle width (cm)	Stem diameter (cm)	1000 grain weight (g)	Grain yield (g)	Stover yield (g)
IP 20381	1.91**	25.03**	1.86**	1.74**	8.41**	5.68**	0.05**	-1.62**	0.23**	0.11**	0.76**	3.02**	69.42**
PT 5665	-1.52**	-19.63**	1.15**	0.50**	-1.32**	-3.93**	-0.23**	1.10**	-0.06**	-0.13**	0.24**	-1.03**	-23.20**
GP 15071	-2.11**	1.68	0.35**	0.23**	-3.46**	1.43**	0.11**	-0.12**	-0.05**	0.01**	-0.63**	0.57**	-17.30**
PT 5600	-0.78**	-12.10**	-0.35**	-0.63**	-4.23**	-1.84**	0.01	-0.79**	-0.27**	-0.06**	-0.79**	-2.78**	-21.45**
PT 5651	-1.45**	-7.23**	-1.55**	-0.96**	-1.59**	-2.48**	-0.22**	-0.60**	-0.05**	-0.06**	0.17**	-1.64**	-34.14**
IP 20334	-1.01**	1.76	-0.73**	-0.58**	-1.49**	-1.23**	0.30**	0.17**	0.01	0.05**	0.90**	0.36**	-19.60**
PT 5136	2.25**	-4.93**	-0.27**	-0.22**	0.75**	-1.98**	-0.07**	0.41**	-0.09**	-0.02**	-1.17**	-0.37**	12.39**
IP 19125	-0.52**	20.12**	0.53**	1.00**	4.89**	4.36**	0.22**	2.26**	0.38**	0.06**	0.43**	2.50**	55.85**
IP 20389	-0.01	-18.89**	-1.05**	-1.27**	-2.37**	-3.49**	-0.21**	-1.49**	-0.15**	0.07**	-0.33**	-0.81**	-37.21**
GP 16239	1.35**	1.78	-0.15**	-0.21**	-1.46**	0.78**	0.10**	-1.26**	-0.12**	-0.06**	-0.18**	-1.30**	12.60**
IP 20350	1.71**	12.41**	0.22**	0.41**	1.86**	2.71**	-0.06**	1.96**	0.17**	0.05**	0.61**	1.48**	2.64**
SE	0.13	1.09	0.03	0.03	0.11	0.07	0.01	0.04	0.01	0.003	0.01	0.06	0.21
Correlation with performance *per se*	0.65*	0.89**	0.94**	0.8**	0.84**	0.69*	0.71*	0.51	0.89**	0.67**	0.89**	0.95**	0.64*

* – Significant at 5% level; ** – Significant at 1% level.

Table 2. Three best dual purpose hybrids selected based on mean performance and *sca* effects.

Characters	Mean performance	*sca* effects
Days to 50 percent flowering	P_2 x P_5, P_3 x P_{11}, P_4 x P_7	P_1 x P_{10}, P_4 x P_7, P_3 x P_{11}
Plant height (cm)	P_1 x P_2, P_1 x P_8, P_1 x P_3	P_1 x P_2, P_5 x P_{10}, P_4 x P_7
No. of tillers plant	P_1 xP_2, P_1 X P_3, P x P $_{11}$	P_1 x P_2, P_1 x P_3, P_6 x P_{11}
No. of productive tillers	P_1 x P_2, P_1 x P_3, P_1 x P_{11}	P_1 x P_2, P_6 x P_{11}, P_1 x P_3
No. of leaves	P_1 x P_3, P_1 x P_2, P_1 x P_{11}	P_1 x P_3, P_1 x P_2, P_8 x P_{10}
Leaf length (cm)	P_8 x P_{10}, P_1 x P_3, P_1 x P_8	P_5 x P_{10},P_8 x P_{10}, P_4 x P_6
Leaf breadth (cm)	P_6 x P_{11}, P_1 x P_3, P_1 x P_2	P_1 x P_2, P_s x P_{10}, P_6 x P_9
Panicle length (cm)	P_7 x P_9, P_2 x P_8, P_5 x P_8	P_7 x P_9, P_7 x P_{10}, P_2 x P_8
Panicle width (cm)	P_1 x P_3 P_1 x P_2, P_8 x P_{11}	P_1 x P_3, P_1 x P_2, P_5 x P_{10}
Stem diameter (cm)	P_5 x P_{10}, P_1 x P_{11} P_6 x P_9	P_5 x P_{10}, P_5 x P_9, P_5 x P_7
1000 grain weight (g)	P_1 x P_{11}, P_6 x P_{11}, P_6 x P_{10}	P_4 X P_7, P_1 X P_{11}, P x P_{11}
Grain yield (g)	P_1 xP_2, P_1 x P_3, P_1 x P_{11}	P_1 x P_2, P_1 x P_3, P_6 x P_{11}
Stover yield (g)	P_5 x p_{10}, P_1 x P_3, P_1 x P_8	P_5 x P_{10}, P_2 x P_6, P_5 x P_7

highest *sca* effect was recorded using crosses in which one parent had good combining ability. The hybrid IP 20381 x GP 15071 (P_1 x P_3) had high *per se* performance for grain cum stover yield along with seven other traits. So, this hybrid could be utilized for selecting a superior dual purpose line.

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Expression and Segregation of Stay-Green in Pearl Millet

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Introduction

Drought stress can occur any time during the crop cycle, but drought stress during flowering through grain fill results in low and unstable yield of pearl millet [*Pennisetum glaucum* (L.) R. Br.] (Yadav et al. 2002). Delayed senescence, or stay-green (Borrell et al. 2003; Mahalakshmi and Bidinger 2002; Thomas and Howarth 2000), is a mechanism of drought tolerance characterized by the retention of green leaf area at crop maturity under water-stressed environments (Borrell et al. 2000). Scientific literature concerning stay-green in pearl millet is minimal, so the sorghum model may provide useful information. In sorghum, stay-green is genetically and physiologically complex, expressing a variety of patterns and environmental sensitivities depending on the background genotype (Thomas and Howarth 2000). Stay-green hybrids partition more carbon and nitrogen to leaves during early growth compared to their senescent types resulting in greater specific leaf nitrogen (Borrell et al. 2003). Higher specific leaf nitrogen after anthesis delays the onset and reduces the rate of leaf senescence. Delayed senescence

effectively increases cereal production under water-limited conditions (Yadav et al. 2002). Under post-anthesis water stress-grain, yield is positively correlated with green leaf area at maturity and negatively correlated with rate of leaf senescence (Borrell et al. 2000). Grain yield in cereals is basically a reflection of starch accumulation, which relies on current photosynthate and a non-senescent canopy (Thomas et al. 2000). Under post-anthesis water stress, stay-green genotypes remain photosynthetically active and continue to fill grain as opposed to the senescent types (Thomas and Howarth 2000), leading to increased grain yield and lodging resistance (Borrell et al. 2003) and disease resistance (Hash et al. 2003). Stay-green sorghums contain more cytokinins and basal stem sugars than do senescent genotypes (Borrell et al. 2000). Increased accumulation of soluble sugar in stay-green types is associated with greater functional leaf area during grain fill, reducing the dependence on stem reserves in grain fill. Higher concentrations of stem sugars improve the digestible energy content of the straw or stalk (Borrell et al. 2000), making stay-green valuable for grain and fodder production (Hash et al. 2003). Since stay-green genotypes remain photosynthetically active during grain fill, their leaves tend to maintain more nitrogen than the senescent types, which may also improve stover quality.

The stay-green trait should have multiple benefits in pearl millet improvement. The objectives of these experiments were to quantitatively compare the chlorophyll content of a putative stay-green and normal senescent pearl millet over time and obtain preliminary information on the inheritance of the stay-green through segregation in an F_2 population.

Materials and Methods

Pearl millet lines developed by the United States Department of Agriculture-Agricultural Research Station (USDA-ARS) were evaluated at Tifton, GA, USA in 2004. 02F266-4 is a putative stay-green inbred. It is resistant or tolerant to prevalent diseases, insect pests and drought in the southeastern U.S., but lacks some desirable agronomic traits so it is not used for hybrid production. 02F266-4 was crossed with a normal senescent, agronomically elite line Tift 454, which is an A, restorer for hybrid production. F_1 and F_2 progenies were produced from crosses between the two parents.

Measurement of chlorophyll content in a putative stay-green. 02F266-4, Tift 454 and their F_1 were planted in June 2004 in a randomized complete block design with four replications. Each genotype was planted in a single 5m long row. Row spacing alternated between 1m and 2m due to planter configuration.

Four plants were marked in each plot as the panicle was emerging from the boot. At stigma emergence, relative chlorophyll content was measured on the top three leaves (with flag leaf taken as leaf 1) of the main tiller with a SPAD 502 Chlorophyll Meter (Minolta, Japan). At each evaluation, three readings were taken on each leaf and the average values for individual leaves were recorded. Data were collected at 7 d intervals for a total of 5 ratings. Data were analyzed by analysis of variance within weeks. Sums of squares were partitioned into effects of replication, genotype, plant within genotype and leaf position.

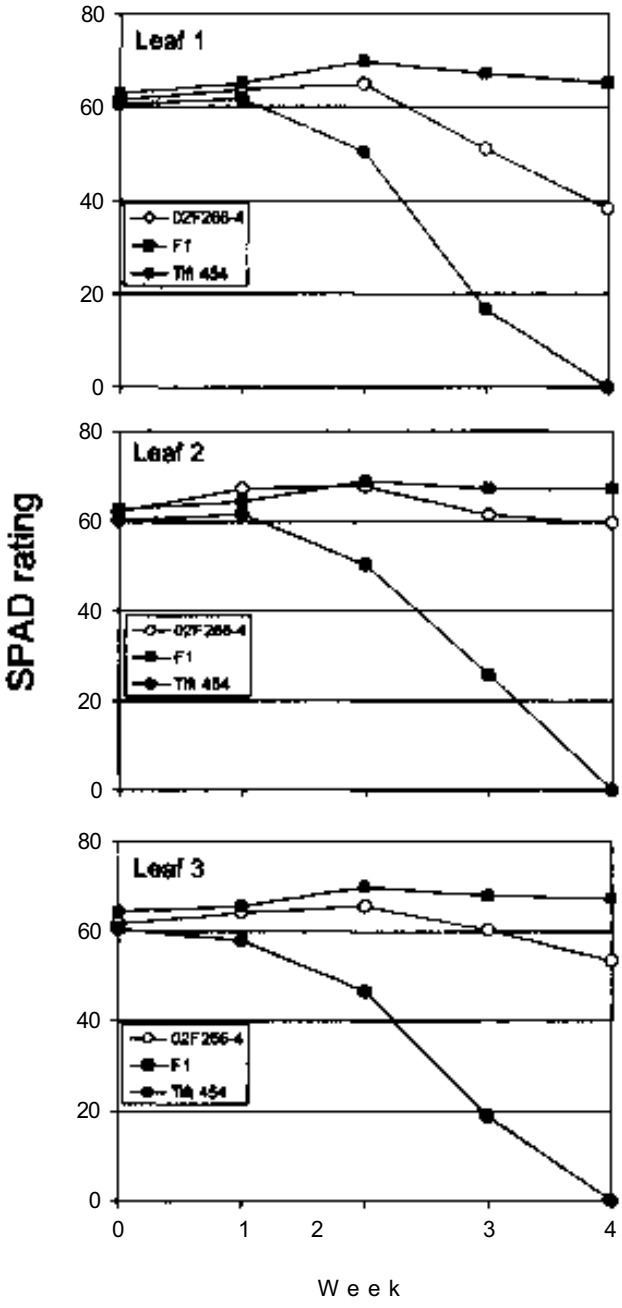

Figure 1. Changes in chlorophyll content, as measured by SPAD ratings, in pearl millet genotypes Tift 454, 02F266-4, and their F1. First reading was taken at stigma emergence. Leaf 1 = flag leaf, Tift 454 = senescent type, 02F266-4 = stay-green type.

Segregation of the stay-green trait. A non-replicated trial consisting of 02F266-4, Tift 454, and their F_1 and F_2 progenies was planted in the field at Tifton in July 2004. Within a 50m long plot, 6 rows of F_2s were flanked by two rows of Tift 454 and (because of limited seed availability) one row each of the F_1 and 02F266-4. Plots were surrounded by two border rows of Tifgrain 102.

Seedlings were thinned to leave at least 1m spacing between plants. During early vegetative growth, 236 F_2s, 19 F_1s, 29 plants of Tift 454 and 10 plants of 02F266-4 were marked at random and monitored for panicle emergence. When half emerged from the boot the main tiller panicle was bagged for self-pollination. Using the SPAD 502 Chlorophyll Meter, relative chlorophyll measurements were taken on the second uppermost leaf (leaf 2) of the main tiller. Data collection proceeded as described above.

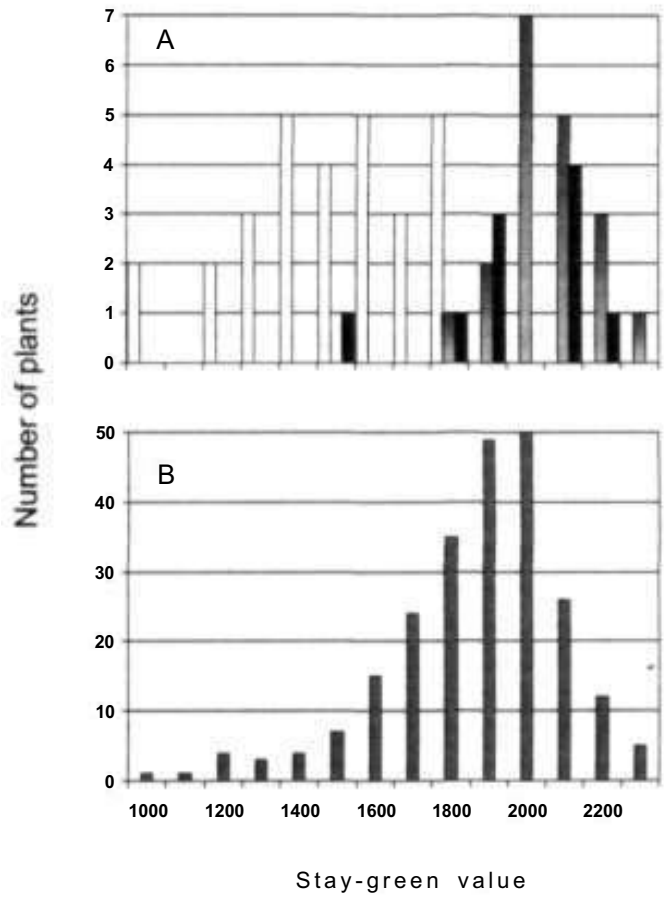

Figure 2. Distribution of the stay-green value in Tift 454 (white bars), 02F266-4 (black bars), and their F_1 (grey bars. A) and F, (grey bars, B) progeny. Stay-green axis labels are the lower boundary for the category class.

A stay-green value which reflected the magnitude and duration of the relative chlorophyll content was calculated for each plant. This stay-green value was calculated as

$$\pounds\ [{}^{1}/_{2}\ (SPAD_{n} + SPAD_{n+7})] * 7$$

where $SPAD_n$ = SPAD rating on day *n*. Differences in mean stay-green value between populations were evaluated by pairwise t-tests.

Results and Discussion

Chlorophyll content in a putative stay-green. Across genotypes, SPAD ratings did not differ *(P* <0.05) by leaf position until week 2. In week 3, SPAD ratings were greater *(P* <0.05) in leaf 2 than in leaf 1. In the same week, there were no differences in SPAD rating between leaf 2 and 3, and between leaf 1 and 3. In week 4, SPAD ratings were greater (*P* <0.05) in leaf 2 and 3 than in leaf 1.

Minor differences in SPAD ratings among genotypes were observed at stigma emergence, but over time, the top three leaves of 02F266-4 and the F_1 maintained greater levels of chlorophyll than Tift 454 (Fig. 1). SPAD ratings of 02F266-4 were similar to that of the F_1, but greater *(P* < 0.05) than that of Tift 454 in weeks 1 and 2. In weeks 3 and 4, SPAD rating of the F_1 was greater *(P* < 0.05) than that of 02F266-4, and ratings of both genotypes were greater *(P* < 0.05) than that of Tift 454 (Fig. 1). The data indicate a level of dominance or over-dominance in the expression of relative chlorophyll content in the F_1.

Segregation of the stay-green trait. Stay-green mean (± standard error) of Tift 454 (1548 + 237) was less than (P<0.001) the means of 02F266-4 (2001 ± 196), the F_1 (2104 ± 113), and the F_2 (1917 ± 227). Stay-green mean of 02F266-4 did not differ (P>0.05) from that of the F_1 or F_2, but the F_1 and F_2 means differed (P<0.001). Although not statistically different, the numerically greater stay-green value for the F_1 suggested overdominance, with degree of dominance = 1.46. Stay-green in the F_2 population was not normally distributed and skewed toward normal senescent types, which may reflect a segregation of homozygous recessive plants with lower stay-green values (Fig. 2). Several F_2 plants had stay-green values exceeding values of 02F266-4 plants but this may be due to the larger F_2 population rather than transgressive segregation.

Use of the SPAD meter to measure relative chlorophyll content provided a quantitative assessment of the stay-green trait. The data confirmed previous observations that 02F266-4 expressed stay-green characteristics. Any of the leaves evaluated were suitable for measurements,

but expression was greatest in leaf 2. Whereas SPAD ratings indicated the magnitude of the relative chlorophyll content at a point in time, a stay-green value could be calculated as a measure of the magnitude and retention of chlorophyll content over time for assessing the distribution of the trait within populations.

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Identification of Drought-Tolerant Inbred Lines of Pearl Millet

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Introduction

The occurrence of drought stress in pearl millet (*Pennisetum glaucum*) is a common feature as it is predominantly grown under rainfed conditions. Terminal drought stress is the most detrimental factor to the growth and development of grain and ultimately results in considerable loss in grain yield (Joshi et al. 1999). The solution to this, to some extent, lies in the development of drought resistant/tolerant hybrids. Selection of parental material based on suitable selection criteria for the moisture-stressed environment was the objective of this investigation.

Materials and Methods

Nine pearl millet lines, four male-sterile (MS) lines (B lines) and five inbreds, were grown at the Millet Research Station, Junagadh Agricultural University, Jamnagar, Gujarat, India, in the summer seasons of 1999 and 2001 in a randomized block design with three replications and plot sizes of 3 m^2. The irrigation was stopped after boot leaf stage until maturity to impose terminal moisture stress. A number of observations were recorded - days to 50% flowering, grain yield, harvest index (HI), leaf relative water content (RWC), threshing percentage and drought susceptibility index (DSI). The data were subjected to analysis of variance, pooled over years and are presented in Tables 1 and 2. For calculating F values for entries, YxE interaction ms was used but for a combination of non-significant interaction and heterogeneous error variance (as evident from F-test), a pooled ms derived from pooled error ss and interaction ss was used as in the case of HI (19.9) and RWC (30.7). DSI was calculated on the basis of aggregate values and standard deviations. The HI (Donald and Hamblin 1976), RWC (Kramer 1969) and DSI (Osmanzai 1994) were calculated as follows:

$$HI\ (\%) = \frac{\text{Grain yield}}{\text{Biological yield}} \times 100$$

$$RWC\ (\%) = \frac{\text{Leaf fresh weight} - \text{Leaf dry weight}}{\text{Leaf turgid weight} - \text{Leaf dry weight}} \times 100$$

$$DSI = S = \left[\ 1 - \frac{Ys}{Yp}\ \right] / D$$

$$\text{Where } D = 1 - \left[\ \frac{\bar{Y}s}{\bar{Y}p}\ \right]$$

Where,

Ys = Mean yield under stress

Yp = Mean yield under full irrigation

Ys = Yield of individual entry under stress

Yp = Yield of individual entry under full irrigation

Results and Discussion

The year x line (Y x E) interactions were not significant for the grain yield, HI and RWC. Pearl millet lines differed significantly for these characters (Tables 1 and 2). The MS line 95444B and the inbred J 2340 had the highest values for grain yield, HI and RWC. The DSI was lowest in 95444B and in J 2340. Although Y x E interactions were significant for days to 50% flowering and threshing (%), 95444B and J 2340 were the earliest to flower and had the highest threshing percentage. Thus, both the lines (95444B and J 2340) were found to be drought resistant/tolerant and all these characters studied were found to be associated with the drought resistance/ tolerance in pearl millet. Significant positive correlations of yield under terminal drought stress with H1 (r=0.86) and significant negative correlations with days to flowering (r=-0.70) and DSI (r=-0.91) were observed. Further, J 108 also appeared to be a drought tolerant inbred. The authenticity of the selection parameters was further proved when MH 1049 (GHB 538), a drought resistant hybrid developed by crossing drought tolerant parents identified in the present investigation, 95444A and inbred J 2340 were released in 2004 for the dryland rainfall zone A1 of India. This hybrid ranked first during all three years of testing in AICPMIP trials and recorded 16.3% and 35.7% higher grain yields than the check hybrids ICMH 356 and HHB 67, respectively. MS line 95444A was developed by ICRISAT and restorer line J 2340 was developed at the Main Millet Research Station, Jamnagar. Partitioning of dry matter to the grains and escaping the drought due to earliness partially explained some of the mechanism of drought tolerance in pearl millet within this experiment.

Table 1. Grain yield and physiological parameters as influenced by terminal moisture stress in B-lines and inbreds pooled over 2 years (1999 and 2001).

Pearl millet lines	Days to flower	Grain yield (kg/ha)	Harvest index	Threshing (%)	Relative water content (%)	Drought susceptibility index
81 B	70.8	136.1	4.7	22.0	74.9	1.196 ±0.06
218B	65.7	234.4	8.7	28.4	63.4	1.259 ±0.12
89111B	59.3	263.3	11.2	26.0	77.2	1.104 ±0.12
95444B	58.7	628.9	22.4"	47.7	74.2	0.812 + 0.06
J-108	57.8	648.9	18.2	40.5	72.0	0.846 ±0.10
J-998	62.8	353.3	9.3	29.7	69.4	1.057 ±0.04
J-2290	68.0	375.0	8.2	31.5	75.7	1.099 ±0.09
J-2296	59.2	376.7	15.4	37.7	75.0	0.880 ± 0.08
J-2340	58.8	806.7	18.1	44.4	77.8	0.761 ± 0.18
LSD (*P* = 0.05)	4.7	304.2	5.2	13.5	6.5	-
CV%	3.9	39.7	36.4	19.0	7.1	-

Table 2. ANOVA for grain yield and physiological parameters mentioned in Table 1.

source	df	Days to flower		Grain yield (kg/ha)		Harvest index (%)		Threshing (%)		Relative water content (%)	
		ms	F value	ms	F value	ms	F value	ms	F value	ms	F value
Rep/year	4	2.8	0.4^{NS}	51894	1.8^{NS}	25.8	1.2^{NS}	15.4	0.4^{NS}	101.0	3.7*
Year	1	647.6	105.3**	2140	0.07^{NS}	127.6	5.8*	4.8	0.1^{NS}	88.6	3.3^{NS}
Entry	8	135.5	9.8**	294932	5.2*	206.9	10.4**	460.9	4.1*	120.6	3.9**
Y × E	8	13.8	2.2*	57045	2.0^{NS}	10.8	0.5^{NS}	113.6	2.7*	45.0	1.7^{NS}
Pooled error	32	6.2		28452		22.3		42.6		27.1	

1. NS – Non Significant; * – Significant at 5% level; ** – Significant at 1% level.

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Evaluation of Pearl Millet Hybrids for their Tolerance to High Temperature and Limiting Soil Moisture at the Seedling Stage

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Introduction

High or low temperature deviations from the optimum can adversely affect the growth and development of plants and they respond to such changes through several mechanisms (Stone 2001, Maestri et al. 2002). The situation gets more aggravated by limiting soil moisture, where heat and drought stresses frequently occur simultaneously. In pearl millet *(Pennisetum glaucum),* grown during the rainy season, high temperature in certain regions adversely affects seedling establishment and plant population (Rai and Anand Kumar 1994), while in summer low temperature during germination (Joshi et al. 1997) and high temperature during flowering and grain development adversely affect the respective processes (Norman et al. 1995). Although, molecular bases of thermotolerance, including the roles of phytohormones, antioxidants, membrane lipids and heat shock proteins, apart from the thermotolerance of translation and thermostability of key enzymes, have been discussed (Maestri et al. 2002), the significance of the morphophysiological consequences in identifying the thermotolerance are very vital for a good crop establishment. In view of the above, this investigation was done to understand the effects of high temperature

Table 1. Physiological parameters in pearl millet hybrids as influenced by high temperature and receding soil moisture at seedling stage (pooled over 2 years, 2003 and 2004).

Entries	Root dry mass (mg/plant)	Shoot dry mass (mg/plant)	Total dry mass (mg/plant)	Root/ shoot ratio (dry wt. basis)	Survival (%) at 19-22 DALI*	Leaf elongation rate (cm/day) index at 15 DALI*	Chlorophyll stability
GHB-558	26.3	30.9	57.1	0.9	44.9	0.35	0.111± 0.016
GHB-559	29.5	26.4	55.7	1.2	68.4	0.48	0.079± 0.007
GHB-316	26.9	25.9	52.8	1.1	61.3	0.47	0.096± 0.019
GHB-526	**38.7**	**37.4**	**76.1**	**1.1**	**76.4**	**0.59**	**0.053± 0.016**
GHB-538	32.8	34.9	67.8	1.0	72.9	0.55	0.058± 0.014
LSD (*P*=0.05)	NS	5.3	NS	NS	16.4	0.09	-
CV (%)	10.5	15.1	10.8	17.9	9.1	17.3	-

*DALI= Days after last irrigation, NS=Not significant

Table 2. ANOVA for physiological parameters influencing different seedling growth traits.

Source		Root dry mass (mg/ plant)		Shoot dry mass (mg/ plant)		Total dry mass (mg/ plant)		Root/shoot ratio (dry wt. basis)		Survival (%) at 19-22 DALI		Leaf elongation rate (cm/day)	
	df	ms	F value	ms	F value	ms	F value	ms	F value	ms	F value	ms	F value
Year	1	756.0	72.0**	355.8	16.2**	74.0	1.7^{NS}	2.81	81.3**	910.6	26.2**	0.722	101.3**
Entry	4	205.5	3.0^{NS}	208.9	7.7**	759.9	5.2^{NS}	0.11	0.7^{NS}	1242.3	8.9*	0.064	7.0**
Y × E	4	69.5	6.6**	26.3	1.2^{NS}	147.4	3.3*	0.15	4.4**	140.4	4.0*	0.011	1.5^{NS}
Pooled error	30	10.5		21.9		44.7		0.04		34.8		0.007	

NS=Not significant, *=Significant at 5% level, **=Significant at 1% level

and receding soil moisture on seedling growth characters of some commercial pearl millet hybrids.

Materials and Methods

Two pot experiments were conducted in the late summer (April-May) of 2003 and 2004, when the temperature was high (38-40°C) at the Millet Research Station, Junagadh Agricultural University, Jamnagar, Gujarat, India. Five pearl millet hybrids - GHB 558, GHB 559, GHB 316, GHB 538 and GHB 526 - were grown in a completely randomized design (CRD) with four replications. Initially, 25 seeds were sown in each pot, which were later thinned to 10, and watering was stopped after emergence. The observations were recorded for shoot dry mass, root dry mass, seedling dry mass, root/shoot ratio, rate of elongation of completely emerged leaf and seedling survival percentage 19-22 days after the last irrigation. The soil moisture by this time receded to around 14%. For estimating the soil moisture, the pot soil was mixed thoroughly and 100 g of the sample was weighed and kept in an oven at 80°C for 48 h. The dried sample was weighed and soil moisture was calculated as percent of dry weight. The data, pooled over years, were subjected to analysis of variance to determine significant effects of the factors influencing seedling growth traits of pearl millet hybrids. The chlorophyll stability index (CSI) was estimated as per Koleyoreas (1958), only for the aggregate leaf samples and hence the same is presented with standard deviation.

Results and Discussion

The hybrid GHB 526, specifically released for summer cultivation in Gujarat, was superior to all the other hybrids with regards to most of the parameters studied (Table 1). GHB 526 also recorded the minimum value of CSI, where lower index values indicate better performance against temperature-stress. Root/shoot ratio, however, was highest in GHB 559 followed by GHB 526 (Table 1). Year x entry interactions were not significant for shoot dry mass and leaf elongation rate (Table 2). CSI showed good consistency across years. Hence, these three parameters appeared to be the most consistent and reliable for indicating thermotolerance at seedling stage.

Genetic variability for thermotolerance was noted in numerous studies including pearl millet and cellular membrane thermostability (CMS) correlated well with seedling thermotolerance (Howarth et al 1997). Ashraf and Hafeez (2004) also related early growth and nutrients with thermotolerance in pearl millet. Further, three commonly used assays of heat tolerance in plants are related to membrane based processes (Maestri et al 2002): plasmalemma assay (CMS assay); mitochondrial membranes (cell viability test of TTC reduction); and, photosynthetic membranes (chlorophyll fluorescence assay). The CSI in the present investigation exhibited excellent promise as a screen for temperature stress tolerance. In a study of more than 1200 Mexican wheat landraces collected from areas with diverse thermal regimes, a highly significant correlation between leaf chlorophyll content and 1000-seed weight was observed (Hede et al 1999).

Thus, the hybrid GHB 526 released for summer cultivation performed best among the hybrids evaluated for thermotolerance and soil moisture stress. The results indicated that seedling growth parameters such as shoot dry mass and leaf elongation rate, and the CSI can be used for screening against high temperature and limited soil moisture stresses.

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Pathology

Resistance in Pearl Millet Male-Sterile and Restorer Lines to Diverse Pathogen Populations of *Sclerospora graminicola*

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Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is an important food and fodder crop of India. The crop, however, is susceptible to many plant pathogens causing various diseases. The common diseases caused by fungal pathogens are downy mildew *(Sclerospora graminicola),* smut *{Tolyposporium penicillariae),* ergot *(Cleviceps fusiformis)* and rust *(Puccinia penniseti).* Downy mildew caused by *S. graminicola* is wide spread and is one of the most destructive in the production of pearl millet. The pathogen adapts its virulence and continues to threaten popular F1 hybrids when these are grown in the same field over many years (Singh 1995; Thakur et al. 1998). Development of new hybrids is a continuous process in India and they are developed both by public and private institutions. Several male sterile (A) lines, maintainer (B) lines and restorer (R) lines are being developed to breed downy mildew resistant high yielding new hybrids. The parental lines of new hybrids should be tested for stability of resistance to diverse pathogen populations at several locations before their use in hybrid development. We tried to identify potential male-sterile and restorer lines that have shown resistance to 5. *graminicola* populations at different locations.

Materials and Methods

A total of 25 test lines comprising 17 A-lines, 4 B-lines and 4 R-lines developed by different public and private sectors were screened against downy mildew pathogen across five locations. A universal downy mildew susceptible inbred line 7042S was included as susceptible check and planted after every fifth test row. Test lines were distributed through AICPMIP and grown in downy mildew sick plot soils at 5 locations; Mandor and Jaipur in Rajasthan, Hisar in Haryana, Gwalior in Madhya Pradesh and Jamnagar in Gujarat state. The lines were evaluated in the rainy seasons of 1999-2003. The disease nurseries were maintained through use of diseased leaf tissues containing oospores from the previous season crop (Singh et al. 1997) and planted with infector rows (Williams et al. 1981). Each test line was grown in 2 rows of 4-5 m length in two replications in a randomized block design. Recommended agronomic practices were followed. Downy mildew incidence was recorded at 30 and 60 days after sowing (DAS), by counting the total and diseased plants in each plot. Due to increase in the number of infected plants in some lines at 60 days compared to 30 days, the 60 days-data were used for analysis.

Results and Discussion

Of the 25 test lines, 19 of 23 lines at Jaipur, 19 at Hisar, 22 at Gwalior and 19 at Jamnagar were resistant (< 10% incidence) to downy mildew. At Mandor 15 of 17 lines were resistant (Table 1). Nine lines (NMS 9A, NMS 11A, NMS 14A, NMS 20A, NM 1024A, 95222A, JMS 112A, JMS 113A and J 2405R) had <10% downy mildew incidence across the locations. Differential disease reactions across locations were observed in A and R lines. For example, MS 38A was susceptible at Jaipur (14% incidence) and Jamnagar (31% incidence), but remained resistant at Hisar and Gwalior. ICMA 98004 was susceptible (22%) at Jamnagar, while it was free from the disease at Hisar and Gwalior. Out of 17 male sterile lines, four lines (MS 42A, NMS 9A, NMS 11A and PM 462A) at four locations, four lines (ICMA 98004, JKMS 2A, JKMS 40A and GK 1015A) at three locations, two lines (MS 38A and AR 1A) at two locations and JKMS 3A at one location were found resistant while the remaining six male sterile lines (95222A, JMS 112A, JMS 113A, NMS 14A, NM 1024A and NMS 20A) were resistant across all the five test locations (Table 1).

Among four restorer lines grown at Junagadh Agricultural University (JAU), Jamnagar, J 2290 was susceptible at Hisar (14% incidence) and remained resistant (< 4.0% incidence) at the other four locations (Table 1). Two R lines (J 2405 and J 2340) were found resistant across all the five locations. Of the four maintainer lines ICMB 93222 and JMS 101B remained susceptible at Mandor and Hisar, respectively, and exhibited resistant reaction at the other four locations. Similarly, ICMB 98004 was scored as resistant at three locations except Jamnagar.

Based on mean downy mildew incidence data from five locations, the 25 test lines were classified into three distinct groups; Group I = highly resistant (< 5% incidence), Group II = resistant (6-10% incidence) and Group III = susceptible (> 10% incidence). Of the 25 test lines, 15 lines including eleven A-lines, one B-line and three R-lines were highly resistant (Table 2). The remaining six

Table 1. Downy mildew reaction of pearl millet A, B and R lines against five populations of *Sclerospora graminicola* during 1999 to 2003.

Line	Source	Downy mildew incidence (%) at 60 DAS				
		Mandor	Jaipur	Hisar	Jamnagar	Gwalior
ICMA 98004	ICRISAT, Patancheru	-	6	0	22	0
95222A	ICRISAT, Patancheru	10	2	0	3	1
JMS 112A	JAU, Jamnagar	0	6	9	5	4
JMS 113A	JAU, Jamnagar	2	3	0	0	2
MS 38A	Mahendra, Jalna	-	14	0	31	8
MS 42A	Mahendra, Jalna	-	8	2[a]	5	6
JKMS 2A	JK Agri, Secunderabad	-	4	0	0	11
JKMS 3A	JK Agri, Secunderabad	-	15	13	26	8
NMS 9A	New Nandi, Ahmedabad	-	5	0	2	6
NMS 11A	New Nandi, Ahmedabad	-	5	0	2	8
NMS 14A	New Nandi, Ahmedabad		0	0	1	2
JKMS 40A	JK Agri, Secunderabad	13	11	5	0	3
NM 1024A	Mahendra, Jalna	2	0	2	1	1
GK 1015A	Ganga Kaveri, Secunderabad	6	-	11	2	10
AR 1A	Amreshwra Seeds	4	-	0	12	39
PM 462A	Ganga Kaveri, Secunderabad	6	0	13	0	6
NMS 20A	New Nandi, Ahmedabad	4	7	0	3	9
ICMB 98004	ICRISAT, Patancheru	-	6	8	14	2
ICMB 95444	ICRISAT, Patancheru	9[a]	14[a]	13[a]	1[a]	17
ICMB 93222	ICRISAT, Patancheru	21	3	10	3	4
JMS 101B	JAU, Jamnagar	4	2	18	0	0
J 2290R	JAU, Jamnagar	0	0	14	0	4
J 2340R	JAU, Jamnagar	3[a]	0[a]	10'	3[a]	6[a]
J 2405R	JAU, Jamnagar	10	7	0	0	5
J 2440R	JAU, Jamnagar	0	7	8	13	4
7042 S Check		91	82	83	93	97
Mean		6	5	5	6	7
SE (m)±		1.2	0.9	1.1	1.7	1.5

DAS - Days after sowing.
JAU - Junagadh Agricultural University.
[a]Mean of two years' data.

and four lines were resistant and susceptible, respectively. J 2290R, J 2340R and JMS 101B, classified into the highly resistant group, and had 14, 10 and 18% incidence in field nursery at Hisar shile JKMS 40A had 13 and 11% incidence at Mandor and Jaipur, respectively.

Downy mildew of pearl millet is still a threat and host plant resistance is the only feasible way to combat the disease. The break down of resistance on farmer's field is a common phenomenon in F1 hybrid cultivars due to their narrow genetic base (Thakur et al. 2003). The nine lines (95222A, JMS 112A, JMS 113A, NMS 9A, NMS 11A, NMS 14A, NMS 1024A, NMS 20A & J 2405R) that were found resistant at 4-5 locations under field conditions provides an opportunity for their direct use in breeding hybrids with more stable resistance to different pathotypes of 5. *graminicola.*

Acknowledgment. We thank the AICPMIP Project Centre pathologists for evaluating the lines at their centres.

Table 2. Classification of 25 pearl millet lines based on average of downy mildew incidence (%) caused by *Sclerospora graminicola* at five locations.

Pearl millet line	Mean disease incidence (%)	Phenotypic group
NMS 14A	1 ± 0.6	Highly resistant
NM 1024 A	1 ± 0.3	Highly resistant
JMS 113A	1 ± 0.5	Highly resistant
95222A	3 ± 1.5	Highly resistant
NMS 9A	3 ± 1.1	Highly resistant
NMS 11A	4 ± 1.3	Highly resistant
J 2290 R	4 ± 2.4	Highly resistant
J 2340 R	4 ± 1.5	Highly resistant
J 2405 R	4 ± 1.7	Highly resistant
JKMS 2A	4 ± 1.9	Highly resistant
NMS 20A	5 ± 1.4	Highly resistant
JMS 101B	5 ± 3.0	Highly resistant
JMS 112A	5 ± 1.3	Highly resistant
PM 462A	5 ± 2.1	Highly resistant
MS 42A	5 ± 1.2	Highly resistant
JKMS 40A	6 ± 2.1	Resistant
J 2440R	6 ± 1.9	Resistant
ICMB 98004	6 ± 2.2	Resistant
ICMA 98004	7 ± 3.8	Resistant
GK 1015A	7 ± 1.9	Resistant
ICMB 93222	8 ± 3.0	Resistant
ICMB 95444	11 ± 2.5	Susceptible
MS 38A	13 ± 5.1	Susceptible
AR IA	14 ± 6.5	Susceptible
JKMS 3A	16 ± 3.8	Susceptible

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Downy Mildew Incidence on Pearl Millet Cultivars and Pathogenic Variability among Isolates of *Sclerospora graminicola* in Rajasthan

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Introduction

Downy mildew (DM), caused by *Sclerospora graminicola* (Sacc.) Schroet, is the most important and widespread biotic constraint to the sustained high productivity of pearl millet [*Pennisetum glaucum* [(L.) R. Br.] hybrids in India. The fungus, S. *graminicola,* is an obligate heterothallic oomycetes (Michelmore et al. 1982) that reproduces by both sexual (oospores) and asexual (zoospores) means and thus produces large genetic variability in the progenies. Because of this large genetic variability in the pathogen, several host-specific pathotypes have been identified (Thakur and Rao 1997; Thakur et al. 2003). Rajasthan is one of the major pearl millet growing states in India and during the past 5 years increased DM incidence has been reported in several of the commercial hybrids. Under the ICAR-ICRISAT partnership project, on-farm surveys for DM incidence were conducted in the major pearl millet growing districts of Rajasthan during the 2001-2004 rainy seasons. Studies were also conducted to understand virulence diversity in *S. graminicola* populations especially from western Rajasthan.

Materials and Methods

On-farm downy mildew survey. Roving field surveys were conducted during the four rainy seasons of 2001 **to** 2004 covering 585 pearl millet fields in 52 taluks (a revenue unit) of 16 districts of Rajasthan (Alwar, Barmer, Bikaner, Churu, Dhaulpur, Dausa, Hanumangarh, Jaipur, Jalore, Jhunjhunun, Jodhpur, Karauli, Nagaur, Pali, Sikar and Tonk). In each field, five random subplots (four at the corners and one in the middle) were selected, and within each subplot, a minimum of 50 plants were counted in 2-3 rows to record diseased and healthy plants. The sum totals of healthy and diseased plants from 5 subplots of each field were used to determine the DM incidence percent. Thirty-seven DM-infected leaf samples as oosporic isolates from highly susceptible (>20% incidence) hybrids and the local cultivars were collected to study their pathogenicity and virulence diversity.

Pathogenic variability. Sporangial inocula were raised on seedlings of a highly susceptible genotype 7042S in isolation chambers in a greenhouse from the 12 selected isolates obtained from four districts (Banner - 4, Bikaner - 1, Churu - 2 and Jodhpur - 5) of western Rajasthan and three controls (one isolate each from Jodhpur, Durgapura and Patancheru). Pot-grown 48h-old seedlings of seven pearl millet differential lines (IP 18292, IP 18293, P 7-4, P 310-17, 700651, 852B and ICMP 451) were spray-inoculated with sporangia] suspension (1 x 10^6 sporangia mL^{-1})of each of the above 15 isolates. The experiment was conducted in a completely randomized design with three replications, 100 seedlings per replication. Data were recorded for disease incidence 14 days after inoculation. The experiment was repeated once to confirm the results.

Table 1. On-farm pearl millet surveyed for prevalence of downy mildew (DM) in 16 districts of Rajasthan during the 2001-04 rainy seasons.

District	Year	No of fields		DM incidence (%)	
		Surveyed	With DM	Mean	Range
Alwar	2002	17	0	0	0-0
Banner	2003-04	55	39	15	0-67
Bikaner	2003	13	13	15	0-54
Churu	2003	92	85	21	0-76
Dhaulpur	2002	7	2	1	0-2
Dausa	2002	20	1	1	0-12
Hanumangarh	2003	59	38	2	0-17
Jaipur	2001-02	103	31	5	0-56
Jalore	2002, 2004	9	8	17	0-41
Jhunjhunun	2001	1	1	2	2-2
Jodhpur	2003-04	84	66	14	0-69
Karauli	2002	21	0	0	0-0
Nagaur	2003-04	25	13	5	0-21
Pali	2004	19	7	2	0-17
Sikar	2003	46	38	15	0-78
Tonk	2002	14	2	1	0-10
Total	-	585	344	7	0-78

Table 2. Downy mildew incidence on pearl millet cultivars in farmers' fields in Rajasthan, 2001-2004.

Cultivar	Downy mildew incidence (%)				
	2001	2002	2003	2004	Range
Pusa 23	0(1)[1]	-[2]	-	0(1)	0-0
Proagro 9444	-	0(12)	0(3)	1(2)	0-1
Pioneer 7688	0(16)	1(27)	-	-	0-1
JKBH 26	2(7)	0(35)	-	-	0-2
PAC 931	-	2(1)	-	0(1)	0-2
HHB 67	<1 (28)	0(3)	5(44)	3(22)	0-5
Proagro 9330	-	-	10(1)	0(1)	0-10
Bioseed 8434	-	5(3)	11(4)	21(5)	5-21
ICMH 451	56 (3)	2(2)	39 (35)	-	2-56
VF 4112	-	-	12(3)	-	12
MLBH 308	-	-	21(1)	-	21
PG 5822	28(6)	-	-	-	28
Eknath 301	-	-	-	19 (7)	19
BK 560	76(5)	-	-	-	76
ICTP 8203	-	1 (9)	-	-	1

1. Number of fields.
2. Not found.

Table 3. Differential virulence reactions of 13 isolates of *Sclerospora graminicola* from western Rajasthan on seven host differential lines evaluated in greenhouse during 2004.

			Downy mildew reactions on host differential lines[1]							
Isolate	Location	Cultivar	IP 18292	IP 18293	P7-4	P 310-17	700651	852B	ICMP451	Ratio (R : S)
Sg 138	Jodhpur	Mixture	S	S	S	S	R	S	S	1 :6
Sg 144	Jodhpur	81A	R	R	R	S	R	S	R	5:2
Sg 145	Jodhpur	HB 3	S	S	S	S	R	S	S	1 :6
Sg 148	Jodhpur	Mixture	S	R	R	R	R	S	R	5:2
Sg 381	Jodhpur	OPY 97	R	S	S	S	S	S	S	1 :6
Sg 382	Barmer	Mixture	S	S	S	S	R	S	S	1 :6
Sg 383	Banner	ICMH 451	S	S	S	S	R	S	S	1 :6
Sg 384	Barmer	Local	S	S	S	S	S	S	S	0:7
Sg 385	Barmer	Local	S	S	S	S	S	S	S	0:7
Sg 388	Chun]	Local	S	S	S	S	R	S	S	1 :6
Sg 406	Bikancr	Local	R	R	R	R	S	R	S	5:2
Sg 407	Churu	Gachri local	R	S	S	S	S	R	S	2:5
Sg 139[2]	Jodhpur	Nokha local	S	S	S	R	R	S	S	2:5
Sg 212[2]	Durgapura	Plant gene	R	R	R	R	R	R	S	6: 1
Sg 409[2]	Patancheru	PMB 11571-2	R	S	S	S	S	R	S	2:5

1. Based on the mean of 2 experimental runs, 3 replications in each experimental run.
2. Controls.

R (resistant)= <20%, S (susceptible)= >20% incidence.

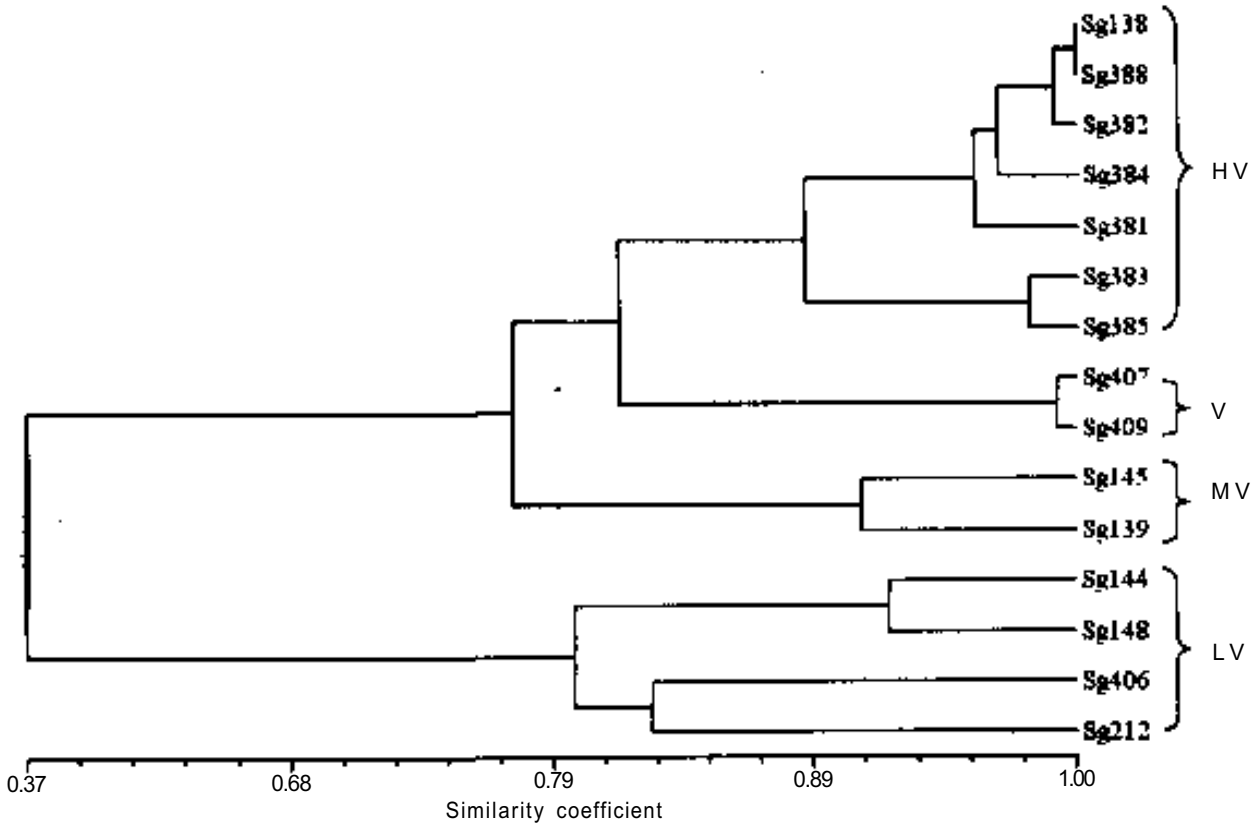

Figure 1. Classification of 15 isolates of *S. graminicola* based on downy mildew incidence on seven host differential lines into highly virulent (HV), virulent (V), moderately virulent (MV) and low virulent (LV) groups.

Data analysis. Data sets were subjected to ANOVA to determine significant differences among different isolates for DM incidence. The DM incidence data from two experimental runs were subjected for homogeneity test using the F-test of significance before pooling them. The DM incidence was subjected to hierarchical cluster analysis using the average linkage method with Euclidian distances to classify the pathotypes.

Results and Discussion

Downy mildew incidence. Of the 585 pearl millet fields surveyed in 16 districts, 59% of these showed DM infection. The mean DM incidence across pearl millet cultivars over four seasons varied from low to moderate (1-21%) in 14 districts, and no DM was recorded in Alwar and Karauli districts (Table 1).

A total of 26 hybrids, one open-pollinated variety (ICTP 8203), and several unknown cultivars were observed during the 4-year surveys. Of the 26 hybrids, only 9 were common in 2-3 years, and six of these (Pusa 23, JKBH 26, Proagro 9444, Pioneer 7688, PAC 931 and HHB 67) were highly resistant with mean DM incidence of < 5% compared with 2-56% incidence on ICMH 451 (Table 2). Thirteen hybrids (Bioseed 8448, GS 7788, ICMH 356, Kaveri 434, MLBH 319, MRB 2210, Nandi 5, VBMH 304, Pioneer 83M54, Proagro 7701, Pusa 23, Sona 288 and Swaminath) grown in any one season were DM-free, while the remaining five hybrids (VF 4112, Eknath 301, MLBH 308, PG 5822 and BK 560) also grown in one season were susceptible with 12 to 76% mean incidence. ICTP 8203 recorded 1% mean incidence while locals and unknown varieties showed 3-21% DM. In general, the DM incidence levels were higher in pearl millet hybrids in Sikar, Churu, Jodhpur and Barmer than in other districts. This could be due to more congenial weather conditions prevailing at the seedling stage in the above districts. Hybrids that recorded more than 20% mean DM incidence (highly susceptible) may be considered for withdrawal from cultivation to avoid the occurrence of epiphytotics in the near future.

Pathogenic variability. All the 12 isolates from western Rajasthan were maintained on 7042S through asexual generations. The DM incidence varied from 2 to 100% across isolate x host differential combinations, with mean DM incidence of 15 isolates across host differentials varied from 20 to 81% (data not shown). The quantitative DM incidence data were defined for qualitative resistant (R) reaction ($\leq$ 20% mean incidence) and susceptible (S) reaction ($\geq$ 20% mean incidence) to understand the virulence pattern of the isolates. Based on the R:S ratios across seven host differentials, isolates Sg 384 and Sg 385 from Barmer were most virulent (0R:7S) while some others, such as Sg 138, Sg 145 and Sg 381 from Jodhpur; Sg 382 and Sg 383 from Barmer; and Sg 388 from Churu were also highly virulent (1R:6S) (Table 3). These isolates were more virulent than the control isolates from Jodhpur (Sg 139) that was known to be highly virulent until recently (Thakur et al. 1999). Using hierarchical cluster analysis of the mean disease incidence data, the 15 isolates were classified into five virulence groups (Fig 1). Seven isolates (Sg 138, 381, 382, 383, 384, 385 and 388) belonged to highly virulent group; two (Sg 407 and 409) to virulent group; another two (Sg 139 and 145) to moderately virulent group; and four (Sg 144, 148, 212, and 406) to low virulent group. Two isolates from Barmer (Sg 384 and 385) were more virulent than those from Jodhpur, Churu and Bikaner, and therefore, one of these isolates should be used in resistance screening of breeding lines targeted for western Rajasthan. Collection and evaluation of more isolates from western Rajasthan may be required to better understand their virulence diversity.

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On-Farm Adaptive Management of the Blast of Finger Millet

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Introduction

Of the several diseases that afflict finger millet [*Eleusine coracana* (L.) Gaertn], blast caused by *Pyricularia grisea* (Cke.) Sacc is widely distributed in almost all the finger millet growing regions of the world and is the most destructive disease. In India, the disease was first reported from the Tanjore delta of Tamil Nadu by Mc Rae in 1920. The average loss due to finger millet blast has been reported to be around 28% and has been reported as high as 80-90% in endemic areas (Bisht 1987; Rao 1990; Ramappa et al. 2002). '

Finger millet is a low value crop and is generally grown as a rainfed crop and most often on marginal soil. Disease control by fungicide application is not economical. Under these circumstances, the most effective way of managing the disease is through host-plant resistance. An experiment was conducted to control the disease by integrating seed treatment and host resistance in an on-farm-adaptive-research (OFAR) program. The OFAR is a process that helps in evaluating newly developed technologies on farmer's fields and involves close cooperation with the farmers. Trials were conducted in Karnataka, Tamil Nadu and Uttaranchal states of India.

Materials and Methods

The trials were evaluated in eight districts of Karnataka (Bangalore, Chamarajnagar, Chitradurga, Haveri, Hassan, Kolar, Mysore and Tumkur), two districts of Tamil Nadu (Dharmapuri and Coimbatore) and one district in Uttaranchal (Tehri) states of India (Table 1). The experiments were conducted on farmer's fields during the *kharif* seasons of 2001-02 and 2002-03 in an area of 4000 m^2 at all locations except Uttaranchal where 100 m^2 was used because of the farms' surface gradient and hilly terrain. There were four treatments on each farm: T_1. Farmers variety + untreated seed; T_2. Farmers variety + seed treated with carbendazim @ 2 g kg^{-1} seed; T_3. Resistant variety (GPU 28/VL 149/CO 13) untreated; and, T_4. Resistant variety (GPU 28/VL 149/CO 13) + seed treated with carbendazim @ 2 g kg^{-1} seed, along with recommended FYM and fertilizer dose. There were 69 trials in Karnataka, 23 trials each in Tamil Nadu and Uttaranchal with a total of 115 farmers in 56 villages. The number of farmers in each district was Bangalore- 15, Chamarajnagar- 5, Chitradurga- 5, Haveri- 2, Hassan -10, Kolar- 12, Mysore-5, Tumkur- 15, Coimbatore-8, Dharmapuri- 15 and Tehri- 23. The trials were undertaken in collaboration with the State Department of Agriculture, Extension Education Units (EEU) and Operational Research Projects (ORP) in the watershed areas of the State Agricultural Universities (SAU). Care was taken in the selection of experimental areas for items such as watershed, fertility level, socioeconomic status, for choosing representative area of the entire tract as per guidelines established by the national agricultural technology project (CRIDA 2001). The experimental plots were periodically monitored and reviewed by the Peer Review Team (PRT) and Site Committee of the respective universities in Karnataka, Tamil Nadu and Uttaranchal. Observations on disease incidence and yield were recorded. Farmer knowledge of blast prior to the OFAR interventions were as follows: finger millet is a staple food for poor people; blast cause serious yield loss

Table 1. Summary of on-farm trials conducted during 2000-03.

State	On station trials	On farm trials	Area covered (Ha)	# of farmers covered	# of villages covered	# of district covered
Karnataka	6	69	27.6	69	30	8
Tamil Nadu	4	23	9.2	23	15	2
Uttaranchal	3	23	0.5	23	11	1
Total	13	115	37.3	115	56	11

and grown under rainfed situation; 20-25% recurring yield loss; sowing at convenience; indiscriminate use of fertilizers; lack of cultural management and lack of awareness of varietal reaction to blast.

The proposed integrated blast management of finger millet (Fig. 1) is aimed at educating and demonstrating to farmers the importance of improved practices such as - optimum time of sowing (before July); creating awareness on the availability of resistant varieties (GPU 28 and VL 149); use of green manure (cowpea); use of balanced nutrition (50:40:25 NPK ha^{-1}); use of Farm Yard Manure (7.5 t ha^{-1}); optimum spacing (30 cm x 10 cm) and maintaining ideal population; early warning and prediction of blast based on epidemiological factors; demonstrating the advantages of integrated blast management and other related practices such as summer ploughing, clean cultivation, three split application of N with 50% as basal dose and the remaining as two equal application one month after sowing and at 50% flowering. Forecasting the occurrence of blast is based on a trap crop method using blast susceptible variety KM 245 as well as weather factors such as morning and evening temperature and humidity and spore count in the air by sticky slide method (>10 spores/microscopic field). Biological control of blast by spraying of cultures of *Pseudomonas fluorescens* and *Trichoderma viride* was also tried in Uttaranchal state. Farmers were provided with all the inputs required such as seed, fertilizers and fungicide.

The incidence of leaf, neck and finger blast were recorded. Leaf blast was scored on a scale of 0-5 where 0=no symptoms on the leaves; l=small brown specks of pinhead size to slightly elongated, necrotic grey spots with a brown margin, less than 1 % leaf area affected; 2=a typical blast lesion elliptical, 5-10 mm long, 1-5% of leaf area affected; 3=a typical blast lesion elliptical, 1-2 cm long, 5-25% of leaf area affected; 4=25-50%. of leaf area affected; and 5=more than 50% of leaf area affected with coalescence of the lesions.

The observations on neck and finger infections were recorded at the milk stage. The percent neck blast incidence was calculated separately for each treatment based on the number of plants infected at the neck region over total plant population of that treatment. In recording finger blast incidence, first stage mean number of fingers per earhead in a treatment is computed by taking average of randomly chosen KM) earheads in each treatment. The percent finger blast incidence was calculated by considering

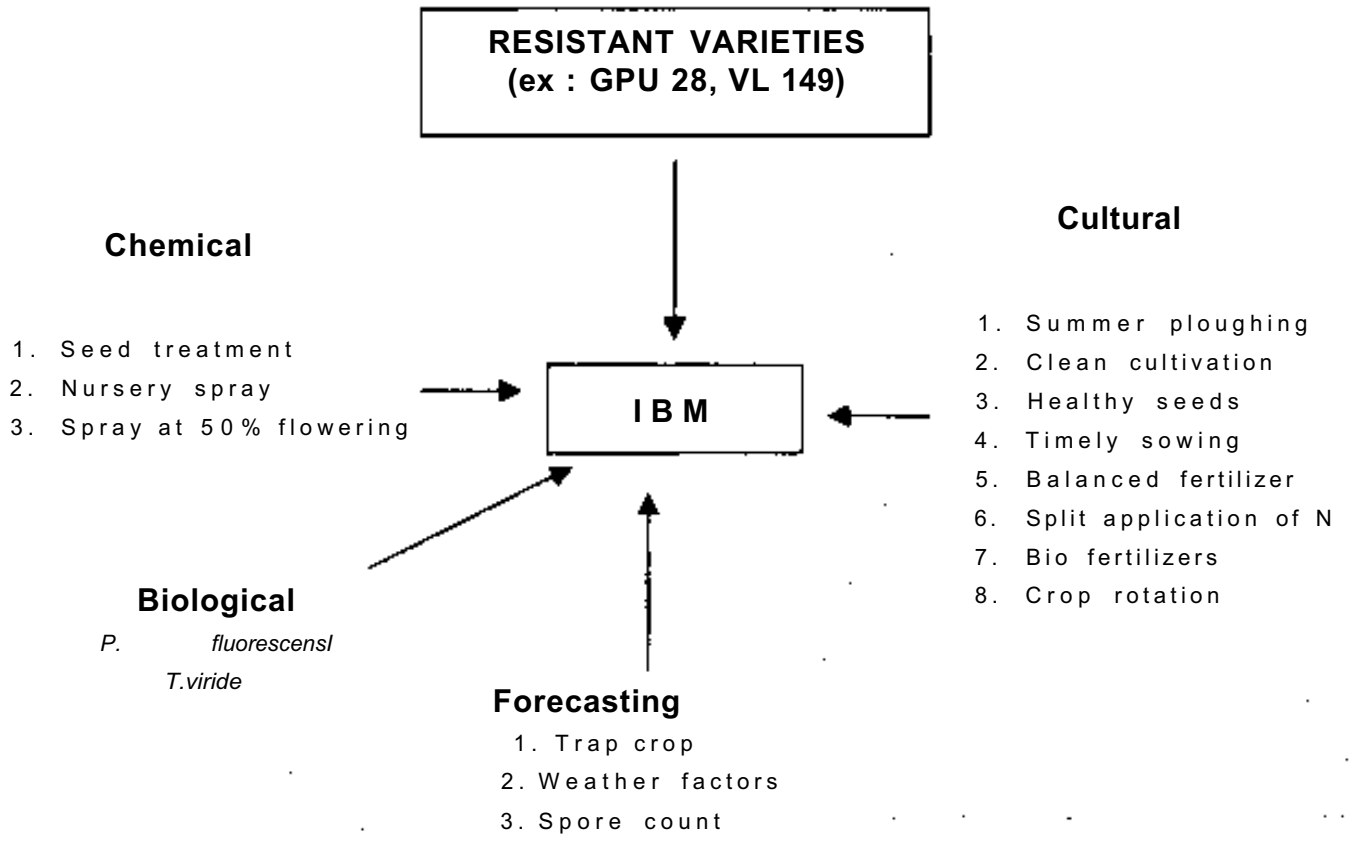

Figure 1. Schematic diagram of Integrated Blast Management (IBM) of finger millet.

Table 2. Consolidated results of on-farm adaptive research trials during 2000-03.

Treatments	Karnataka & Tamil Nadu (2000–03)				Tamil Nadu (2001–02)				Uttaranchal (2000–03)			
	LB (G)	NB (%)	FB (%)	Yield (kg/ha)	LB (G)	NB (%)	FB (%)	Yield (kg/ha)	LB (G)	NB (%)	FB (%)	Yield (kg/ha)
T_1: Farmers variety + Untreated	4.0	6.1	6.8	1676	4.0	8.4	9.1	1399	NR	29.9	31.7	2050
T_2: Farmers variety + Seed treatment with Carbendazim @ 2 g kg^{-1} seed (Seed treatment with Tricyclazole @ 2 g kg^{-1} seed in case of Tamil Nadu during 2001–02)	0.2	3.1	3.9	1827	2.4	7.4	7.9	1447	NR	29.4	30.6	2196
T_3: GPU 28/VL149 (CO 13 in case of Tamil Nadu during 2001-02) + Untreated	4.0	0.4	1.3	2524	2.3	5.3	8.3	2985	NR	0	1.3	2667
T_4:GPU 28/VL149 (CO 13 in case of Tamil Nadu during 2001–02) + Seed treatment with Carbendazim @ 2 g kg^{-1} seed (Seed treatment with Tricyclazole @ 2 g kg^{-1} seed in case of Tamil Nadu during 2001–02)	0.2	0.1	0.6	2708	1.7	4.0	4.7	3137	NR	0	1.1	2761
SEm ±	0.2	0.1	0.2	39.23	0.1	0.1	0.1	27.42	-	-	0.04	29.31
CD at 5%	0.5	0.4	0.5	127.49	0.3	0.3	0.4	89.11	-	-	0.13	95.12

LB – Leaf blast; NB – Neck blast; FB – Finger blast; G – Grade; NR – Not recorded.

number of infected fingers over total number of fingers (number of earheads x mean value of fingers/earhead). The opinions of the farmers were recorded after the trial.

Results and discussion

Seed treatment of finger millet gave good control of leaf blast regardless of the varieties compared to the untreated control. The incidence of leaf blast was almost uniform in Karnataka and Tamil Nadu with a severity score of 4.0 on a 0-5 scale on untreated farmers (Indaf/HR911/Local) as well as on the improved variety GPU 28. Seed treatment with carbendazim @ 2g kg^{-1} showed only mild infection (0.2). The results of the OFAR trials helped in illustrating to farmers the importance of resistant varieties in helping to manage the disease compared to local variety/ improved susceptible varieties. Farmers also appreciated the advantages of simple and low cost technology such as seed treatment with carbendazim in preventing blast. Seed treatment was used to protect the young seedlings from leaf blast since all the available finger millet cultivars are susceptible including GPU 28, which is resistant only to neck and finger blast. All existing resistant varieties are resistant only against ear and finger blast, but invariably susceptible to leaf blast. The seed treatment technique to control leaf blast proved to be effective.

The resistant variety GPU 28 was readily acceptable to farmers in both the states of Tamil Nadu and Karnataka because of its high level of resistance against ear and finger blast as well as high yield potential. In Uttaranchal, the variety VL 149 was the most suitable. The majority of finger millet growers are small and marginal farmers, who cannot afford to take up chemical control or buy costly inputs for management. Thus integrated management technology where blast resistant cultivars were the major component of the program is a viable option for these farmers (Table 2).

The OFAR trials also served the purpose of validating improved technologies and provided hands-on experience to the farmers in the adoption of these technology at the farm level. The trial plots not only served as research plots but also as demonstration plots that showed how some simple technologies could boast farmers yields. The interactive sessions between scientists and farmers during field days and training programs was helpful in demonstrating the usefulness of these new technologies and convincing farmers of their benefits.

In India, blast is one of the major diseases causing recurring yield losses in all the states (Seetharam 1983). Viswanath and Seetharam (1989) have researched the etiology and management of blast along with other diseases of ragi, but management of finger millet blast through an integrated approach was done for the first time.

The wide dissemination of integrated blast management technologies in finger millet was very important as susceptible varieties were predominantly grown largely in finger millet growing states (DE&S 2004). The OFAR management trials were a success story of how a technology is capable of bringing change in agriculture production in dryland situation. The training program sessions were also quite helpful for farmers and their feedback on the program will be used to further refine the technology package for future use.

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Entomology

Evaluation of Pearl Millet for Resistance to Millet Head Miner in Niger

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Introduction

In the Sahel, pearl millet (*Pennisetum glaucum)* suffers significant yield loss because of poor soil fertility, scarce and erratic rainfall, warm temperatures, and insect pests (FAO and ICRISAT 1996). While drought has been the major cause of low yields of pearl millet in West Africa, damage by various pests decreases yield potential (Nwanze and Harris 1992). Millet head miner (*Heliocheilus (=Rhagava) albipunctella),* the most important insect pest of millet in the Sahel, causes severe crop loss and poor grain quality.

In Niger and other Sahelian countries, the emphasis has been on finding pearl millet genotypes resistant to millet head miner. Genotypes of pearl millet resistant to millet head miner are: Souna, 3 /4 HK-78, ICMS-7819, JBV-8004, H24-38, Nigerian composite, HKB-Tif, C1VT, HKP, Zongo, Nieluva, Boudouma, JBM-8302, JNMG-52, ITMU-5001, Sadore, Totini, and Haini Kirei (Gahukar et al. 1986, Gahukar 1987). Gahukar (1987) reported that early or late-flowering pearl millet was more resistant to millet head miner. This mechanism of resistance (pseudoresistance) was thought to be asynchrony in times of vulnerability of millet plants and peak abundance of millet head miner (Youm and Kumar 1995).

Plant resistance offers good potential for managing millet head miner. Availability of resistant or tolerant pearl-millet is important for farmers in the Sahel where the ecosystem does not support the use of chemicals. The objective of this research was to identify resistant genotypes of pearl millet and incorporate them into an integrated pest management and/or millet breeding program.

Materials and Methods

Pearl millet developed at INRAN and ICRISAT Niger was evaluated for resistance to millet head miner during the 2004 cropping season at the Regional Agricultural Research Center in Maradi in East-Central Niger (15°26' North and 8°33' East) about 640 km east of Niamey, Niger. The mean annual rainfall there is 400-500 mm. The genotypes of pearl millet evaluated were ANKOUTESS, ICMH 2003, SOSAT-C88, ICMH 2104, TMK, 1A x TMK, 1A x KBH, KBH, ICMV IS 99001, ICMV IS 90311, ICMV IS 92326, HKP-GMS, % HK B-78, and ZAT1B (local check). The experimental design was a completely randomized block with four replications. Each 12 m^2 sub-plot had 5 rows 3 m long. Seeds were planted with 1 m between rows and 1 m between hills.

A cage 70-90 cm long and 30 cm diameter constructed of a wire frame covered by fine cotton mesh was placed over a spike exserted 5-10 cm (1/3 exsertion). Four spikes of each genotype of pearl millet were used. A sticker with 40 millet head miner eggs collected in farmers' fields was pinned onto each spike 2 to 3 days later. An iron bar was used to support each caged spike to prevent breakage by wind or other natural conditions.

Five days after infestation, each spike was inspected, and the number of hatched eggs was counted. At maturity, the spikes were cut and damage was assessed using a 1-9 scale (Youm and Kumar 1995). The spikes from each plot were weighed to determine yield. Data on scores of damage by millet head miner and on yields of pearl millet were analyzed using SAS. The Student t-test was used to compare means of measured variables and determine genotypes resistant to millet head miner.

Results and Discussion

The genotypes of pearl millet did not differ significantly by damage score. The genotypes had damage scores of 1.0-3.3 (Table 1). ICMH 2104, SOSAT-C 88, and ICMV IS 99001 were most damaged (20-30%), with damage scores of 3.3, 2.5, and 2.3, respectively. TMK, 1A x KBH, ICMH 2003, ICMV IS 92326, ZATIB, ICMV IS 90311, and KBH had damage scores of only 1.0 to 1.5.

Yields differed among the genotypes (F = 0.38; df = 3,13; P = 0.0961). Greatest yields of 1.1, 1.1, 1.1, 1.0, and 1.0 t ha^{-1} were calculated for 1A x TMK, ICMV IS 99001, HKP-GMS, TMK, and 1A x KBH, respectively (Table 1). KBH, ANKOUTESS, ICMV IS 90311, SOSAT-C 88, $^3/_4$ HK B-78, and ZATIB yielded least (0.6, 0.7, 0.7, 0.8, 0.8, and 0.9 t ha^{-1}, respectively).

Yield (1.0 t ha^{-1}) of the pearl millet hybrids, ICMH 2003 and ICMH 2104, was not correlated with damage score (1.3 and 3.3, respectively). This suggested tolerance to millet head miner. Damage scores were 3.8 and 4.3 for these hybrids during another experiment at ICRISAT Niger (Kadi Kadi et al. 2004).

Three improved varieties, TMK, HKP-GMS, and ICMV IS 99001 had some tolerance to millet head miner. Damage scores were 1.0, 2.1, and 2.3 and yields were 1.0, 1.1, and 1.1 t ha^{-1} for TMK, HKP-GMS, and ICMV

Table 1. Mean (± SE) scores of damage by millet head miner and yields (t ha^{-1}) of pearl millet, Maradi, Niger, 2004.

Genotype	Damage score	Yield (t ha^{-1})
1A x TMK	2.0 ± 0.4ab	1.1 ± 0.3a
ICMV IS 99001	2.3 ± 0.8ab	1.1 ± 0.3a
HKP-GMS	2.1 ±0.6ab	1.1 ± 0.3a
TMK	1.0 ± 0.0b	1.0 ± 0.1a
1A x KBH	1.3 ± 0.3b	1.0 ± 0.3a
ICMH 2003	1.3 ± 0.3b	1.0 ± 0.4b
ICMH 2104	3.3 ± 0.9a	1.0 ± 0.9b
ICMV IS 92326	1.5 ± 0.5b	0.9 ± 0.9b
ZATIB	1.4 ± 0.4b	0.9 ± 0.3b
$^3/_4$ HK B-78	1.8±0.8ab	0.8 ± 0.3b
SOSAT-C 88	2.5 ± 0.9ab	0.8 ± 0.6b
ICMV IS 90311	1.5 ± 0.5b	0.7 ± 0.3b
ANKOUTESS	2.0 ± 0.7ab	0.7 ± 0.2b
KBH	1.0 ± 0.0b	0.6 ± 0.8c
Mean	1.8	0.91
LSD	1.6	70.6
CV	0.644	0.542

Means followed by the same letter in a column are not significantly different (Student t-test, $P < 0.05$).

IS 99001, respectively. Yields of only 0.7, 0.8, 0.8, and 0.8 were calculated for ANKOUTESS, ICMV IS 90311, ICMV IS 99001, and ICMH 2104, respectively, at ICRISAT Niger (Kadi Kadi et al. 2004).

Some genotypes of pearl millet were tolerant to millet head miner. Tolerance might be caused by compensation and non-preference. Antixenosis resistance could not be determined because millet head miners caged on spikes of pearl millet do not have alternate hosts to attack. Attributes of antibiosis (e.g., weight loss or larval mortality) were not measured.

Pseudoresistance was observed in $^3/_4$ HK B-78 and ZATIB used as checks. The two varieties yielded 0.8 and 0.9 t ha^{-1}, respectively. Damage scores were 1.8 and 1.4, less than those of the hybrids ICMH 2003 and ICMH 2104.

Sources of resistance to millet head miner may be obtained from local, improved, and newly developed genotypes available at local and international agricultural institutions. Some genotypes have been evaluated and accepted for adaptation in the pearl millet-growing zone. An intensive evaluation program is needed for quick identification and development of West African millets resistant to millet head miner.

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Assessment of Opportunities to Map Pearl Millet Tolerance to Salinity during Germination and Early Seedling Growth

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Introduction

Pearl millet, *Pennisetum glaucum* (L.) R. Br., tolerates drought, low soil fertility, low soil pH and responds well to water and favorable soil conditions. Soil salinity greatly hampers pearl millet productivity, delaying germination, reducing seed germination percentage, and severely affecting subsequent growth (Ashraf and Idrees 1992).

This study was undertaken to assess opportunities for using existing pearl millet mapping populations (Hash and Witcombe 1994, Hash et al. 2001) and other pearl millet genetic stocks available at ICRISAT-Patancheru to generate molecular markers for genomic regions contributing to salinity stress tolerance during germination and early seedling growth. Salinity tolerance during these early growth stages is critical to crop establishment in saline soil conditions and soil-free *in vitro* screens can be used to assess this on the large numbers of entries required for phenotyping a mapping population progeny set.

Materials and Methods

Twenty-eight inbred pearl millet genotypes (LGD 1-B-10. ICMP 85410-P7. Tift $23D_2B_1$-P1-P5, WSIL-P8,81B-P6, ICMP 451-P8, ICMP 451-P6, H 77/833-2-P5(NT), H 77/833-2, PRLT 2/89-33, W504-1-P1, P310-17-Bk, PT732B-P2, P1449-2-P1, ICMB 841(=841B)-P3, 863B-P2; IP 18293-P152, Tift $238D_1$-P158, Tift 186, Tift 383, ICMB 89111. ICMB 90111, ICMB 92666. ICMB 95333. 843B, ICMB 98004, ICMB 99022 and ICML 22) obtained from ICRISAT were tested for salt stress tolerance over a range of salt concentrations (0 mM to 150 mM NaCl). The first 18 lines are nine parental line pairs of ICRISAT pearl millet mapping population progeny sets (Hash and Witcombe 1994, Hash et al. 2001); Tift 186 and Tift 383 are forage hybrid pollinators from Tifton, Georgia, USA, used as control lines; and the following four inbreds are maintainer lines of male-sterile lines used as testers in line x tester trials assessing the opportunities to use the ICRISAT pearl millet mapping populations to map various secondary target traits (Hash et al. 2001). The final four lines are ICML 22, derived from an oasis landrace accession (IP 2696) from Chad that was expected to possess some degree of salt tolerance; 843B. and two lines near-isogenic to 843B (ICMB 98004 and ICMB 99022) derived by backcrossing ICML 22 (as the donor of oligogenic downy mildew resistance) to recurrent parent 843B (CT Hash unpublished).

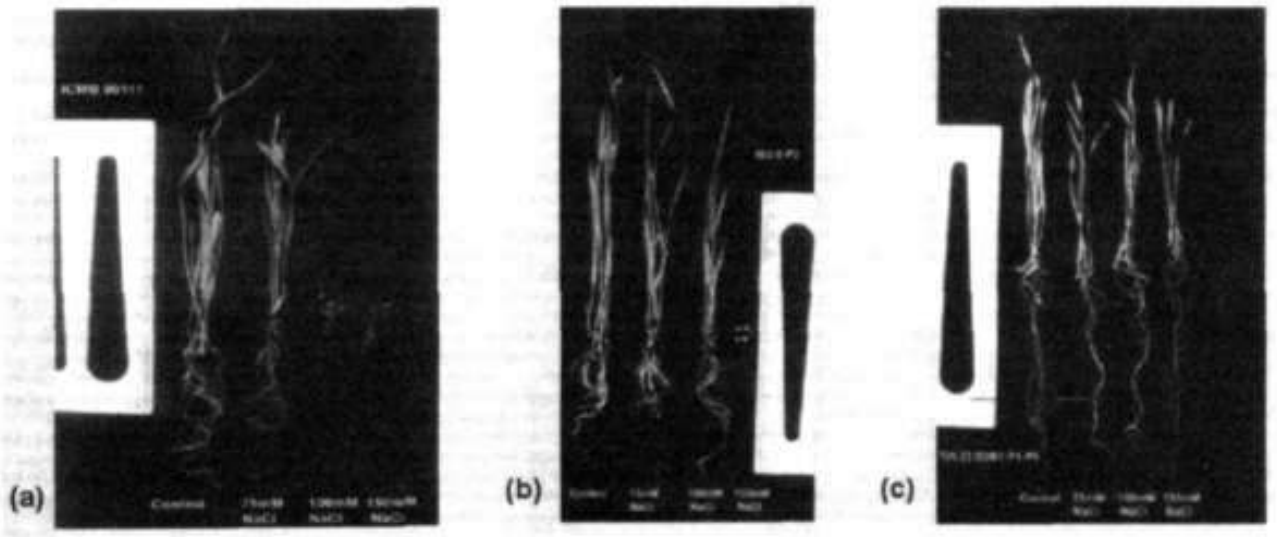

Figure 1. Ten-day-old seedlings of pearl millet inbreds ICMB 90111 (a). 863B-P2 (b) and Tift $23D_2B_1$-P1-P5 (c) grown in 0 mM, 75 mM, 100 mM and 150 mM NaCl media. ICMB 90111 was classified as sensitive to salinity stress during germination, whereas 863B-P2 was classified as moderately tolerant, and Tift $23D_2$ B_1-P1-P5 was classified as tolerant.

Seeds of these 28 inbred pearl millet lines were surface-sterilized and germinated on filter-paper boats in balanced nutrient solutions (Hoagland and Amon 1938) of pH 6.7 at 20°C, containing four different concentrations of NaCl (0 mM, 75 mM, 1(X) mM and 150 mM) in triplicates for each RCBD experiment. Fifteen seeds were germinated in each culture tube and the seedlings were allowed to grow for 10 days at 25°C under continuous fluorescent light (30 µE m^{-2} S^{-1}) in the same nutrient solutions. The experiments were repeated 4 times for each line and the genotype x salinity treatment means of each experiment were taken for the statistical analysis.

The seed germination percentage of each pearl millet inbred was determined for each treatment 10-days after sowing. For genotype x salinity level treatment combinations, shoot and root length (cm), fresh weight (mg), and dry weight (mg) were recorded for 10-day old seedlings. Experimental data were analyzed statistically using the GenStat software package (GenStat 1995) to ascertain the levels of significance for each source of variation (replications, genotypes, salinity levels, genotype x salinity level interactions, and error) in the experiment.

Table 1. Best linear unbiased predictions (BLUPs) for germination percentage of 28 pearl millet inbreds screened *in vitro* in Hoagland's solution in three treatments varying in NaCl concentration and in a non-saline control.

Entry name	0 mM NaCl	75 mM NaCl	100 mM NaCl	150 mM NaCl
LGD 1-B-10	96.8	88.3	75.3	0.0
ICMP 85410-P7	100.0	99.3	0.0	0.0
Tift $23D_2B_1$-P1-P5	91.8	89.3	90.3	78.3
WSIL-P8	95.3	92.5	0.0	0.0
81B-P6	100.0	92.0	1.3	0.0
ICMP 451-P8	92.5	89.0	87.5	0.0
ICMP 451-P6	99.8	81.3	75.3	0.0
H 77/833-2-P5(OT)	99.0	94.8	87.5	1.3
H 77/833-2	100.0	92.0	68.3	0.8
PRLT 2/89-33	73.8	36.8	4.3	0.5
W 504-1-P1	86.3	75.3	68.0	0.0
P310-17-Bk	96.0	96.0	90.0	72.8
PT 732B-P2	87.0	72.3	75.8	12.3
P1449-2-P1	95.8	75.3	0.0	0.0
841 B-P3	100.0	97.0	91.0	76.5
863B-P2	93.8	93.0	82.0	0.0
IP 18293-P152	23.3	0.0	0.0	0.0
Tift $238D_1$-P158	90.3	87.5	0.0	0.0
Tift186	97.5	70.0	73.0	0.0
Tift 383	99.5	94.8	91.8	10.0
ICMB 89111	52,5	43.3	19.0	0.0
ICMB 90111	100.0	33.0	0.0	0.0
ICMB 92666	97.3	79.5	88.3	0.0
ICMB 95333	99.3	98.8	98.0	53.0
ICML 22	85.0	79,8	74.5	63.5
843 B	93.8	92.3	89.0	0.0
ICMB 98004	80:0	73.5	56.0	0.0
ICMB 99022	89.5	81.8	47.0	0.0
Salinity level grand mean	89.83	78.49	54.74	13.17
SE	±1.69	±4.71	±1.30	±1.19
CV(%)	3.77	11.99	4.74	18.11
F-ratio	95.84	24.71	867.08	508.19
$h^2.1$	0.96	0.86	1.00	0.99
$h^{2.2}$	0.99	0.96	1.00	1.00

$h^{2.1}$ = operational heritabilities calculated on entry mean basis

$h^{2.2}$ = operational heritabilities calculated on plot basis

Results and Discussions

Genotype x salinity level interactions were significant for all observed traits, indicating that the genotypes differed in their tolerance to the salinity treatments (Tables 1 and 2). The pearl millet inbreds were categorized as sensitive, moderately tolerant and highly tolerant to salinity based on their relative abilities to maintain high germination levels and good early seedling growth (Fig. 1) across NaCl levels of 75 mM, 100 mM and 150 mM, respectively. Seven of the pearl millet inbred lines were categorized as sensitive (ICMB 90111, PRLT 2/89-33. P1449-2-P1, Tift $238D_1$ -P152, 81B-P6, WSIL-P8 and ICMP 85410-P7), fifteen as moderately tolerant, and five as highly tolerant (Tift $23D_2B_1$-P1-P5, ICMB 841-P3, P310-17-Bk, ICML 22 and ICMB 95333).

Large differences in germination salinity tolerance (Table 1) were detected between members of several pearl millet mapping population parental line pairs (including Tift $23D_2B_1$-P1-P5 and WSIL-P8, ICMB 841-P3 and 863B-P2, and P310-17-Bk and W 504-1-P1 at 150 mM NaCl; and ICMP 451-P8 and 81B-P6, LGD 1-B-10 and ICMP 85417-P7, and PT 732B-P2 and P1449-2-P1 at 100 mM NaCl), indicating that their previously skeleton-mapped pearl millet mapping population progeny sets can be phenotyped to map genomic regions contributing

Table 2. Best linear unbiased predictions (BLUPs) for shoot and root lengths of 28 pearl millet inbreds screened *in vitro* in Hoagland's solution in three treatments varying in NaCl concentration and in a non-saline control.

	Shoot Length (cm)				Root Length (cm)			
Entry name	0 mM NaCl	75 mM NaCl	100 mM NaCl	150 mM NaCl	0mM NaCl	75 mM NaCl	100 mM NaCl	150 mM NaCl
LGD 1-B-10	9.1	8.5	5.5	-	6.4	6.3	2.5	-
ICMP 85410-P7	8.8	11.9	-	-	6.7	7.3	-	-
Tift $23D_2B_1$-P1-P5	10.1	7.9	8.9	9.5	10.7	11.9	10.7	8.4
WSIL-P8	10.7	11.1	-	-	6.9	7.6	-	-
81B-P6	9.7	9.9	0.6	-	4.7	8.2	0.6	-
ICMP 451-P8	14.5	10.7	10.6	-	7.7	11.6	16.9	-
ICMP 451-P6	10.0	4.4	8.4	-	12.8	10.2	11.1	-
H 77/833-2-P5(OT)	11.7	8.6	9.2	2.8	12.3	10.5	9.4	3.2
H 77/833-2	5.8	4.3	4.4	0.5	6.3	5.7	9.4	1.1
PRLT 2/89-33	8.6	13.9	2.3	0.4	4.3	7.3	1.5	0.2
W 504-1-P1	8.8	8.8	8.2	-	9.3	9.6	9.4	-
P31()-17-Bk	9.4	6.9	8.6	7.1	10.2	7.2	9.2	8.5
PT 732B-P2	10.2	9.0	8.1	4.2	10.5	8.3	8.5	7.1
P1449-2-P1	13.6	11.4	-	-	11.4	12.4	-	-
841B-P3	12.1	9.1	8.4	7.6	7.6	9.6	7.3	5.9
863B-P2	10.4	9.9	7.6	-	8.7	6.1	8.7	-
1P 18293-P152	4.3	-	-	-	1.0	-	-	-
Tift $238D_1$-P158	8.4	7.8	-	-	4.9	4.7	-	-
Tift 186	8.3	8.8	9.4	-	8.6	10.0	9.5	-
Tift 383	10.3	8.5	9.2	2.5	8.7	9.9	12.6	1.9
ICMB 89111	8.5	8.3	7.9	-	9.2	9.9	8.3	-
ICMB 90111	9.8	7.8	-	-	8.9	6.7	-	-
ICMB 92666	10.3	9.6	6.6	-	8.9	9.5	10.1	-
ICMB 95333	10.9	9.8	7.7	8.5	5.1	9.4	10.9	8.5
ICML 22	7.6	7.1	6.4	5.0	6.2	5.7	7.1	4.6
843B	10.3	7.3	8.2	-	5.4	8.7	9.4	-
ICMB 98004	12.2	13.0	8.7	-	10.4	10.0	13.6	-
ICMB 99022	11.5	9.7	6.6	-	8.8	7.5	9.5	-
Salinity treatment grand mean	9.9	9.0	7.3	4.8	7.9	8.6	8.9	4.9
SE '	±0.2	±0.3	±0.2	±0.6	±0.2	±0.3	±0.3	±0.4
CV(%)	3.01	5.73	5.90	24.44	3.94	5.92	6.21	17.32
F-ratio	200.38	71.08	119.70	31.36	293.77	63.03	177.45	59.83
$h^{2.1}$	0.98	0.95	0.97	0.88	0.99	0.94	0.98	0.94
$h^{2.2}$	0.99	0.96	1.00	1.00	1.00	0.99	0.99	0.97

$h^{2.1}$ = operational heritablities calculated on entry mean basis
$h^{2.2}$ = operational heritablities calculated on plot basis

to these differences. Differences between mapping population parental line pairs were also detected for salinity tolerance of early seedling growth (Table 2).

This preliminary study indicates the potential for mapping genomic regions contributing to genetic variation in tolerance of pearl millet to salinity during germination and early seedling growth by combining existing marker data sets with phenotypic data sets produced by screening progeny sets from currently available pearl millet mapping populations. Such QTL mapping could be the next step towards identification of genes contributing to these components of salinity tolerance.

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Long-Term Regeneration in Callus Culture of Paisa (*Echinochloa frumentacea* Link.)

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Introduction

Paisa (*Echinochloa frumentacea* Link.) is an annual fodder cultivated in central Russia's Evropian region (near the city of Orel). The yield of paisa has been reported as high as 60 t ha^{-1} and seed production of 2 t ha '. Vegetative growth period ranges from 110 to 125 days, which is too long to obtain mature seed. No significant difference for this trait was found in our plant breeding material. Therefore, the use of biotechnological tools is being exploited to assist in the improvement of the crop. Regeneration in the genus *Echinochloa* has been based on deriving shoots in callus cultures from mature *E. oryzicola* seed (Maeda and Sugiura 1976), regenerated plants in cultures of *E. oryzicola* leaves (Takahashi et al. 1984), from segments of young *E. crusgalli* and *E. colonum* (L). Link, inflorescences (Wang and Yan 1984; Tyagi et al. 1985), from the mesocotyle of *E. crusgalli* var. *oryzicola* and *E. muricata* (Cobb et al. 1985), and in cultures of immature *E. glabrescens* Munro ex Hook F. inflorescences and leaves (Wang and Zapata 1987). This research was undertaken to evaluate the long-term regeneration of paisa through callus culture.

Materials and Methods

Mature seed of the paisa cultivar *Udalaya* were sterilized in 70% ethanol (1 min) and 0.5-2.0% chlorhexidine sodium digluconate (10 min) and were placed on 2KC media which contained salts according to the MS protocol (Murashige and Skoog 1962), vitamins according to the B5 media (Gamborg and Eveleigh 1968), and 100 mg L^{-1} myo-inositol, 4 g L^{-1} sucrose, 2 mg L^{-1} glycine, 6 g L^{-1} agar and 2 mg L^{-1} 2,4-D. Various levels of 2,4-D (0.5-3.0 mg L^{-1}) was also used in the 2KC media. The MSB media was similar to 2KC without 2,4-D. Subcultivation of the calli with regenerated plants was performed on the 2KC media with 2 mg L^{-1} 2,4-D and maintained for 6 years. Regenerated plants with roots were transferred into soil media. Seed of $R_{()}$ plants were harvested and made available to the laboratory of millet plant breeding for use in their breeding and selection programs.

Results and Discussion

Seed sprouted in the 2KC media, while on other seed, formation of calli (12.2%) was observed. Calli were formed due to de-differentiation of tissues from sprouts. Calli, with few exceptions, had no embryogenic properties. After transfer of non-embryogenic calli onto fresh media embryogenic calli (62.5%) with various tissue types (including embryogenic) were formed. On the surface of non-differentiated callus masses, segments with regenerated shoots were observed, along with hairs and white compact tissues.

Segments of calli with white, compact tissue and with regenerated shoots (type A) were transferred onto fresh 2KC media. As a result of their transfer and development on fresh media, callus tissues of 4 types were formed: A. Calli with non-differentiated tissue on the border with the media, white compact tissues on the surface and presence of the process of regeneration; B. Calli with non-differentiated tissue and presence of the regeneration process; C. Calli with predominance of the regeneration process and lack of proliferation of callus mass; and, D. Calli with predominance of proliferation of a non-differentiated tissue (Table 1). It is extremely important to pay attention to the transfer onto fresh media of the organized callus parts of type B (Fig. 1) so that callus tissues of all the indicated types are formed.

Transfer of type C and D callus from the 2KC media onto an MSB media (without growth regulators) resulted in a decrease of callus mass proliferation and an increase of shoots without roots. Plants without root systems, obtained on MSB, did not tolerate transfer to unsterile conditions. As a rule, regenerated plants with a weak root system obtained on 2KC were transplanted to soil. To develop plants with roots suitable for transfer into non-sterile conditions, replanting was done at intervals exceeding 35 days.

Periodic transfer of the organized segments of type A and B calli onto fresh 2KC has maintained the calli and regeneration potential to regenerate plants over a long

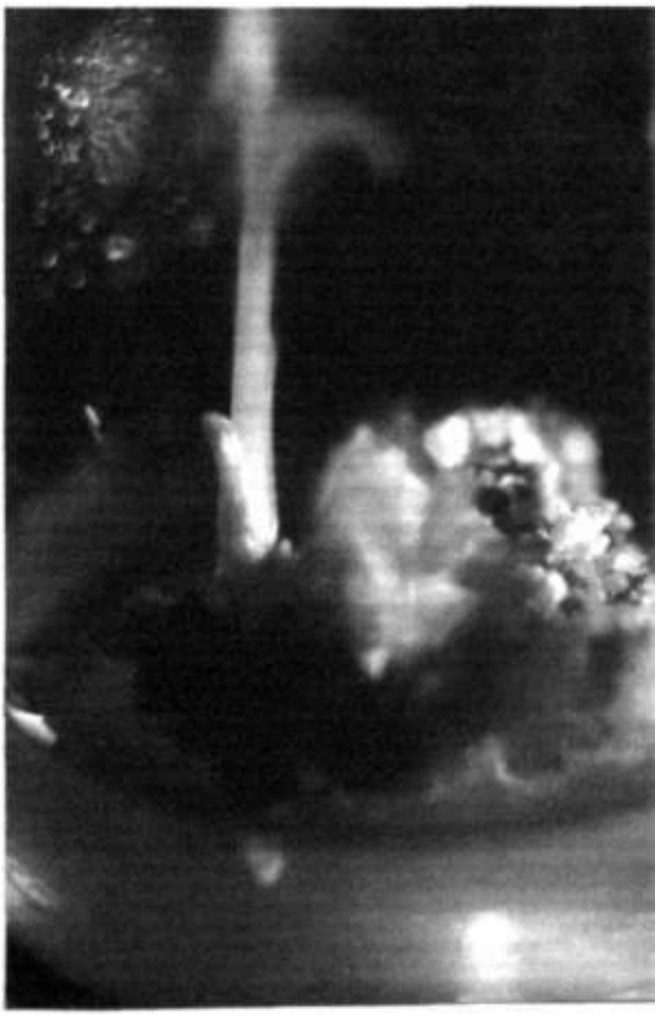

Figure 1. Emhryogenic callus (type B) with regeneration segments and regenerated plant.

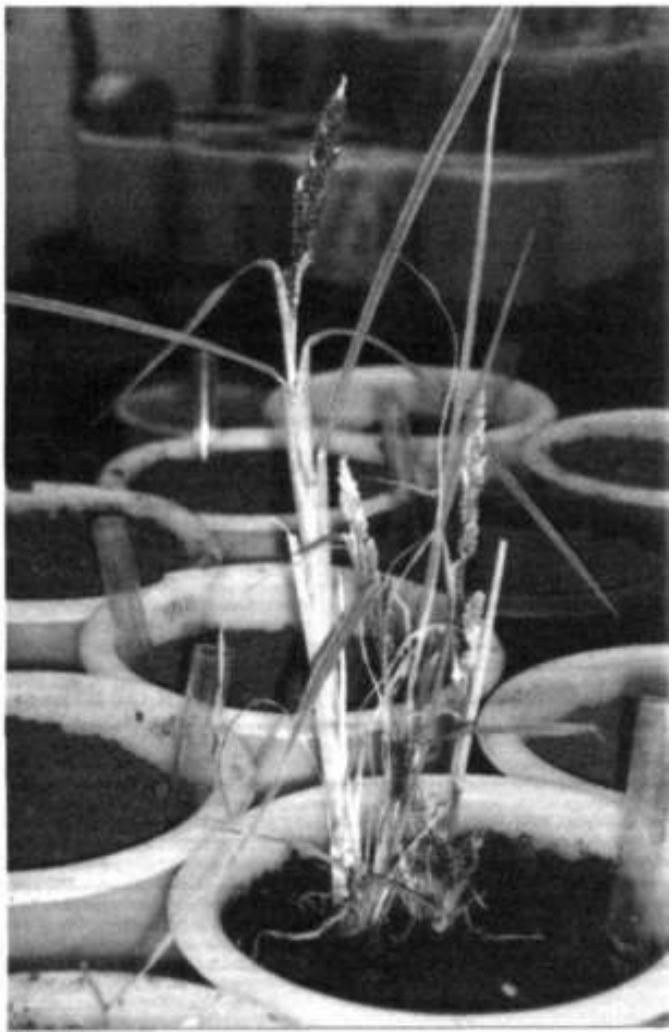

Figure 2. Regenerated plants of paisa R_0 in hothouse box.

period of time (6 years). Regenerated R_0 plants were transferred into soil, grown in the greenhouse (Fig. 2), flowered, and produced seed. Regenerated callus tissues produced seed from more than 100 regenerated plants, which were transferred to the laboratories of millet plant breeding of the All-Russia Research Institute. The plants were used to detect somaclonal variants for use in various plant breeding programs. Among regenerated plants, families of R1-R4 early maturing lines were identified. Some of the somaclonal lines matured two weeks earlier than the control cultivar Udalaya (Fig. 1). The plants of these lines had short stem lengths and are expected to be used for hay production.

The most common model for plant regeneration in the genus *Echinochloa* was based on the use of several types of media, callusogenics for regeneration and other media for maintaining the regeneration potential of calli for long periods of time. Using this model, regeneration potential was exhausted after a short time. Use of the media to maintain calli resulted in the loss of regeneration potential and regenerated plants.

The method suggested here was based on obtaining embryogenic calli in culture from mature seed and establishing a regeneration callus culture. As a rule, during subculturing onto fresh 2KC, the organized segments of embryogenic calli should be transferred. During their development, the interlayer of proliferating, non-differentiated tissue was formed on the border with the media. White compact and regenerating tissues on the surface of explants underwent the process of de-differentiation. In 30-40 days after transfer of explants onto fresh media, the intensity of de-differentiation noticeably drops and newly generated calli are divided into 4 types: A, B, C and D.

It was determined that the possibility of long-term cultivation of regenerating calli tissues was caused by the initial concentration of 2,4-D. The initial content of 2 mg L^{-1} 2,4-D in 2KC determined a difference of concentrations in calli tissues, ensuring a heterogeneous process. De-differentiation and active growth of a calli tissue (high 2,4-D concentration on the border with the media) and differentiation and regeneration (reduction of 2,4-D concentration in a direction from media to the callus tissue surface). Reduction of the initial concentration of 2, 4-D in the medium resulted in a drop in intensity of proliferation of non-differentiated tissue. On the media with 1 mg L^{-1} 2,4-D, the process of de-differentiation completely stopped for 2/3 of the calli, which before was cultivated on the medium with 2 mg L^{-1} 2,4-D. On the media without growth regulators, simple growth of shoots was observed. The augmentation of 2,4-D in media over 2 mg L^{-1} resulted in intensification of de-differentiation and reduction of embryogenic calli. We showed that tissue culture can be optimized to maintain viable callus for many years of paisa, which can lead to the regeneration of clones for use in breeding programs and can generate somoclonal variation for use in those programs.

Table 1. Development of embryogenic parts of callus tissues of type A after their transfer from medium 2KC onto 2KC and MSB, %.

	Type of callus			
Medium	A	B	C	D
2KC	12.50	64.06	17.19	6.25
MSB	-	-	100	-

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Sequence of ITS-2 Amplified from Pearl Millet Downy Mildew Samples

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Introduction

Sclerospora graminicola is the causal agent of downy mildew of pearl millet (*Pennisetum glaucum).* It is an obligate oomycete that reproduces asexually to produce sporangia (that release motile zoospores) and sexually through the production of soil-borne oospores. At least 15 different pathotypes of *S. graminicola* have been defined in India based on the use of 7 host cultivar differentials (Thakur 2000). Considerable effort has been applied to the development of resistant cultivars, especially in India. Widespread use of hybrids resistant to specific pathotypes has generated changes in the pathogen population. Similarly, cultivars identified as resistant in India often turn out to be susceptible in African locations at or very soon after introduction. Consequently, studies to examine genetic variability in the pathogen have also been undertaken. In India, where samples can be collected and expanded by re-infection on greenhouse grown plants in isolation, relatively pure *S. graminicola* DNA can be extracted and used for analysis of variability. Under these conditions it has been possible to identify RFLPs that show association with unique pathotypes (Sastry et al. 1995) and to cluster isolates in groups related to mating type based on AFLP patterns (Singru et al. 2003). AFLP utilizes PCR to amplify subsets of restriction fragments, leading to highly repeatable and easily scored banding patterns that have revealed a high level of polymorphism among *S. graminicola* isolates sampled across India. DNA-based comparisons of African to Indian isolates would be of great interest for assessing the relatedness of the comparative populations and perhaps of value in predicting resistant pearl millet genotypes for local deployment. However, phytosanitary concerns and regulations prevent the international transfer of viable *S. graminicola.* Also, the limited facilities available in Africa prevent the expansion of field-collected samples in isolation under greenhouse conditions. Thus for this study, only asexual spores collected from single infected leaves and fixed in alcohol were available for DNA extraction for samples from Africa; the tiny amounts of DNA available from these samples dictated that PCR-based techniques be employed for comparisons. Here we show that targeted DNA amplification revealed contamination of some samples, but also allowed sequence and phylogenetic comparisons among others.

Materials and methods

DNA samples were provided directly for isolates from India. Sporangial samples collected on patches of cheesecloth from infected plants in Burkina Faso, Mali, Nigeria and Niger were placed in microfuge tubes filled with alcohol for shipment to the US. On arrival, the spores were collected by centrifugation, lyophilized and DNA extracted using a Phytopure® (Nucleon) kit, as directed.

Nested PCR was used to amplify internal transcribed spacer (ITS) region 2 of the ribosomal RNA encoding genes (Fig. 1). The first primers (ITSI & 6) were in conserved regions of the large and small subunit rRNA and the second pair (ITS 3 & 4) flanked just the ITS-2 segment.

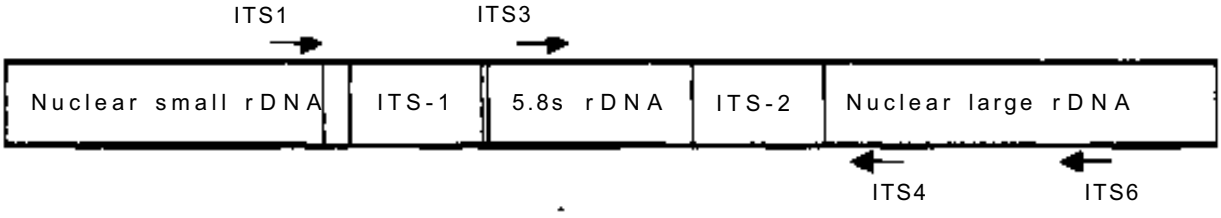

Figure 1. Locations of PCR primers for internal transcribed spacers on the nuclear ribosomal DNA map.

ITS-1: 5'-TCCGTAGGTGAACCTGCGG-3'
ITS-3: 5'-GCATCGATGAAGAACGCAGC-3'
ITS-4: 5'-TCCTCCGCTTATTGATATGC-3'
ITS-6: 5'-CACTTTTCAAAGTGCTTTTCATC7TTC-3'

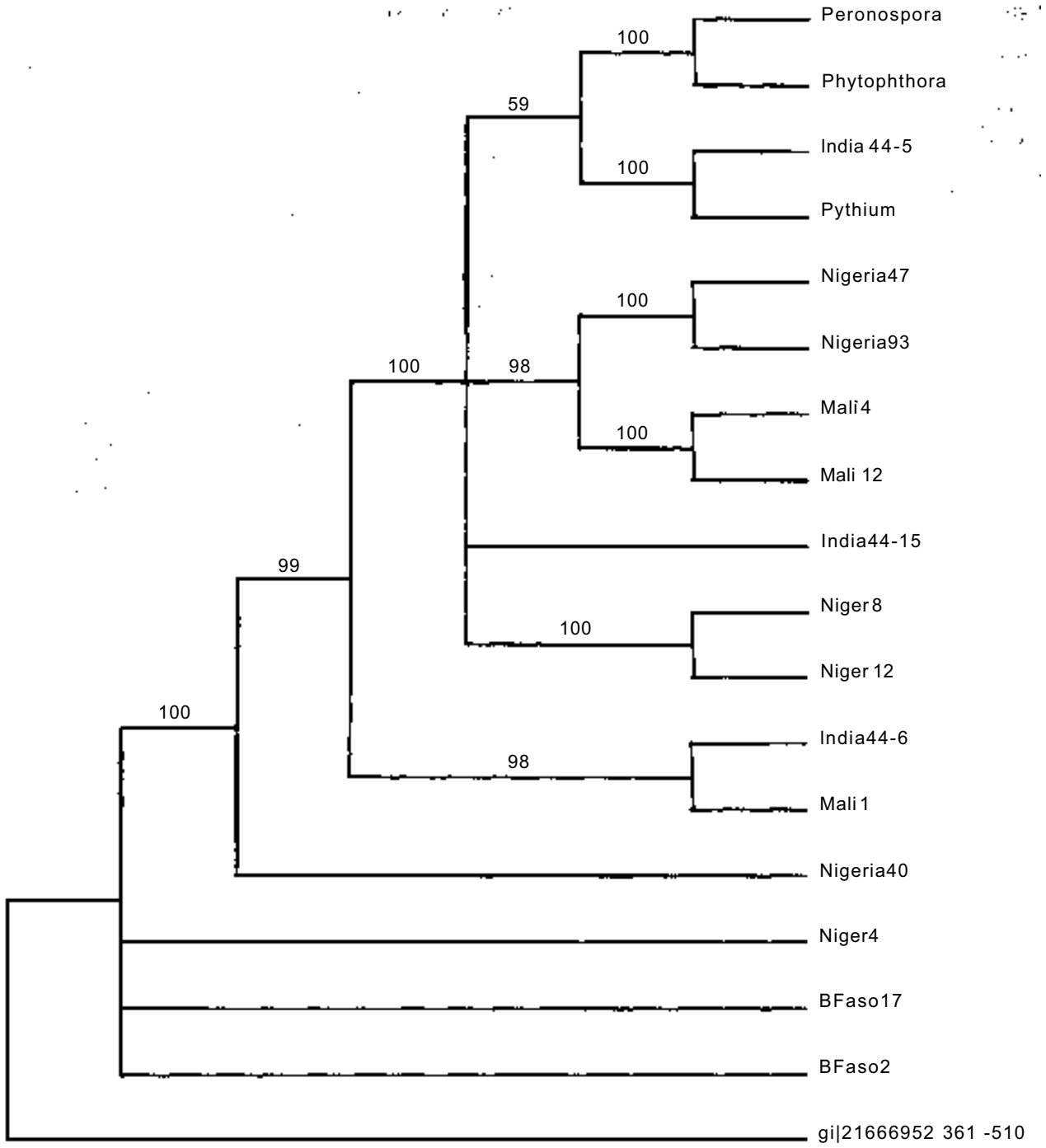

Figure 2. A bootstrap consensus of a parsimonious tree based on 400 base-pair sequences amplified from the ITS-2 region of rDNA from samples as indicated. Numbers above represent the bootstrap values. The outgroup used for constructing this tree was *Cladosporium herbarum.*

Standard PCR reactions were used, with the following parameters: initial denaturing for 3 min at 94°C followed by 28 cycles of 1 min for denaturing at 94°C, 1 min for primer annealing at 50°C and 2 min for primer extension at 72°C. The product from the first PCR reaction was diluted 1:100 and 1 µL of it was substituted for template DNA in the second PCR reaction. The amplified ITS-2 band was cloned into using TA-cloning (Invitrogen) and sequenced at the TAMU Gene Technologies Laboratory. Phylogenetic analysis used PAUP* v4.0.

Results and discussion

DNA sequence differences can be used all the way from defining kingdoms to unique identification of individuals. When DNA amplification is involved, as is often the case when the amount of starting material is limited, either very pure samples or primers of known specificity are essential to avoid amplifying contaminating DNA. In the case of *S. graminicola* samples from Africa neither of these conditions was met, meaning that differences detected by RAPDs or AFLP would be questionable. The transcribed spacers of rDNA genes are present in multiple copies in each genome. Their non-coding function means that changes are unlikely to have detrimental effects on survival. On the other hand, concerted evolution tends to maintain a common sequence in the multiple copies within a given species (Atkins and Clark 2003). Amplification of the ITS 2 region from presumed *S. graminicola* samples gave products of about 400 base pairs that were sequenced. BLAST searches of GenBank showed those from India and some African samples to be similar to ITS 2 from other oomycetes, including *Peronospora* spp. and *Phytophthora* spp. Other African samples were clearly derived from other species. For example, Mali sample 1 was almost identical to *Cryptococcusflavus* (P value for mismatch = 0) and Nigeria sample 69 matched ITS 2 of *Pseudzyma paraantarctica* at $P=e^{-160}$. A tree made using ITS 2 of *Cladosporium herbarum,* an ascomycete, as the "outgroup" is shown in figure 2. It shows the similarity of most isolates to other oomycetes, that in general the isolates collected from nearby locations are most similar and that isolates from India are similar to those from African countries. However, it also shows that neither of the ITS 2 sequences amplified from samples collected in Burkina Faso is from *S. graminicola* and thus serves to emphasize the caution that must be used when PCR products are the basis for measurements of diversity when pure samples are not available.

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Notes

Information for ISMN Contributors

Publishing objectives

The International Sorghum and Millets Newsletter (ISMN) is published annually by the Sorghum Improvement Conference of North America (SICNA) and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). It is intended as a worldwide communication link for all those interested in the research and development of sorghum [*Sorghum bicolor* (L.) Moenchl, pearl millet [*Pennisetum glaucum* (L.) R. Br.J, and minor millets, and their wild relatives. Though the contributions that appear in ISMN are reviewed and edited, it is expected that the work reported will be developed further and formally published in refereed journals.

What to contribute?

- Contributions should be current, scholarly and well justified on the grounds of new information.
- Results of recently concluded experiments, newly released varieties, recent additions to germplasm collections, registration notes for newly developed trait-specific breeding lines/germplasm, etc.
- Genome maps and information on probe-availability and sequences, and populations synthesized for specific traits being mapped.
- Short reports of workshops, conferences, symposia, field days, meetings, tours, surveys, network activities and recently launched or concluded projects.
- Details of recent publications, with full bibliographic information and 'mini reviews' whenever possible.
- Personal news (new appointments, awards, promotions, change of address, etc.).

Deadline for submission: 15 August 2006

How to format contributions

- Keep the items brief up to **6 pages (double-spaced) including data tables and figures.**
- Table should be separated from the text and placed upright (not landscape). Supply only the essential information; round off the data-values to just one place of decimal; use suitable units to keep the values small (eg, tons instead of kg).
- Keep the list of references short - not more than five references, all of which should have been seen in the original by the author. Provide all the details including author/s, year, title of the article, full title of the journal, volume, issue and page numbers (for journal articles), and place of publication and publishers (for books and conference proceedings) for every reference. **Cite references as in this issue.**
- Black-and-white photographs are welcome. Send disk-files whenever you submit line figures and maps.
- Express all quantities only in SI units. Spell out in full every acronym you use.
- Give Latin name of every crop, pest, or pathogen at the first mention.
- Submit one hard copy of the manuscript in the correct format to the Scientific Editor of the respective region at the address given below. Also send the manuscript MS Word file as email attachment.
- Include full address of all authors, and provide telephone, fax and e-mail of the **corresponding** author.

ISMN will carefully consider all submissions and will accept only those that conform to its scientific standard and requirements. The language of the Newsletter is English, but we will do our best to translate articles submitted in other languages. **Authors should closely follow the format and style of the articles in this issue to prepare the manuscripts.**

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About ICRISAT

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) is a nonprofit, non-political organization that does innovative agricultural research and capacity building for sustainable development with a wide array of partners across the globe. ICRISAT's mission is to help empower 600 million poor people to overcome hunger, poverty and a degraded environment in the dry tropics through better agriculture. ICRISAT belongs to the Alliance of Future Harvest Centers of the Consultative Group on International Agricultural Research (CGIAR).

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