Chapter 1.

Sorghum (*Sorghum bicolor*)

*This chapter deals with the biology of sorghum (*Sorghum bicolor*). It contains information for use during the risk/safety regulatory assessment of genetically engineered varieties intended to be grown in the environment (biosafety). It includes elements of taxonomy, morphological characteristics, centre of domestication, current geographic distribution and cultivation practices, reproductive biology, genetics; outcrossing and gene flow, ecology, common pests and pathogens; and biotechnological developments.*

This chapter was prepared by the OECD Working Group on the Harmonisation of Regulatory Oversight in Biotechnology, with South Africa and the United States as the co-lead countries. It was initially issued in June 2016. Updates have been made on production and yield data from FAOSTAT, including Tables 1.2-1.4.

Taxonomy

Classification and nomenclature

The word "sorghum" typically refers to cultivated sorghum (*Sorghum bicolor* [L.] Moench subsp. *bicolor*), a member of the grass family Poaceae, tribe Andropogoneae, and subtribe Sorghinae (Clayton and Renovoize, 1986) that is grown for its grain (grain sorghum), its sugary sap (sweet sorghum) or as a forage (forage sorghum) (Figure 1.1). A variety of common names are used in different regions to refer to cultivated sorghum, including great millet, guinea corn, broomcorn, kaffir corn, durra, mtama, milo, jowar or kaoliang (FAO, 1995).

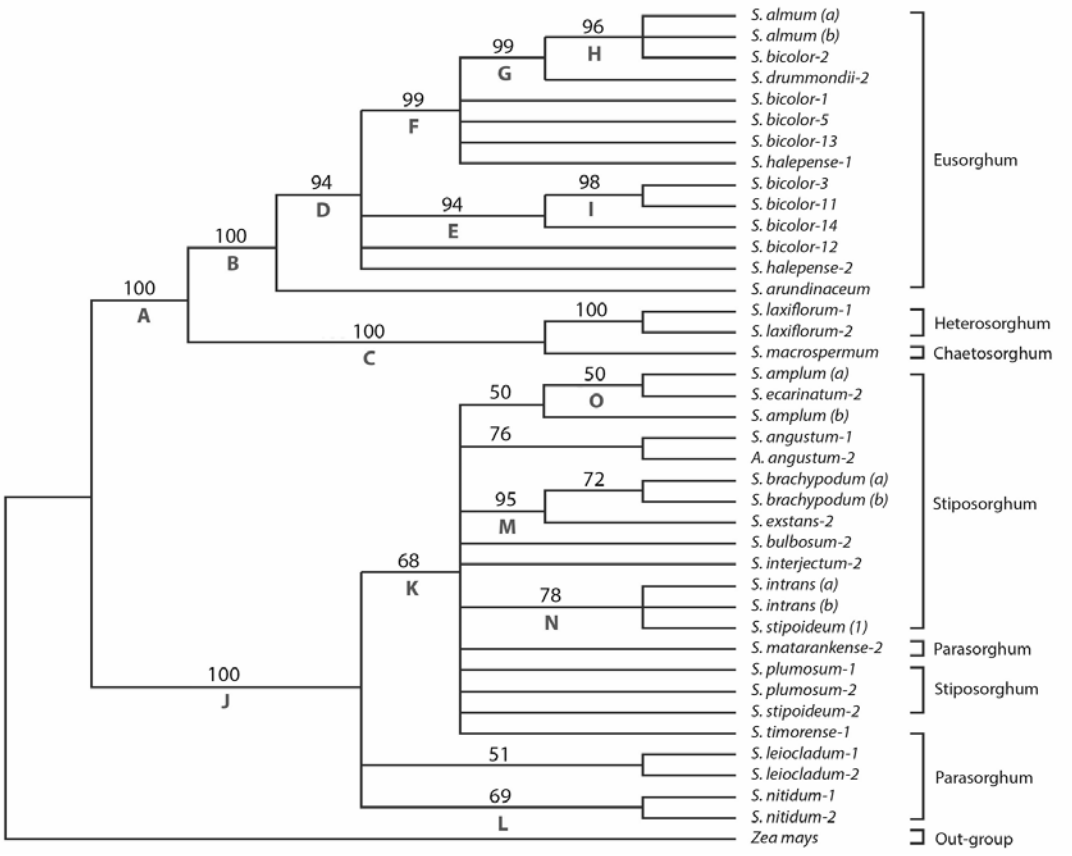
Figure 1.1. **Grain sorghum (upper-left), sweet sorghum (upper-right) and forage sorghum (bottom)**

Source: Upper-left: Daniel Georg Döhne, licensed under CC BY-SA 3.0. Upper-right: Judgefloro, licensed under CC BY-SA 4.0. Bottom: courtesy of Alex Stelzleni, University of Georgia College of Agricultural and Environmental Sciences.

Cultivated sorghum is only one member of the genus sorghum, made up of 25 species (USDA-ARS, 2012) and separated into five taxonomic sections: Chaetosorghum, Heterosorghum, Parasorghum, Stiposorghum and Eusorghum (Garber, 1950). Agronomically important Eusorghum species are listed in Table 1.1 and include cultivated sorghum, its wild progenitor (*Sorghum bicolor* [L.] Moench subsp. *verticilliflorum* [Steud.] de Wet ex Wiersema & J. Dahlb.), Sudan grass (*Sorghum bicolor* nothosubsp. *drummondii* [Steud.] de Wet ex Davidse), and weedy relatives such as Johnsongrass (*Sorghum halepense* [L.] Pers.), shattercane (a feral form of *Sorghum bicolor* nothosubsp. *drummondii* [Steud.] de Wet ex Davidse) and *S. propinquum* (Kunth) Hitchc.

Ng'uni et al. (2010) published a phylogenetic analysis showing the relationships between the taxonomic sections based on four regions of the chloroplast DNA (*trn*Y-*trn*D, *psb*Z-*trn*G, *trn*Y-*psb*M and *trn*T-*trn*L) and the internal transcribed spacer region of the 18S-5·8S-26S nuclear ribosomal DNA from 21 sorghum species. The results are shown in Figure 1.2. Germplasm accessions used in their study include wild sorghum species and several cultivated sorghums obtained from the Australian Tropical Crops Genetic Resource Centre, Biloela, Queensland, Australia; and the Zambian National Plant Genetic Resources Centre.

Figure 1.2. **Phylogenetic analysis of 21 sorghum species based on 4 regions of chloroplast DNA and internal transcribed spacers of nuclear ribosomal DNA**



* Clades are indicated by letters below the branches. Bootstrap values ≥ 50%, indicating the percentage likelihood that subgroups differ, are located above the branches.

Source: Ng'uni et al. (2010).

The division of cultivated sorghum into subspecies and races over the past century has been somewhat archaic, with many competing classifications that are not properly validated. Synonyms are often used even at the species level. One such example is the ongoing use of *Sorghum caffrorum* and *Sorghum vulgare* by agencies throughout the world to indicate *Sorghum bicolor*.

The nomenclature of cultivated sorghum and its wild and weedy relatives was thoroughly reviewed by Wiersema and Dahlberg (2007). Competing names and priorities were considered and three subspecies were validated for *S. bicolor*: *S. bicolor* subsp. *bicolor*, *S. bicolor* subsp. *verticilliflorum* and *S. bicolor* subsp. *drummondii*. *S. bicolor* subsp. *bicolor* comprises the cultivated sorghums; *S. bicolor* subsp. *verticilliflorum* comprises annual wild relatives of cultivated sorghum native to Africa, Madagascar, the Mascarenes, and introduced varieties to India, Australia and the Americas; *S.* subsp. *drummondii* comprises annual weedy derivatives arising from hybridisation of cultivated sorghum and *S. bicolor* subsp. *verticilliflorum*. A complete listing of the names of all known subspecies plus homotypic species names is provided in Wiersema and Dahlberg (2007).

Section Eusorghum also includes the rhizomatous taxa Johnsongrass and *S. propinquum* (de Wet, 1978). Although Johnsongrass is native to southern Eurasia and India, its introduction to temperate regions and introgression with cultivated sorghums has caused it to become a troublesome weed (de Wet, 1978). *S. propinquum* is generally restricted to Sri Lanka, southern India, and Burma east toward Southeast Asia (de Wet, 1978; Doggett, 1988). By natural crossing with cultivated sorghums in the Philippines, *S. propinquum* has also become a geographically isolated noxious weed (de Wet, 1978).

Of the 25 recognised species of *Sorghum*, 17 are native to Australia and Southeast Asia, of which 14 are endemic to Australia (Lazarides et al., 1991). Basic chromosome numbers vary from 10-40, and in some cases, such as within *S. timorense* (Kunth) Buse, there are multiple ploidy levels. These species are not within the Eusorghum section and previously were regarded as sufficiently distant to be sexually incompatible with cultivated sorghum. Recent studies have demonstrated that *S. bicolor* × *S. macrospermum* crosses are not only possible (Price et al., 2005), but that there is significant genomic introgression of the wild germplasm into the cultivated species after backcrossing the hybrids (Price et al., 2005; Kuhlman et al., 2010).

Description

Cultivated sorghum is a cane-like grass with diverse morphology (NRC, 2004). Plant height ranges from 0.5 metres (m) to 6 m. Culms (stalks) are erect and range from slender to stout. Tillers (adventitious stems originating from the plant base) can range in quantity from none to profuse. Leaf blades vary from linear to lanceolate, and can be smooth or hairy, measuring up to 100 centimetres (cm) long and 10 cm wide with smooth to thinly pilose sheaths. The inflorescence consists of a single panicle with many racemes. Panicles may be either compact or open up to 50 cm long and 30 cm wide; panicle branches are stiffly ascending or spreading and pendulous, with the bottom branch being almost half as long as the panicle. At maturity, racemes have one to eight nodes and can be either fragile or tough. Spikelets may be glabrous or hirsute, elliptic to obovate, and up to 6 mm long. Glumes (bracts) range from leathery to membranous, often with winged keels. Lower lemmas are approximately 6 mm long while upper lemmas are slightly shorter and often awned. Both upper and lower lemmas of sessile spikelets are somewhat ciliate and translucent (Doggett, 1988).

For many years, sorghum breeders have classified cultivated sorghum into races (Snowden, 1936) or working groups (Murty and Govil, 1967) according to morphological characteristics. De Wet et al. (1970) described the various groups of cultivated sorghum and identified their historical geographic distribution. A system was then developed dividing cultivated sorghum into five basic interfertile races (Bicolor, Kafir, Caudatum, Durra and Guinea) and ten intermediate races, based on floral morphology (Harlan and de Wet, 1972). This classification system was widely adopted. An integrated classification of cultivated sorghum was proposed by Dahlberg (2000) following the morphological guidelines outlined above and simplifies their classification systems by presenting working groups numerically. Brown et al. (2011) and Morris et al. (2013) provide molecular support for classification of the races. A more detailed description of the characteristics of each of the five main races of cultivated sorghum can be found in Table 1.1. Diagrams of spikelet and head types of the races are in Figure 1.3.

Table 1.1. **Cultivated sorghum race characteristics**

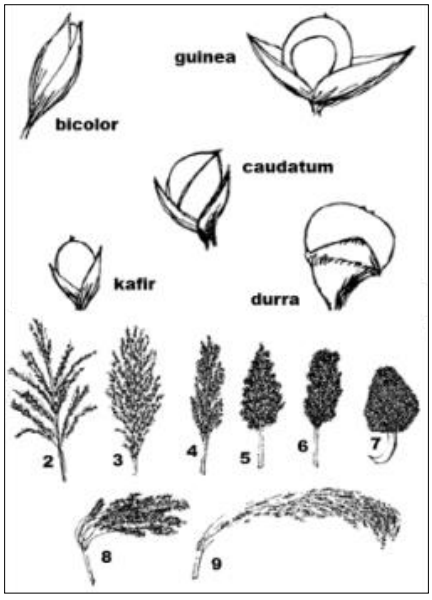
Race	Distinct characteristics
Bicolor	– Open inflorescences with pendulous branches – Long, clasping glumes – Elliptic grain
Kafir	– Moderately compact, cylindrical inflorescences – Elliptic spikelets – Tightly clasping, long glumes
Caudatum	– Compact to open inflorescences – Grains with one side flat, opposite side curved – Shorter glumes that expose grains
Durra	– Compact inflorescences – Flat, ovate shaped sessile spikelets – Middle-creased lower glume – Distinct texture on tip of lower glume
Guinea	– Large, open inflorescences with pendulous branches – Long, separated glumes that expose grains – Obliquely twisted grains

Source: Doggett (1988).

Sorghum grain is a staple food for millions of people in the semiarid regions of Africa and Asia where it is used to make food products such as tortillas, breads, cakes, noodles, couscous, beer and porridge (Rooney and Waniska, 2000). Sweet sorghum sap can be processed into sweeteners for the food industry or fermented into ethanol. Nearly all sorghum production (97%) in the western hemisphere is for livestock feed and forage because it is a lower cost alternative to maize and requires less water to grow (Hancock, 2000). Developing countries also use sorghum plant products for cooking fuel, construction materials, leather dyes and as physical support for vining crops like yams (NRC, 2004).

Cultivated sorghum ranks fifth in worldwide cereal crop production behind maize, rice, wheat and barley (FAOSTAT, 2017). It is a widely adapted species capable of growing in semiarid, subtropical, tropical and temperate climates. An extensive root system and the ability to become dormant during water stress make cultivated sorghum drought-resistant (Whiteman and Wilson, 1965), typically requiring only one-half to two-thirds the amount of rainfall as maize (Hancock, 2000). Plants are primarily self-pollinated, but some wind pollination occurs. Cultivated sorghum is physiologically a perennial that is typically grown as an annual. In some environments a second ratoon (resprouted) crop is produced from the unharvested roots and stolons of the first crop.

Figure 1.3. **Spikelet types of the five races of cultivated sorghum and their associated head types**



* Head type 1 (not shown) is reserved for wild races and is more diffuse than Type 2. Types 2, 3 and 4 have Bicolor and Guinea spikelets; Types 5, 6 and 7 have Kafir and Durra spikelets; many head types have Caudatum spikelets; and Broomcorn (Type 9) has Bicolor spikelets. Type 8 spikelets were not specified by the authors.

Source: Harlan and de Wet (1972).

In 2014, approximately 68.9 million tonnes (mln t) of sorghum was produced on almost 45 million hectares in 112 countries (FAOSTAT, 2017). In the same year, the leading sorghum-producing countries included the United States (11.0 mln t), Mexico (8.4 mln t), Nigeria (6.7 mln t), Sudan (8.4 mln t) and India (5.4 mln t) (Table 1.2). Africa is the world regional leader in total production of sorghum at 29.2 mln t (Table 1.3). Although Africa leads in total production, its average yield per hectare is the lowest, at 994 kg ha^{-1} (Table 1.4). This disparity may be attributed to the relative prevalence of subsistence agriculture in Africa as opposed to other regions.

Table 1.2. **Leading sorghum grain-producing countries, 2014**

Country	Total production (10^6 t)
United States	11.0
Mexico	8.4
Nigeria	6.7
Sudan	6.3
India	5.4
Ethiopia	4.3
Argentina	3.5
China (People's Republic of)	2.9
Brazil	2.3
Burkina Faso	1.7
Niger	1.4
Australia	1.3

Source: FAOSTAT (2017).

Table 1.3. **Sorghum grain production by region, 2014**

Region	Total production (10^6 t)
Africa	29.2
North America	11.0
Asia	9.7
Central America	8.8
South America	7.5
Europe	1.4
Oceania	1.3

Source: FAOSTAT (2017).

Table 1.4. **Average sorghum grain yield by region, 2014**

Region	Average yield (kg/ha^{-1})
Europe	3 525
North America	4 242
Central America	3 954
South America	3 308
Oceania	2 413
Asia	1 298
Africa	994
World average	1 533

Note: kg/ha^{-1} = kilograms per hectare.

Source: FAOSTAT (2017).

Geographic distribution, domestication and cultivation

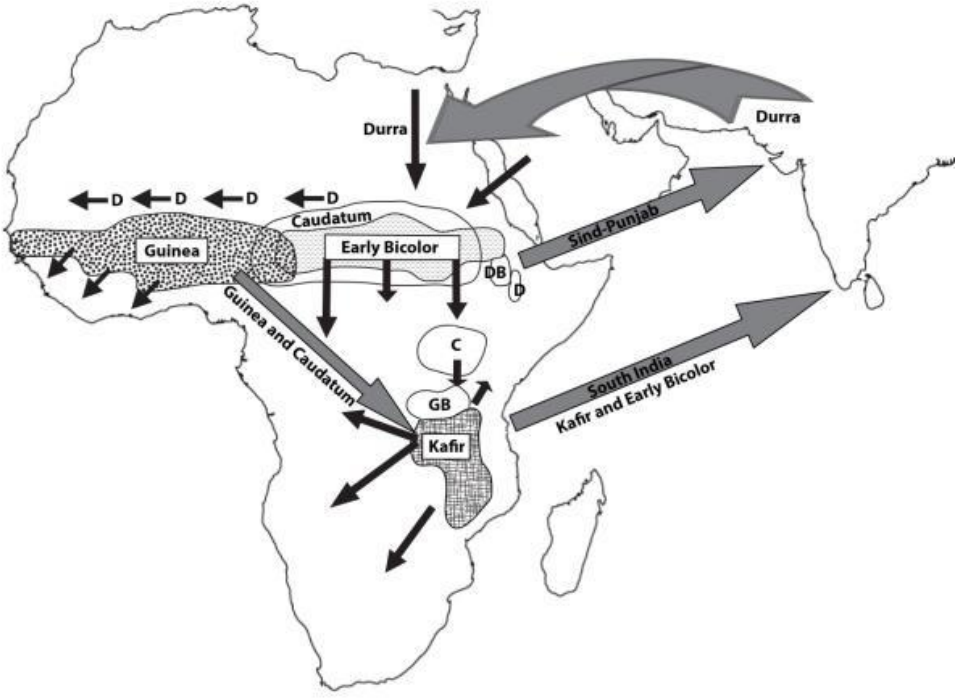
Centre of domestication and ancient geographic distribution

Sorghum's centre of domestication is likely the Ethiopia-Sudan region in north-east Africa because the greatest plant diversity and variation in ecological habitats occurs there (Doggett, 1988). Archaeological evidence suggests sorghum was originally cultivated around 5000 B.P. (Krzyzaniak, 1978). Studies comparing the morphology of ancient and modern grain (Dahlberg and Wasylikowa, 1996) and data from molecular markers (Deu et al., 1995) agree that the different races be classified as the same biological species. It is possible that a single domestication event occurred and that the various races were derived from it. Alternatively, multiple domestication events may have occurred, leading to the development of different races that subsequently anastomosed into the current, extant *S. bicolor* lineage. Regardless, distinct cytoplasmic markers have been identified in race Guinea (Deu et al., 1995), including alleles specific to the margaritiferum subrace (Deu et al., 2006; Figueiredo et al., 2008), whose grain is cooked and eaten like rice.

Following domestication in east Africa, humans moved cultivated sorghum across much of sub-Saharan Africa. The germplasm was diversified through selection and introgression with sympatric wild populations according to the needs of different ecological conditions and desired crop uses (Doggett, 1988). Grain size and the ability to withstand dry or wet conditions became important selection criteria leading to diversity within the germplasm. For example, race Guinea was bred for grain production in wetter conditions with open panicles that would prevent seed moulding. Conversely, race Durra was adapted to drier conditions by developing more compact panicles as humans

expanded the crop into the southern Sahara (Doggett, 1988). Smith and Frederiksen (2000) illustrated the movement and domestication of sorghum races in Figure 1.4.

Figure 1.4. **Origins and movements of the five races of *S. bicolor***



Source: Smith and Frederiksen (2000), Harlan and Stemler (1976), Harlan (1976), Doggett (1988), and Ehret (1988).

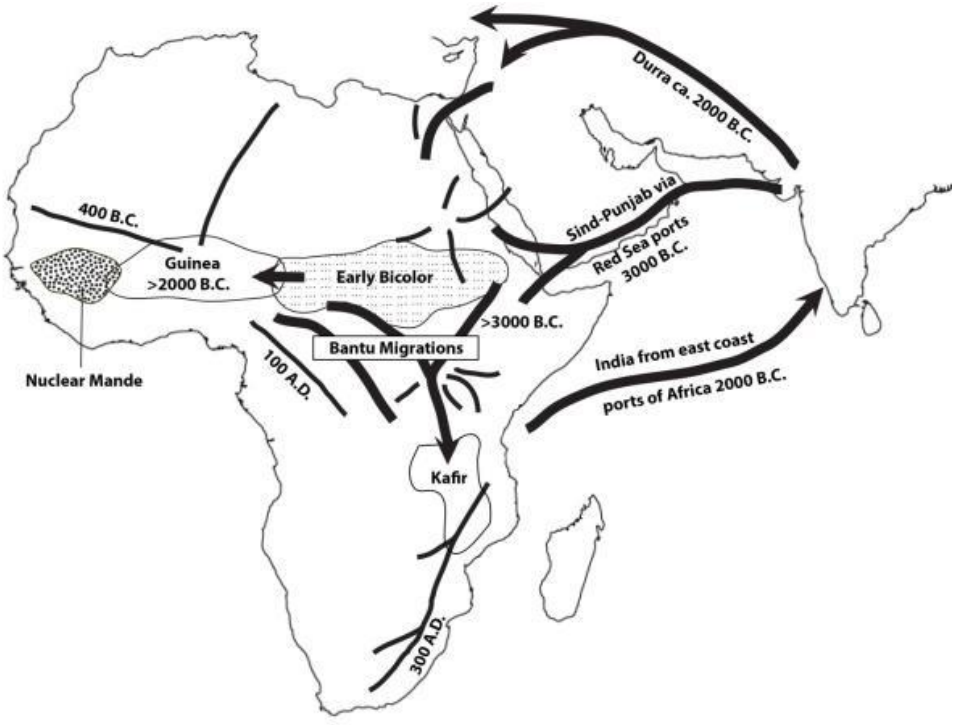
Cultivated sorghum was transported from Africa to India via trade routes over the Arabian Peninsula and the Indian Ocean (Figure 1.5). Durra varieties began emerging in India as the crop was adapted to the environmental conditions and needs of people. The earliest archaeological evidence of domesticated sorghum in India is dated around 4000 B.P. (Kimber, 2000). Domesticated sorghum continued to be spread from India to the People's Republic of China (hereafter "China") along overland trade routes. In China, the crop was adapted to tolerate temperate conditions and varieties known as the Kaoliangs were developed that are tolerant of cooler early season temperatures (Doggett, 1988). Sorghum came from Africa to America relatively recently through the slave trade. In the United States, the crop has been bred for commercial purposes since its introduction, resulting in the development of dwarf hybrids which are easier to cultivate on a commercial scale (Smith and Frederiksen, 2000).

Contemporary geographic distribution and methods of cultivation

Sorghum's adaptability to a range of environmental conditions allows it to be cultivated in multiple regions around the world with substantially varied climates (Figure 1.6). There are currently two main belts of cultivation in Africa. The northern belt ranges from the Ivory Coast north to the Sahara, and east towards Sudan and Ethiopia. The races Bicolor, Durra, Guinea and Caudatum are primarily grown in this belt. The second African sorghum belt runs north to south from Ethiopia to South Africa. Races grown

include Kafir, Bicolor and Caudatum. In India, sorghum is mainly cultivated on the Deccan Plateau, with only minor production in northern India. Sorghum is produced throughout China but the core of production is in the northern region, especially the areas north of the Qinling Mountains, and between the Yellow and Yangtze Rivers (de Wet et al., 1970; House, 1985; House et al., 2000).

Figure 1.5. **Early human migrations and associated diffusion of *S. bicolor* races**



* Wide arrows, postulated early sorghum routes; narrow arrows, diffusion of iron-making technologies; dots, earliest centres of iron making.

Source: Smith and Frederiksen (2000); Murdock (1959); Harlan et al. (1976); Doggett (1988); and Shillington (1989).

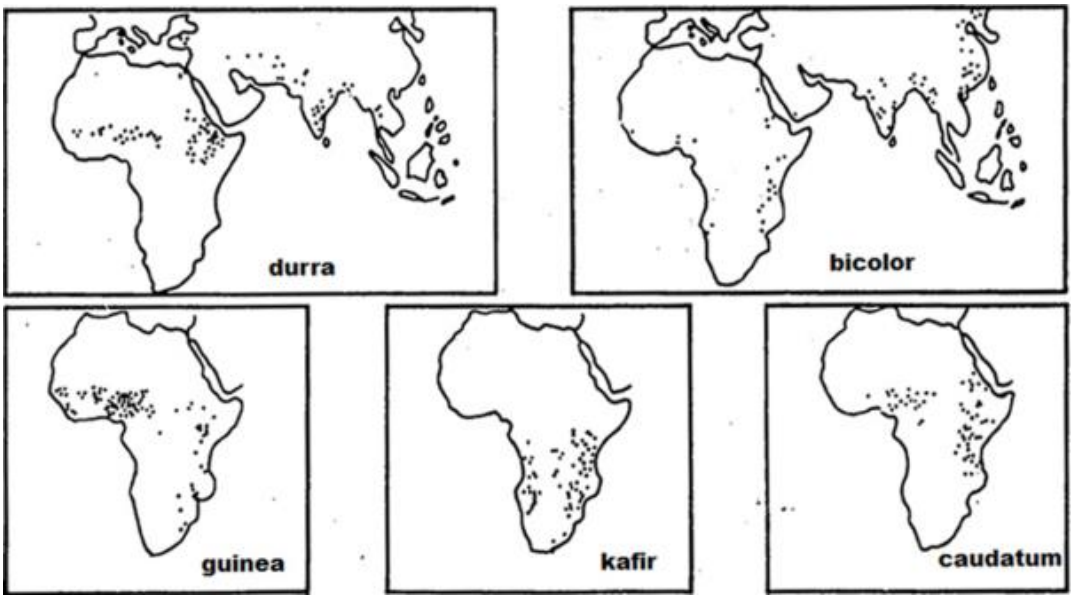
Regardless of where it is grown, required annual rainfall ranges from 400 mm to 750 mm, making it an important crop for areas too dry for maize. Although primarily known for its drought resistance, cultivated sorghum can also withstand temporary water logging. Altitudinal range is from sea level to 3 000 m, while latitudinal range is from 50°N in Germany to 40°S in Argentina. Favourable temperatures range from 10°C to 35°C for germination, with 30°C being optimal. Plant breeding has developed some cultivars to grow in lower temperatures. Tolerated soil types vary widely from heavy or cracked clay to light or deep sand. Soil acidity may range from pH 5.0 to 8.5. Cultivated sorghum possesses some tolerance to soil salinity.

Argentina, Brazil, Bolivia, Uruguay and the Bolivarian Republic of Venezuela lead sorghum production in South America. Cultivation in Central America is located in parts of Mexico, El Salvador, Nicaragua, Honduras, Guatemala, Belize and Panama (FAOSTAT, 2017). In the United States, an area extending from South Dakota to Texas and from Colorado to Mississippi is the primary sorghum-producing region (USDA-NASS, 2012). Most of Europe's sorghum production occurs in France, Italy, Ukraine and the

Russian Federation (FAOTAT, 2017). More than 95% of Australia's sorghum cultivation takes place in the states of Queensland and northern New South Wales (ABS, 2012).

Sorghum is a highly domesticated crop plant that does not generally survive outside of cultivation; however, its weedy relatives do survive in the wild. These weeds are feral in road ditches, stream banks, field margins or abandoned areas. Sorghum's progenitor, S. *bicolor* subsp. verticilliflorum, originated in Africa but has been introduced wherever sorghum is cultivated and occurs alongside the crop as an annual weed (Doggett, 1988). Johnsongrass has become a noxious weed since being naturalised in temperate regions. It is especially difficult to control due to its rhizomes (Warwick et al., 1984). Shattercane is a weed wherever it is sympatric with cultivated sorghum, and is a prolific self-sower due to its shattering panicle which facilitates seed dispersal (Doggett, 1988; Dahlberg, 2000; Hoffman and Buhler, 2002).

Figure 1.6. **Historical geographic distribution of the five basic races of cultivated sorghum in Africa, India and China**



Source: House (1985) and de Wet et al. (1970).

Cultivation and management practices of sorghum differ by region, particularly between the commercial-scale production in Australia, Europe and the United States and the smaller-scale production in Africa and Asia. In order to optimise yields, commercial producers consider soil quality, moisture content, nutrient availability and pest prevalence when developing plans for crop management. Most commercial cropping system strategies involve crop rotation for soil conservation or to reduce weed, pest and disease pressure (Cothren et al., 2000).

Land preparation in commercial-scale operations begins immediately following harvest by shredding the stalks of the harvested sorghum and disking or plowing the land. Mixing the soil and stalk residue aids decomposition, improves soil nutrient and water content, and reduces erosion. The land is then bedded and controlled for weeds through chemical herbicides, rotary hoeing or bed reshaping (Cothren et al., 2000). Insects may be controlled through insecticides, biological predators or cultural methods such as crop

rotation, variety selection and seed treatment (Teetes and Pendleton, 2000). Planting of the selected hybrid or cultivar is mechanised and the rows are evenly spaced, with spacing between rows determined by the moisture content (optimal yield requires adequate moisture, and if planting density is too high, there may not be enough moisture for each plant). Recommended plant populations range from 30 000 plants ha^{-1} in parts of Australia with marginal rainfall to 250 000 plants ha^{-1} under irrigation in the United States. Irrigation may be used to achieve higher yield despite sorghum's water efficiency characteristics. To achieve proper soil nutrient values, fertilisers are applied at planting and sometimes later as a broadcast application (Cothren et al., 2000).

The methods of subsistence food production in Africa and Asia vary according to climate, degree of mechanization, availability of improved varieties and fertilisers, methods of pest control, size of land holdings, moisture, and soil type. Land holdings are typically smaller with limited mechanization (House et al., 2000). Land is prepared by hand hoe, animal-drawn equipment, disks or plows. Planting is done by hand by placing several seeds in each hole to help plants emerge through the soil crust and resist wind. Row spacing is farther apart in relatively drier areas and closer together where more moisture is expected (House et al., 2000). In temperate climates, planting occurs in the spring or summer, and sowing early can help protect against pervasive pests like shoot flies or stem borers. In tropical climates planting is done in the wet season such that moisture is abundant during plant growth and restricted during grain harvest (Doggett, 1988; House et al., 2000). In some tropical climates, two cropping seasons are possible, depending on precipitation patterns. The second crop is seeded or ratooned. Manure is the primary fertiliser used, although phosphorous and potassium solutions are applied if available. Weed control is performed by hand with tools specialised for different soil types. Farmers usually do not have access to chemicals to control insects, weeds or diseases, due to cost and/or lack of availability (House et al., 2000). Few opportunities exist for surface irrigation.

Reproductive biology

Generation time and photoperiodism

A broad range of generation times, associated with high levels of genetic variance, has been introduced among cultivated sorghums through adaptation to different climates and cropping systems. The lifecycle is typically 90-120 days post-germination. Flowering begins at 60-90 days (Quinby, 1974) and lasts about a week (Stephens and Quinby, 1934). Photoperiod and temperature sensitivity play major roles in determining the time between sowing and panicle initiation for adapted genotypes. Thus, generation times may be considerably longer in parts of Africa and Asia where tall photoperiod-sensitive landraces are cultivated.

Photoperiod-insensitive cultivated sorghum hybrids are typically used in commercial agriculture to boost production through more consistent growth durations. Such systems include cultivars tailored to a particular duration of growing season, pest pressure and response to inputs like fertilisers, pesticides and opportunities for irrigation (Doggett, 1988; Folliard et al., 2004; Kouressy et al., 2008). However, photoperiod sensitivity is a crucial environmental adaptation for subsistence farmers in tropical climates who must wait until precipitation has softened the ground before land preparation can begin. This makes the time of sowing inconsistent and potentially spread out over several weeks. However, regardless of when seeds were sown, once the appropriate photoperiod threshold has been reached, floral initiation begins uniformly, coinciding with the most favourable time for seed production in varieties adapted to that climate (Doggett, 1988).

In West Africa, floral initiation occurs at the end of the rainy season, thus minimising the potential for damage caused by grain mould, insects or birds in early-maturing varieties; and by water shortage in late-maturing ones (Folliard et al., 2004; Kouressy et al., 2008). In the Ethiopian highlands, for example, the crop has been bred to grow through two wet seasons, with anthesis as late as 160-180 days (Shewayrga et al., 2008).

Plant growth (the vegetative phase) occurs primarily under decreasing day length, and floral development (the reproductive phase) typically requires photoperiod to fall below a specific day length to initiate (Folliard et al., 2004), although this threshold varies widely. Many traditional varieties require 12 or more hours of darkness to initiate flowering. Ellis et al. (1997) identified three developmental stages of sorghum undergone during the vegetative phase, and differentiated according to photoperiod and temperature responses. The first stage is photoperiod-insensitive, with a temperature-sensitive response that will delay floral initiation if the temperatures are too hot or too cold (optimum temperature is around 30°C). The second stage features a photoperiod-sensitive, temperature-insensitive response period where the stalk and leaves develop. The third stage occurs immediately prior to the reproductive phase and is insensitive to both photoperiod and temperature. The reproductive phase begins with panicle initiation, followed approximately 21 days later by anthesis (pollen shed) (Doggett, 1988). Grain development begins after fertilisation and maximum dry weight is reached approximately 25-55 days after anthesis. Harvest is often conducted 10-20 days after this point to reduce moisture content (Doggett, 1988).

Cultivated sorghum is typically produced as an annual crop with one generation per growing season, but some types are non-senescent and often associated with substantial seed production from tillers. In semi-arid areas of Australia and India, the "staygreen" trait has been introduced to improve post-anthesis drought tolerance (Borrell et al., 2000). Plants bearing this trait may be ratooned provided the climate and soil fertility are favourable. Harvests from the ratoon crop may be more substantial than harvests from the seeded crop in some areas. In Uganda, for example, multiple ratoons may be harvested from the same plants due to two rainy seasons each year (Downes, 1968; Escalada and Plucknett, 1975; Doggett, 1988).

Reproductive biology

Floral morphology and pollination

The many racemes and spikelets that compose the panicle develop about 21 days after the onset of the reproductive phase. A single panicle may bear 6 000 florets in total (Karper and Quinby, 1947). Spikelets along the racemes occur in sessile and pedicelled pairs. The sessile spikelets are fertile while the pedicelled spikelets are staminate and are formed on a short pedicel. The glumes of the sessile spikelets encase two florets. While the lower floret is sterile and consists of a lemma only, the upper floret is perfect, consisting of a lemma, two lodicules flanking the lemma, three stamens and a single-celled ovary with two plumose stigmas (Doggett, 1988).

Anthesis can begin as soon as the panicle begins to emerge from the culm, but usually begins several days later after the peduncle has reached its maximum growth. The first flower to bloom is the uppermost flower, and blooming continues down the panicle in a regular pattern. Sessile spikelets bloom first with pedicelled spikelets blooming two to four days later. Temperature, size of panicle and variety are the primary factors that determine duration of flowering, which is typically about one week, but varies from 2 to

15 days (Stephens and Quinby, 1934; Quinby, 1974). Reports in the literature on bloom time over a 24-hour cycle are inconsistent. Doggett (1988) stated that the time of blooming is affected by darkness and temperature, but generally occurs between 10 pm and 8 am. This is supported by observations in hot and dry Bellary, Karnataka, India (Ramanathan, 1924); Chillicothe, Texas (Stephens and Quinby, 1934); and at other subtropical locations (Ball, 1910; Robbins, 1917; Nafziger, 1918; Vinall, 1926). However, cool nights with heavy dews in more temperate latitudes are associated with delayed flowering, from 8 am to 4 pm (Graham, 1916; Patel and Patel, 1928; Ramanathan, 1924; Ayyangar and Rao, 1931). The flower opens in about ten minutes, allowing the stigmas and anthers to emerge. Pollen may dehisce from the anthers immediately upon emergence or may delay shortly depending upon environmental conditions. The time between the opening and closing of the glumes ranges from half an hour to four hours, but averages about two hours (Doggett, 1988).

Cultivated sorghum panicles may produce up to 24 million grains of pollen (Karper and Quinby, 1947), which is sensitive to desiccation (Lansac et al., 1994) and remains viable for only three to six hours (Stephens and Quinby, 1934; Doggett, 1988). However, in one study, pollen kept at 4°C and 75% relative humidity remained viable for 94 hours, and pollen stored in pollination bags in the shade in the field in Davis, California remained viable for over 20 hours (Sanchez and Smeltzer, 1965). While stigmas can be receptive two days before and up to a week after flowering, the optimal timeframe for pollination is within the first 72 hours (Ross and Webster, 1957; Doggett, 1988). Pollen germinates immediately upon reaching a receptive stigma and fertilisation of the egg cell occurs about two hours later to initiate seed development.

Cultivated sorghum is primarily self-pollinating; however, wind-mediated cross-pollination does occur. Schmidt and Bothma (2005) suggested that insect pollination may also occur, based upon their observations of honey bees, wild bees (sometimes known as solitary bees) and one species of beetle visiting several sorghum flowers consecutively. Upon collection of the insects, pollen grains identical to the grains collected from the sorghum anthers were found on all of the insects, with the honey bee carrying the most and the beetle carrying the least. However, no attempt was made to determine if insect movement resulted in cross-pollination.

Nunes-Silva et al. (2010) reported that the flower fly *Toxomerus politus* (Say) visits cultivated sorghum flowers to feed on pollen during the time that stigmas are receptive. The authors suggested that the flies may also contribute to pollination, but only in a minor way, since the relationship of *T. politus* with cultivated sorghum was similar to mutualisms between pollinators and their host plants wherein the larvae of pollinators also consume the reproductive organs of the host plants. Thus, the observation of insects associated with pollen is not necessarily indicative of the insect's efficiency as a pollinator.

More studies are called for to determine the extent of insect pollination in cultivated sorghum. Further information on outcrossing and gene flow can be found in the following section.

Seed dormancy, dispersal and viability of cultivated and weedy sorghum species

Pre-harvest sprouting due to low seed dormancy is an important challenge in cultivated sorghum, especially when grain maturation occurs under high humidity and rainfall conditions (Maiti et al., 1985); however, considerable variability in the level of seed dormancy exists among varieties (Lijavetzky et al., 2000; Rodríguez et al., 2012; Steinbach et al., 1995). In general, seed dormancy release is under the control of the

hormones abscisic acid (ABA) and gibberellin (GA) and varies greatly in how it is regulated within plant tissues (Finch-Savage and Leubner-Metzger, 2006). In a study of two inbred lines, Rodríguez et al. (2012) found that low seed dormancy was associated with a loss of embryo sensitivity to ABA and greater accumulation of GA, whereas greater seed dormancy was associated with increased embryo sensitivity to ABA and suppression of GA synthesis genes. Currently more than 130 forms of GA have been identified in plants and fungi. Thus, variations in genes affecting different GAs or their associated metabolic pathways, and the overall regulation of these pathways, may account for wide variations in pre-harvest sprouting susceptibility both between and within species. Furthermore, since ABA and GAs are associated with many physiological and developmental features of plants, including environmental sensing (de Lucas et al., 2008), breeding for reduced pre-harvest sprouting is challenging due to its polygenic nature and the hormones' systematic and environmentally modified effects within the plant.

The seed of cultivated sorghum does not shatter and must be transported by wind, water, animals or humans (Andersson and de Vicente, 2010). Seed viability was evaluated for 36 483 germplasm accessions stored under controlled conditions at the International Crops Research Institute for the Semi-Arid Tropics (Sastry et al., 2008). The reported storage period, which ranged from 5 to 21 years, demonstrated a greater than 85% viability, meeting the minimum standard for conservation in international gene banks (FAO, 2014). By contrast, in Sudan where there are few opportunities for controlled seed storage conditions, Ahmed and Alama (2010) reported viability under 85% after only one or two years in a brick warehouse, a corrugated iron warehouse or an underground pit. Evans et al. (1961) studied germination in ten cultivated sorghum genotypes and emphasised the significance of interactions among genotypes, soil moisture and germination temperature in modifying germination outcomes.

Shattercane, as its name implies, is able to disperse its seeds through shattering of the panicles. Its persistence in the seed bank is due to seed dormancy and seed longevity mechanisms (Burnside et al., 1977; Fellows and Roeth, 1992; Kegode and Pearce, 1998). Reports regarding the longevity of shattercane seeds in the soil range from 2 years (Teo-Sherrell and Mortensen, 2000) to 13 years (Burnside et al., 1977). Cold, wet soil conditions contributed to the two-year longevity estimate, such that 80% of seeds died in the first winter and virtually none survived the second (Teo-Sherrell and Mortensen, 2000). However, the authors point out that even a few survivors may be enough to ensure persistence of shattercane in the field due to its high rate of seed production.

Johnsongrass produces large numbers of shattering seeds that also may be carried by the wind, on animals or may remain dormant in the soil for up to 30 months (Holm et al., 1977) with some variability in the level of dormancy (Taylorson and McWhorter, 1969; Ghersa et al., 1992). Its seed does not survive as long at shallow soil depths, but large seed banks can be accumulated in the upper layer of soil by frequent seed input each year. Johnsongrass seeds are much more adapted to survival at depths greater than 22 cm, meaning persistent seed banks can accumulate when they are sufficiently buried (Andersson and de Vicente, 2010). While Johnsongrass primarily reproduces through seed, its invasiveness is also due to its rhizomes (Holm et al., 1977).

Genetics

Considerations for plant breeders

Gene pools

Cultivated sorghum is a genetically diverse diploid (2n = 2x = 20) with 200 classified phenotypic, genotypic and cytogenetic trait genes (Rooney, 2000). It is sexually compatible with some of its wild or weedy relatives, and the level of cross-compatibility determines its primary and secondary gene pools. The primary gene pool lies within section Eusorghum and includes the other diploid species *S. propinquum*, S. *bicolor* subsp. verticilliflorum, and shattercane. Crosses within this gene pool are fully interfertile. The high level of fertility and spontaneous outcrossing of the primary gene pool leads to frequent introgression when distributions overlap and conditions are favourable (Doggett and Majisu, 1968; Baker, 1972; Ejeta and Grenier, 2005).

The secondary gene pool consists of the tetraploid (2n = 4x = 40) members of Eusorghum: Columbus grass (*Sorghum almum* Parodi) and Johnsongrass. Domesticated sorghum is capable of outcrossing with members of the secondary gene pool despite ploidy level differences, producing either sterile triploids or somewhat fertile tetraploids (Arriola and Ellstrand, 1997, 1996; Morrell et al., 2005).

The tertiary gene pool includes species from other sections of sorghum. Outcrossing of cultivated sorghum with members of this gene pool is highly unlikely under natural conditions, and crosses produced through human intervention are anomalous, lethal or almost completely sterile (Ejeta and Grenier, 2005). However, crosses have been made with some of the Australian native sorghum species under controlled conditions using embryo rescue (Price et al., 2005).

The cultivated sorghum genome has been sequenced (Paterson et al., 2009). The haploid genome size is approximately 730 Mega base pairs (Mbp), larger than both *Arabidopsis* and rice (155 Mbp and 510 Mbp, respectively). However, these three plants have similar numbers of gene families. Molecular analysis of the genus has identified relatives of the species with novel traits, endosperm structure and composition that may be used to expand upon its currently known gene pool (Dillon et al., 2007).

Two traits, maturity and male sterility, are considered the most relevant when considering management of gene flow to wild or weedy relatives within cultivated sorghum's gene pools and vice versa. First, genes controlling maturity and their nuanced interaction with day length and temperature are critical for the timing of floral initiation in cultivated sorghum, and, consequently, reproductive success. Second, genes affecting male sterility can significantly modify the ability to cross-pollinate. Further examination of these traits and the potential for gene flow is given below. However, it is important to note that both traits are subject to modification by extremely high temperatures or drought such that flowering or early flowering may still occur under unusual circumstances in otherwise non- or late-flowering backgrounds, respectively; and self-pollination and seed set may occur in otherwise male-sterile backgrounds.

Traits affecting maturity

Time-to-maturity traits are polygenic with the Ma1, Ma2, Ma3 and Ma4 maturity loci containing 13, 13, 16 and 12 alleles, respectively, thus modulating a wide range of floral initiation dates (Rooney, 2000). Maturity is subject to significant genotype × environment (G × E) interactions. Numerous studies have reported a complex relationship between

maturity genotype, day length and temperature (Quinby, 1967; Miller et al., 1968; Caddel and Weibel, 1971; Hammer et al., 1989; Craufurd et al., 1999; Tarumoto, 2011; Tarumoto et al., 2003).

Quinby (1967) focused on 11 varieties of cultivated sorghum and their days to flowering according to genotype (Table 1.5), revealing substantial information about the role of each locus in determining the time from germination to flowering. Specifically, the use of various combinations of alleles may extend the time to flowering over long periods within a single environment.

Day length is a critical factor in the expression of maturity genes. Lane (1963) observed four varieties of cultivated sorghum under both 10-hour (short) and 14-hour (long) days (Table 1.6). "SM90" and "60M" are considered temperate varieties, while "80M" and "100M" are considered tropical varieties. The short day length hastened floral initiation in all varieties; however, the photoperiod-sensitive tropical varieties exhibited more delayed floral initiation than the less photoperiod-sensitive temperate varieties (Lane, 1963). The critical day length required to cause a delay in floral initiation was an additional indicator in determining how influential photoperiod is on floral initiation. For example, a difference of only one hour of day length between "SM90" and "100M" resulted in a dramatic delay of over 30 days to floral initiation (Lane, 1963).

Table 1.5. **Genotypes and time to flowering among 11 cultivated sorghum varieties in Plainview, Texas (United States), 1964**

Variety	Genotype	Time to flowering (days)
"100-day Milo (100M)"	Ma1Ma2Ma3Ma4	90
"90-day Milo (90M)"	Ma1Ma2ma3Ma4	82
"80-day Milo (80M)"	Ma1ma2Ma3Ma4	68
"60-day Milo (60M)"	Ma1ma2ma3Ma4	64
"Sooner Milo (SM100)"	ma1Ma2Ma3Ma4	56
"Sooner Milo (SM90)"	ma1Ma2ma3Ma4	56
"Sooner Milo (SM80)"	ma1ma2Ma3Ma4	60
"Sooner Milo (SM60)"	ma1ma2ma3Ma4	58
"44-day Milo (44M)"	Ma1ma2ma3 RMa4	48
"38-day Milo (38M)"	ma1ma2ma3 RMa4	44
"Hegari (H)"	Ma1Ma2Ma3ma4	70

Source: Quinby (1967).

Table 1.6. **Influence of photoperiod on four cultivated sorghum varieties**

Variety	Critical day length (hours)	Time to flowering (days)	
		10-hour days	14-hour days
"Sooner Milo" (SM90)	13.0	19	35
"60-day Milo" (60M)	12.5	19	38
"80-day Milo" (80M)	12.5	19	44
"100-day Milo" (100M)	12.0	19	70

Source: Lane (1963).

The influence of temperature on maturity can be observed when cultivated sorghum is grown at the same latitude to control for day length but at different elevations, where high elevation is associated with lower night-time temperatures. Quinby (1967) evaluated five varieties grown at both Chillicothe, Texas and Plainview, Texas. The Plainview location

was about 500 metres above the Chillicothe site, corresponding to approximately 2°C lower at night. The observed differences in time to flowering varied among varieties: three varieties were hastened, one was delayed and one hardly changed at the Plainview site (Table 1.7). These deviations indicate a maturity genotype × temperature interaction.

In addition to temperature and day length effects, specific combinations of alleles are used to delay or prevent the onset of the reproductive phase in temperate growing regions. For example, use of the Ma5/Ma6 genotype has recently been proposed for the production of late- or non-flowering cultivated sorghum hybrids (Mullet et al., 2010). Cropping systems using the non-flowering trait may focus solely on biomass or sugar production, as opposed to grain production, and have the potential to lower the likelihood of gene flow.

Table 1.7. **Influence of temperature on five cultivated sorghum varieties**

Variety	Time to flowering (days)		Influence of lower temperature on flowering
	Chillicothe	Plainview (2°C lower)	
"Hegari" (H)	78	68	Hastened
"Early Hegari" (EH)	71	62	Hastened
"100-day Milo" (100M)	100	90	Hastened
"60-day Milo" (60M)	66	66	Minor difference
"Sooner Milo" (SM60)	52	60	Delayed

Source: Quinby (1967).

Traits affecting male and female sterility

Cultivated sorghum possesses genes affecting both male and female sterility. Factors causing male sterility can be divided into two groups: nuclear male genetic sterility (commonly called genetic male sterility) and cytoplasmic-nuclear male sterility (commonly called cytoplasmic male sterility, or CMS). The genes ms1, ms2 and ms3 are associated with genetic male sterility due to the production of normal anthers but dysfunctional pollen (Ayyangar and Ponnaiya, 1937; Stephens, 1937; Webster, 1965). Other genetic male-sterile lines lack either pollen or anthers (Rooney, 2000). Genetic male sterility is used by some breeders to facilitate crossing, but since the genes are recessive, only the homozygous recessive individuals are male-sterile. Genetic male sterility systems are not used to produce commercial hybrids, and new varieties or lines generated using these systems to facilitate making crosses should be fixed back to the homozygous dominant (fertile) condition prior to deployment.

Female sterility is also a nuclear trait and has been observed in the dominant action of genes Fs1 and Fs2, which in the heterozygous condition result in viable pollen but only rudimentary development of stigmas, styles and ovaries, such that no seed set occurs (Casady et al., 1960).

CMS depends on the interaction of nuclear and cytoplasmic genes and renders the production of commercial F_1 cultivated sorghum hybrids economically viable (Stephens and Holland, 1954). The CMS system was first discovered in cultivated sorghum in a "Day" (race "Milo") × "Texas Blackhull" (race Kafir) cross and has been designated the A1 CMS system (Rooney, 2000). The genetics are reviewed in detail by Rooney (2000) and are more complex than summarised here. Briefly, three types of lines are involved: A-lines, B-lines and R-lines. A-lines contain cytoplasmic and nuclear genes that interact to produce male-sterile plants. B-lines contain the same nuclear genes, but not the

cytoplasmic genes for sterility, such that fully fertile plants are produced. A given B-line is backcrossed to an A-line and eventually the B-line is recovered in the A-line cytoplasm. This process yields an A/B-line pair that is essentially identical, except that the A-line is male-sterile and the B-line is male-fertile. The latter is used as a pollen source to "maintain" its A-line pair.

R-lines contain nuclear genes that override CMS and restore male fertility in the F_1 of an A-line × R-line cross. Other similarly functioning male sterility systems have been identified in alternate cytoplasms and are classified as the A2, A3 and A4 CMS systems (Schertz and Ritchey, 1978; Schertz, 1983; Kishan and Borikar, 1989). Most commercial F_1 hybrids of sorghum are currently produced using the A1 CMS system. Protocols utilising CMS to reduce the likelihood of gene flow through pollen were proposed by Pedersen et al. (2003).

Outcrossing

Cultivated sorghum is primarily self-pollinating; however, wind-mediated cross-pollination resulting in gene flow can occur in sorghum crop-weed complexes if the crop and wild or weedy relatives are sexually compatible, sympatric and flower simultaneously. This is often the case wherever cultivated sorghum is grown (de Wet and Harlan, 1971, 1975; Arriola and Ellstrand, 1996).

Outcrossing rates in cultivated sorghum are estimated at 5-30% under field conditions, based upon multiple methods of calculation (Ellstrand and Foster, 1983; Dogget, 1988; Pedersen et al., 1998; Djè et al., 2004). Significant variation exists between varieties and lines (Pedersen et al., 1998; Djè et al., 2004). The more compact panicles of race Durra, which is commonly used in commercial production, outcross at the lower end of the spectrum, about 7% (Djè et al., 2004).

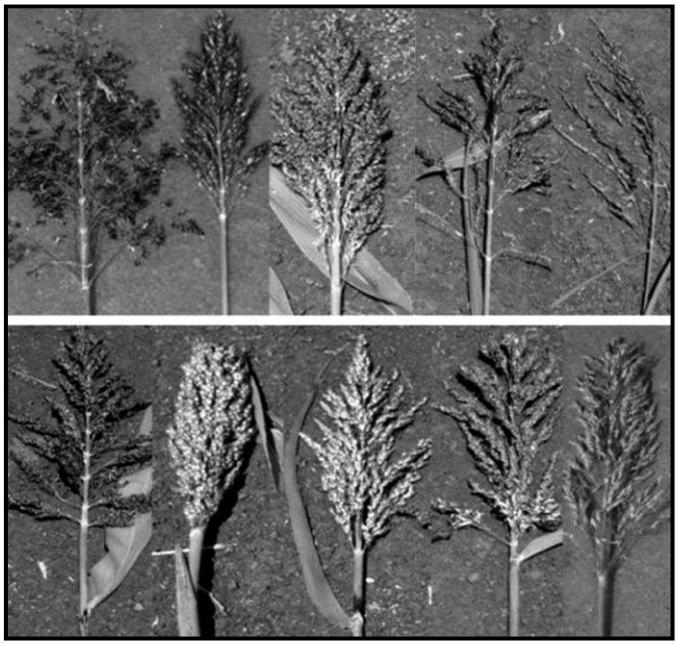
Interspecific crosses have likely occurred since cultivated sorghum's domestication. Crop-specific alleles have been found in samples of wild and weedy sorghum taxa that were genetically analysed for progeny segregation, allozymes and restriction fragment length polymorphisms (RFLP)s (Doggett and Majisu, 1968; Aldrich and Doebley, 1992; Aldrich et al., 1992; Paterson et al., 1995; Morrell et al., 2005). Morrell et al. (2005) surveyed RFLP allelic diversity in five Johnsongrass accessions from different parts of the United States. Among them, the frequency of individuals carrying at least one crop-specific allele ranged from 0.91 to 0.79 in Texas and Nebraska where cultivated sorghum is more frequently grown, and from 0.47 to 0.27 in New Jersey and Georgia where it is less frequently grown. These results suggest that when Johnsongrass is in close proximity to cultivated sorghum, higher rates of crop-to-weed gene flow are likely in the absence of management practices designed to avoid it, despite ploidy levels varying between the two species. Thus, the introduction of cultivated sorghum genes may persist in wild Johnsongrass populations if natural selection favours their presence in the genome. Factors affecting the fitness of crop alleles in wild sorghum populations are discussed in greater detail in the next section.

Phenotypic evidence of crop-to-weed introgression was observed by Tesso et al. (2008), who studied the geographic distribution of wild sorghum species in Ethiopia and Niger. A wide variety of phenotypic variation was observed within different regions, locations and individual fields (Figure 1.7), although the number of subspecies was not identified. The differing phenotypes varied according to habitat and proximity to cultivated sorghum: wild plants most similar in phenotype to cultivated sorghum occurred within crop habitats, while wild plants exhibiting smaller stature, thinner culms and very loose

panicles were found primarily in disturbed habitats, suggesting previous hybridisation and introgression events (Tesso et al., 2008).

Sudan grass is thought to be a hybrid between cultivated sorghum and *S. bicolor* subsp. *verticilliflorum*, and is found throughout Africa wherever cultivated sorghum is grown (Ejeta and Grenier, 2005; Andersson and de Vicente, 2010). Pedersen et al. (1998) investigated *in situ* crossing between Sudan grass individuals, with particular emphasis on the effects of flowering date combined with floret location on the panicle, and their influence on the rate of hybridisation. Two genotypes of Sudan grass were planted twice in two years in a checkerboard pattern and tagged to indicate the approximate date of pollination. The plants were harvested at maturity and the panicles were divided into three parts to be analysed: the upper, middle and bottom thirds. Pollination date was a major factor affecting the level of outcrossing, with the middle pollination date in both years having the highest rate of outcrossing (57.1% in 1991 and 38.9% in 1992). The middle date coincides with the time frame in which the most plants were observed to be entering anthesis resulting in the highest pollen density of the three time frames studied (early, middle and late). Conversely, the early pollination time period which generated the lowest pollen density exhibited the smallest amount of outcrossing in both years (36.0% in 1991 and 20.6% in 1992). Floret location on the panicle influenced outcrossing rates during the middle and later pollination dates in 1992; outcrossing occurred at higher levels in the upper third than in the lower third of the panicle with 48.7% and 30.3%, respectively, at the middle pollination date (Pedersen et al., 1998). Similar observations of outcrossing rate due to the location of florets on the panicle have been made in sorghum; additionally, the more compact grain sorghum panicles outcross at lower rates (10-15%) than the more open Sudan grass panicles (Maunder and Sharp, 1963; Ellstrand and Foster, 1983; Schmidt and Bothma, 2006).

Figure 1.7. **Phenotypic diversity of wild Sorghum species in a single field in Wollo, Ethiopia**



* At the time of peak cultivated sorghum flowering in 2005. The number of species or subspecies was not identified.

Source: Tesso et al. (2008).

Agriculturally important weedy relatives within cultivated sorghum's gene pool

Weedy *Sorghum* species exist either as rhizomatous perennials or as annuals resulting from hybridisation events with cultivated sorghum (Ejeta and Grenier, 2005). Johnsongrass and shattercane (Figure 1.8) are the primary weedy relatives of interest to agriculture due to their invasiveness and propensity to evolve resistance to herbicides (Holm et al., 1977; Heap, 2012). In parts of Southeast Asia, *S. propinquum* also readily crosses with cultivated sorghums (Ejeta and Grenier, 2005).

Rhizomatous relatives of cultivated sorghum are likely derived from the highly rhizomatous *S. propinquum*. Its classification in cultivated sorghum's primary gene pool indicates that it is fully interfertile (Andersson and de Vicente, 2010). In most instances, geographic isolation has prohibited cultivated sorghum from outcrossing with *S. propinquum* due to differing environmental adaptations (Dahlberg, 1995); however, that has not been the case in the Philippines, where frequent crosses with cultivated sorghum have produced progeny that have become noxious weeds (Ejeta and Grenier, 2005).

Figure 1.8. **Two agriculturally important weedy relatives of cultivated sorghum: Johnsongrass (left) and shattercane (right)**

Source: Courtesy of Pamela B. Trewatha, Missouri State University.

Johnsongrass is an aggressive, rhizomatous perennial grass recognised as one of the world's worst weeds (Holm et al., 1977). It is generally considered self-compatible with less than a 10% outcrossing rate (Warwick and Black, 1983; Burke et al., 2007). Regardless, the ability of Johnsongrass to cross with cultivated sorghum is well-documented (Arriola and Ellstrand, 1997, 1996). *S. almum*, also known as Columbus grass, is genetically similar to Johnsongrass. It grows taller and has larger stems and leaves than Johnsongrass, but it has shorter rhizomes and is less troublesome a weed (Magness et al., 1971). *S. almum* and Johnsongrass both belong to cultivated sorghum's secondary gene pool such that F_1 progeny from cultivated sorghum ($2n$) × Johnsongrass ($4n$) crosses are usually completely sterile triploids and progeny from cultivated sorghum ($2n$) × *S. almum* ($6n$) crosses are partially fertile tetraploids (Endrizzi, 1957; Warwick and Black, 1983; Sangduen and Hanna, 1984). However, reports of fertile tetraploid offspring

from cultivated sorghum × Johnsongrass crosses exist and are reviewed in Warwick and Black (1983). Further information about cultivated sorghum × Johnsongrass crosses and gene flow between these species is found in the next section.

Shattercane resembles cultivated sorghum but differs in that it grows taller because it has no dwarfing genes; it is able to disperse seeds through seed shattering, and its seeds exhibit greater dormancy and longevity in the soil (Quinby and Martin, 1954; Burnside et al., 1977; Fellows and Roeth, 1992).

Gene flow and fitness of crop × weed hybrids

In a study investigating crop-to-crop gene flow in race Kafir, Schmidt and Bothma (2006) observed that outcrossing rates among pollen receptors decreased as their distance increased from pollen donors. The experiment was laid out with the pollen donors (male-fertile B-line "Redlan") grown in a 30 × 30 metre block from which eight arms of the pollen receptors (male-sterile A-line "Redlan") radiated out at distances ranging from 13 metres to 158 metres. The average outcrossing rate, across directions, was 2.54% at 13 metres, less than 1% at or beyond 26 metres, and 0.06% at 158 metres. Mathematical models estimated maximum gene flow distance to be 200-700 metres. These values were in agreement with observations by cultivated sorghum breeders, who use isolation distances of 100 metres to achieve less than 1% gene flow from neighbouring fields. Distance and wind direction were found to be the primary factors determining the rate of gene flow. The authors suggested that outcrossing rates under natural conditions would be expected to be lower than what they observed because the use of male sterile receptors eliminated pollen competition and allowed the female flowers to remain receptive longer in the absence of pollination. Female flowers can remain receptive up to 16 days in the absence of pollination even though flowering is typically complete in 4-7 days (Schertz and Dalton, 1980). Under natural circumstances, fully fertile plants are about 70-95% self-pollinated (Ellstrand and Foster, 1983; Pedersen et al., 1998; Djè et al., 1999, 2004; Smith and Frederiksen, 2000).

Crop-to-weed gene flow has been observed between cultivated sorghum and Johnsongrass. Arriola and Ellstrand (1996) investigated the level of spontaneous hybridisation between Johnsongrass and cultivated sorghum at two test sites over a two-year period. They planted a central plot of sorghum (diploid pollen source) surrounded by pots of Johnsongrass (tetraploid maternal plants) at distances of 0.5, 5, 50 and 100 metres. Results indicated a trend toward decreased hybrid production as distance from the crop increased, but crop-to-weed hybrid seedlings were detected at the furthest distance at both sites. No weed-to-crop hybrid seedlings were detected. Measured rates of hybridisation ranged from 0% to 100% per plant, with hybridisation levels as high as 2% at a distance of 100 metres. Like Schmidt and Bothma (2006), an increase in relative pollen flow was needed to produce hybrids at further distances. The triploid hybrids generated in this experiment were capable of being pollinated by diploid sorghum to restore partial self-fertility. Arriola and Ellstrand (1996) concluded that hybrid formation between cultivated sorghum and Johnsongrass was highly variable and somewhat unpredictable, as the observed hybridisation rates in this study varied according to the distance between the weed and crop plants, the location of the study site, and the year the study was performed. The highly variable results were attributed to the large degree of morphological and genetic variation seen within Johnsongrass that influences the hybridisation abilities of different plants and their dynamics in differing systems. In summary, the study concluded that distance was the primary factor affecting relative gene flow, with many more hybrids being produced closer to the pollen source.

Sangduen and Hanna (1984) also evaluated cultivated sorghum × Johnsongrass hybrids. Although not used in cultivation, tetraploid *S. bicolor* has been experimentally produced and was used in their experiments. Two such tetraploid sorghum lines and a tetraploid Johnsongrass were used as both maternal and paternal parents in crosses. Hybrid seeds were produced by covering the flowering panicles with bags after being dusted with pollen. Seeds subsequently produced were then planted for observation. Results revealed that interspecific hybrids were produced at a higher frequency when Johnsongrass served as the female parent than when cultivated sorghum served as the female, with 71-83% of seeds being hybrid compared to 0-33%, respectively. This variation was likely due to specific responses to the crossing technique, cross-incompatibility or a mixture of both. The hybrid plants morphologically resembled Johnsongrass due to their perennial and rhizomatous growth, open inflorescence, seed shattering, seed shape, and seed colour. Stem thickness, number of rhizomes, leaf width and seed size were traits expressed as intermediate between both parents. Hybrid plants were more leafy and vigorous with longer and larger inflorescences than either parent. The high rate of outcrossing is not especially concerning from an agroecological viewpoint. Although tetraploid sorghum exists as an experimental tool, it is not cultivated.

Schmidt (2011) evaluated gene flow between cultivated sorghum and shattercane, using cultivated sorghum as a pollen source and shattercane as a pollen receptor. Cross-pollination ranged from 4% to 16% among shattercane plants placed directly within the area occupied by pollen donors, and decreased to nearly 0% at 200 metres downwind.

In a separate study, crosses between a single shattercane inbred line and cultivated sorghum were produced by Sahoo et al. (2010) in order to assess the fitness components of hybrids. Fitness components evaluated were temperature requirements for germination, rate of germination, dormancy, vegetative growth and seed production. For components of fitness affecting seeds, temperature was a strong modifier of the proportion of seeds able to germinate, their rate of germination and the length of dormancy prior to germination. Overall, the response of F_1 hybrids was similar to shattercane at lower temperatures and to cultivated sorghum at higher temperatures. This could be attributed to the position of the seed within the glumes of the F_1 hybrids; shattercane exhibits a great deal of dormancy and seed protection due to the seed's complete encapsulation by the glumes, whereas cultivated sorghum seeds are not encapsulated. The hybrids were morphologically intermediate with their seed only partially encapsulated by the glume, thus potentially weakening their protection to extreme heat and humidity.

For components of fitness affecting vegetative growth and seed production, Sahoo et al. (2010) observed that shattercane grew taller than cultivated sorghum and that F_1 hybrids exhibited hetorosis, growing taller and producing more biomass than both parents. Cultivated sorghum had the largest leaf area index and shattercane had the smallest, but the F_1 hybrid was intermediate and closer to sorghum. Leaf emergence was greater for sorghum and the hybrid than for shattercane, but seed size and production were more similar to shattercane, which produced many small seeds, than to cultivated sorghum, which produced fewer, larger seeds. When considering these traits together, F_1 hybrid fitness was similar to that of shattercane, suggesting that crop genes that are either neutral or beneficial to shattercane would persist in populations within agro-ecosystems.

Arriola and Ellstrand (1997) measured fitness components of Johnsongrass × cultivated sorghum hybrids relative to the Johnsongrass parents, including time to flowering, pollen viability, seed production, panicle production, tiller production and biomass. The only observed difference between genotypes was a slightly higher level of pollen sustainability

(an estimate of viability) in the hybrid plants; however, overall performance of the hybrids was indistinguishable from Johnsongrass. Therefore, it is expected that hybrid fitness in these crosses is equal to that of the weedy parent.

Methods to mitigate crop-to-weed gene flow

As the above studies show, distance is the primary factor mitigating crop-to-weed gene flow because greater distances are associated with a reduction in pollen density from the source (Arriola and Ellstrand, 1996; Schmidt and Bothma, 2006). Isolation distances for sorghum in OECD countries have been designated as 200, 300 or 400 metres, depending upon seed category and climatic conditions and in many cases have proved sufficient to reliably achieve less than 0.1% outcrossing; however, these distances may not be enough under all circumstances (Andersson and de Vicente, 2010). Gene flow may be substantially influenced by wind strength and direction, genotype, plant morphology and topography.

Where physical separation is not feasible, ensuring different flowering times is the most effective way to reduce opportunities for gene flow (Ellstrand, 2003). In a study of six cultivated lines of rice, the degree of outcrossing with wild relatives was shown to be the highest in the cultivar with the longest overlapping flowering period with the wild relatives (Langevin et al., 1990). However, it must be noted that extreme temperatures or drought may induce flowering among late- or non-flowering cultivated sorghum lines.

Population size and structure also influence pollen density, as does spatial arrangement: Ellstrand and Foster (1983) observed a higher rate of outcrossing in plants grown in a dispersed arrangement than plants grown in a stratified arrangement.

Sexual compatibility influences the possibility of gene flow, but does not prevent it completely. Cultivated sorghum is sexually compatible with the entire section Eusorghum (Ejeta and Grenier, 2005). Hybridisation across gene pools can produce sterility or reduced fertility. In the case of hybrids between tetraploid Johnsongrass and diploid cultivated sorghum, some reduced fertility was observed; however, through backcrossing with diploid parents, partial self-fertility was restored (Arriola and Ellstrand, 1996).

Other genetic barriers to outcrossing have also been proposed. Namely, genetic or cytoplasmic male sterility in cultivated sorghum could be used to create barriers to outcrossing as there is no viable pollen available to initiate spontaneous hybridisation (NRC, 2004). Pedersen et al. (2003) proposed a scheme to take advantage of this in sorghum by using a source of cytoplasmic male sterility with few known fertility restoring R-lines and including a low percentage of fertile pollinators in seedlots. However, pollen competition may be a confounding factor in such systems. Muraya et al. (2011) showed that self-pollination results in higher rates of seed set than cross-pollination, and suggested that the use of male-sterile bait plants in gene flow studies may overestimate gene flow rates and that pollen competition may be a significant factor in reproductive success. Furthermore, extreme temperatures or drought may cause otherwise sterile plants to regain fertility. A recent summary of current strategies to mitigate crop-to-weed gene flow, from crop management to molecular level, and those proposed for future deployment are outlined in detail in Oliver and Li (2012).

Ecology

Potential for increased weediness among wild sorghum species due to gene flow

Wild and weedy sorghum species have the ability to outcompete cultivated crops for nutrients and light, and are also carriers of harmful pests and diseases, such as sorghum ergot caused by the fungus *Claviceps africana* (Ejeta and Grenier, 2005). Mechanised farming practices do not involve the hand pulling of weeds, making it possible for the seeds that survive winter to spread uncontrolled (Ejeta and Grenier, 2005). Weeds rely on traits such as seed dormancy, variable germination, vegetative plasticity and increased fecundity to enhance their ecological fitness (Sahoo et al., 2010), whereas most crops are bred to remove these traits to enhance uniformity and control. If weedy relatives inherit crop traits intended to eliminate seed dormancy or reduce vegetative growth, the new traits are not expected to confer survival or invasiveness advantages (Linder and Schmitt, 1995). The above studies confirmed that cultivated sorghum × Johnsongrass and shattercane hybrids were no more problematic than their weedy parent (Arriola and Ellstrand, 1997; Sahoo et al., 2010). Crop traits expected to confer an advantage to weedy relatives, such as herbicide resistance, are of more concern (Arriola and Ellstrand, 1997, 1996; Schmidt and Bothma, 2006). Hokanson et al. (2010) outlined strategies to mitigate any potential risks that may be associated with the introduction of transgenic plants to Africa, although their suggestions are applicable to policy makers everywhere. Oliver and Li (2012) provide further discussion of the issue of containment.

Improved sorghum has been deployed throughout the world for over a century and many genotype interactions have been studied (Ejeta and Grenier, 2005). Evidence of domestic alleles that are present and persistent in wild populations suggests that crop-to-weed hybridisation is the rule rather than the exception (Ellstrand et al., 1999). These studies indicate that hybridisation with wild relatives has the potential for weed evolution and gene introgression, but little risk of extinction.

Hybridisation between crops and their wild and weedy relatives may confer neutral, detrimental or beneficial selective advantages. These modulate a hybrid's fitness, and consequently a gene's potential to introgress and persist in the environment. Outbreeding depression occurs when detrimental traits in the hybrid confer a selective disadvantage, potentially leading to extinction (Ellstrand et al., 1999). Genetic swamping occurs when continued introgression of neutral or beneficial traits causes hybrids and their progeny to assimilate into the dominant parent population. This is also a form of extinction (Levin et al., 1996). Both forms of extinction are of particular concern in Africa (Doggett, 1988; Schmidt and Bothma, 2006). Although sorghum readily hybridises with its wild and weedy relatives, so far there has been no evidence of genetic swamping or extinction amongst its wild relatives. The only known instances of genetic erosion have been due to habitat change (Ejeta and Grenier, 2005).

It is important to note that even beneficial alleles may not persist following crop-to-weed introgression because other genetic and environmental factors influence subsequent propagation. For example, volunteer cultivated sorghum plants do not typically survive winter in temperate regions (Andersson and de Vicente, 2010). Other genetic factors may also counterbalance a new beneficial allele. Keeler (1989) predicts that a single beneficial trait is unlikely to cause significant increased weediness or invasiveness; however, a single trait like herbicide resistance has obvious consequences in increased weed fitness (NRC, 2004). Nevertheless, a trait's potential to confer increased fitness must be evaluated in

combination with relevant environmental factors to be accurately assessed (Arriola and Ellstrand, 1997, 1996; Sahoo et al., 2010).

Interactions in natural and managed ecosystems

Weedy relatives can be carriers of diseases and pests that can cause significant damage to natural and agroecosystems alike. The potential for increased weediness due to crop-to-weed introgression of herbicide resistance further exacerbates this problem by increasing the number of surviving weeds (Ellstrand et al., 1999). Shootfly and sorghum midge are two notorious pests whose control relies in large part on the time of planting, since these pest populations decrease significantly in the absence of sorghum hosts during winter or the rainy season. Wild, weedy or cultivated sorghum volunteers serve as hosts for these pests between cropping seasons, such that pest populations accumulate (Doggett, 1988). *Claviceps africana* is an ergot-causing fungal parasite that lives only in the flowers of certain grasses and survives in wild sorghum species like Johnsongrass (Ejeta and Grenier, 2005). This pathogen has spread rapidly around the world, and concern exists that it could become endemic on Johnsongrass if it becomes established (Odvody et al., 1999). *Claviceps africana* is a threat to grain sorghum production, as it infects unfertilised ovaries of cultivated sorghum (Frederiksen, 2000). A list of sorghum's common pests and pathogens can be found in Annexes 1.A1 and 1.A2. Bailey (2007) in particular provides a review of pests specific to Australia.

Impact on animals in the environment

Certain factors can render sorghum forage toxic to grazing animals. Environment, genetics, plant part and growth stage are important modifiers of sorghum forage toxicity. Like other C4 forage plants, including maize and pearl millet, cultivated sorghum accumulates nitrates (Pedersen and Fritz, 2000), but at higher rates (Sidhu et al., 2011). Several factors can contribute to increased nitrates in sorghum forage, including environmental conditions, nitrogen fertiliser use, growth stage and plant part (Sidhu et al., 2011). Drought and frost severely interfere with the crop's normal growth, slowing development and allowing higher concentrations of nitrate to accumulate in plant tissues (Pedersen and Fritz, 2000; Sidhu et al., 2011). Young plants have a higher rate of nitrate uptake and generally contain higher levels than mature plants (Sidhu et al., 2011). Stems have the highest concentration of nitrate, followed by roots and leaves, and concentrations in flowers and grain are considered negligible (Sidhu et al., 2011). Excess nitrate in sorghum forage can be toxic to ruminants and other grazing animals through the production of methemoglobin (Wright and Davison, 1964).

Cyanogenic glycosides are secondary products that are produced in a range of plant species, including sorghum (Ganjewala et al., 2010). These compounds are believed to be largely involved in defence against predators, most particularly insects. Excess cyanogenic glycosides can be toxic to ruminants and other grazing animals through the production of cyanoglobin (Vough, 1978). When present, cyanogenic glycosides are mainly found in germinating seeds, sprouts and the leaves of immature sorghum plants. The most abundant of these is dhurrin, which may comprise 3-4% of the leaves of germinating seeds (Newton et al., 1980; Doggett, 1988). Cyanogenic glycosides may be converted in the rumen or nonruminant stomach into prussic acid (also known as hydrocyanic acid, HCN, the aqueous form of cyanide). Environmental stresses including drought and frost are major environmental conditions resulting in higher HCN levels (Pedersen and Fritz, 2000). Frost releases HCN quickly in frozen leaves and may kill the top of the plants, causing new shoots and leaves at the bottom to be high in prussic acid (Vough, 1978).

Drought stunts the growth of the plant preventing it from growing out of the young plant stage, which generally has higher levels of HCN (Vough, 1978). Cyanogenic glycosides are not found in mature grain. Modern screening methods based on near-infrared spectroscopy have been developed to monitor levels of cyanogenic glycosides where the technology exists (Fox et al., 2012).

Sorghum varieties developed specifically for grazing such as Sudan grass have reduced levels of cyanogenic glycosides.

OECD (2010) provides pertinent detailed information for the management of anti-nutrients and toxicants for food and feed.

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Annex 1.A1.
Common insect pests

Common insect pests of cultivated sorghum include:

1. Chinch bug – *Blissus* spp.
2. Corn leaf aphids – *Rhopalosiphum maidis* (Fitch)
3. Greenbugs – various species within order Homoptera, especially *Shizaphis graminum* (Rondani)
4. Soil cutworm – various species within family Noctuidae
5. Wireworms – various species within family Elateridae
6. Seedcorn maggot – *Delia platura* (Meigen)
7. Seedcorn beetle – *Stenolophus lecontei* (Chaudoir)
8. Sorghum midge – various species within family Cecidomyiidae, especially *Stenodiplosis sorghicola* (Coquillett)
9. Fall armyworm – *Spodoptera frugiperda* (J. E. Smith)
10. Stalk and stem borers – various species within order Lepidoptera, especially *Busseola fusca* (Fuller), *Chilo partellus* (Swinhoe), *C. orichalcociliellus* (Strand), *Sesamia calamistis* (Hampson), *Eldana saccharina* (Walker), *Diatraea saccharalis* (Fabricius), *D. lineolata* (Walker) and *D. grandiosella* (Dyar)
11. Shoot fly – *Atherigona soccata* (Rond.)
12. Lesser cornstalk borer – *Elasmoplapus lignosellus* (Zeller)
13. Corn earworm – *Helicoverpa zea* (Boddie)
14. Sorghum webworm – *Nola sorghiella* (Riley)
15. Stink bug – various species within Genera *Nezera*, *Euschistus* and *Oebalus*
16. Billbug – *Sphenophorus* spp.
17. Sugarcane beetle – *Euetheola humilis rugiceps* (LeConte)
18. Yellow sugarcane aphid
19. White grub – *Phyllophaga crinita* (Burmeister)

Annex 1.A2.
Common pathogens

Cultivated sorghum is susceptible to bacterial, fungal, nematode, plant, phytoplasma and viral diseases. Those of greatest agronomic importance are listed below. A complete list may be found in Frederiksen (2000).

1. Grain mould – *Fusarium thapsinum* and various other *Fusarium*, *Alternaria* and *Cochliobolus* spp.
2. Ergot – *Claviceps africana*
3. Sorghum downy mildew – *Peronosclerospora sorghi*
4. *Fusarium* stalk rot – *Fusarium proliferatum* and other *Fusarium* spp.
5. Bacterial stalk rot – *Erwinia chrysanthemi*
6. Charcoal rot – *Macrophomina phaseolina*
7. Anthracnose – *Colletotrichum sublineolum* and *C. graminicola*
8. Rust – *Puccinia purpurea*
9. Zonate leaf spot – *Gloeocercospora sorghi*
10. Head smut – *Sporisorium reilianum*
11. Sooty stripe – *Ramulispora sorghi*
12. Gray leaf spot – *Cercospora sorghi*
13. Sorghum mosaic virus
14. Witchweed – *Striga* spp.

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Annex 1.A3. Biotechnological developments

Sorghum has proven to be highly recalcitrant to genetic transformation (Andersson and de Vicente, 2010), but improvements have been achieved. *Agrobacterium*-mediated modification and particle bombardment are two proven methods for introducing transgenic traits to sorghum. Recent reports have indicated that in some genotypes, transformation efficiencies in excess of 20% are achievable (Liu and Godwin, 2012). There is currently no commercially available genetically modified sorghum, but research has progressed in the four following areas.

Biofortification

A major obstacle to cultivated sorghum use as food is its nutritional deficiency: it has low protein digestibility and lysine content. The Africa Biofortified Sorghum (ABS) Project aims to create nutritionally enhanced transgenic lines with increased lysine content, protein digestibility and bioavailability of iron and zinc (Zhao, 2007). Iron-deficiency anaemia in particular is a problem in many rural areas of Africa. Using *Agrobacterium*-mediated methods of genetic transformation, suppression of kafirin protein synthesis has resulted in compensatory synthesis of other proteins with higher lysine content and increased digestibility (Zhao, 2007; Taylor and Taylor, 2011). ABS #1, a first-generation line with 50% more lysine, was developed based on transgenes originally developed for maize (Zhao et al., 2003). Subsequently, creation of a second generation, ABS #2, was successful and has been crossed with African varieties. The second generation has improved protein quality and digestibility, as well as increased levels of iron, zinc, and vitamins A and E (AHBFI, 2007). Taylor and Taylor (2011) reported that transgenic cultivated sorghum had 52-115% more lysine and 23-102% greater protein digestibility. Furthermore, foods prepared from these grains had improved protein quality.

Insect resistance

Bacillus thuringiensis (*Bt*) genes have been deployed experimentally to confer Lepidopteran insect resistance. Girijashankar et al. (2005) created transgenic cultivated sorghum via particle bombardment of shoot apices with a synthetic *Cry1Ac Bt* gene controlled by *mpiC1*, a promoter from the maize protease inhibitor gene. The resulting transgenic plants were grown in a greenhouse and artificially infested with *Chilo partellus* larvae (spotted stem borer) to assess the degree of insect resistance. In non-transgenic control plants leaves, larvae consumed over 80% of the material within five days whereas transgenic plants showed less than 50% leaf damage, 40% larval mortality and a 36% reduction in surviving larval weight. Assays of shoots indicated no significant decrease in larval weight, which suggests a lower level of *Bt* transgene expression in stem tissue than in leaf tissue. These results document partial resistance in *Bt* sorghum (Girijashankar et al., 2005).

Disease resistance

Transformation of cultivated sorghum for resistance to anthracnose, a fungal disease caused by *Colletotrichum sublineolum*, and to stalk rot-causing fungi like *Fusarium thapsinum,* has achieved some success (Krishnaveni et al., 2001; Kosambo-Ayoo et al.,

2011). Genes encoding chitinase or chitosanase hydrolyse fungal cell walls, rendering them osmotically sensitive. Kosamboo-Ayoo et al. (2011) used particle bombardment to create lines that were significantly more tolerant to anthracnose than non-transgenic control plants. Krishnaveni et al. (2001) used biolistic transformation to introduce rice chitinase into cultivated sorghum. Five to 50% of transformed seedlings demonstrated moderate resistance to stalk rot caused by *F. thapsinum*, but transgene expression varied.

Bioenergy

Grain sorghum and sweet sorghum are excellent candidates for bioenergy use due to high biomass and sucrose production, and the ability to grow them in a wide range of environments with minimal inputs. Grain sorghum can be used in the production of grain ethanol, while sweet sorghum's high sucrose content can be used to produce ethanol from saccharine juice through fermentation (Saballos, 2008). Potential traits for improvement as a bioenergy crop include increased yield and biomass quality such that cultivated sorghum becomes even more cost-effective to process into usable energy (Saballos, 2008). Lignin modification is important to increase the bioenergy production efficiency of sorghum (Saballos, 2008; Basu et al., 2011). Producing ethanol requires the hydrolysis of cellulose polymers, but lignin hinders the enzymatic process and inhibits conversion of lignocellulose (Dien et al., 2009). Two lines of transgenic sorghum with altered lignin composition have been created through *Agrobacterium*-mediated transformation (Basu et al., 2011). These transgenic plants had 28% less total lignin with significant increases in cellulose content and soluble sugars, which would increase the efficiency of fermentation when processing sweet sorghum for bioenergy.

Wu et al. (2007) provide information about seed composition, seed structure and other physical features that either help or hinder conversion of sorghum grain to ethanol based on the analysis of 70 genotypes and elite hybrids. In particular, the authors observed that the major factors having a positive effect on the bioconversion of elite genotypes included high starch content, rapid liquefaction, low viscosity during liquefaction, high fermentation speed and high fermentation efficiency. Major adverse factors included tannin content, low protein digestibility, high mash viscosity and an elevated concentration of amylose-lipid complexes in the mash. A more detailed review of sorghum's potential for ethanol production may be found in Serna-Saldívar et al. (2012).

The United States Environmental Protection Agency announced in December 2012 that sorghum grain qualified as an advanced biofuel. The Environmental Protection Agency's analysis found that ethanol produced from grain sorghum has an estimated lifecycle greenhouse gas emissions reduction of 32% when produced at dry mill ethanol facilities that use natural gas, producing on average 92% wet distillers grains; and a reduction of 52% when produced at dry mill ethanol facilities that use only biogas for process energy and obtain from an off-site supplier 0.15 kWh of electricity per gallon of ethanol produced, compared to the baseline gasoline fuel it would replace. Therefore, grain sorghum ethanol produced at dry mill ethanol facilities using natural gas met the minimum 20% greenhouse gas emissions reduction threshold for conventional biofuels, and grain sorghum ethanol produced at plants using only biogas for process energy and obtain from an off-site supplier no more than 0.15 kWh of electricity per gallon of ethanol produced, and met the 50% greenhouse gas emissions reduction threshold for advanced biofuels as required by the Energy Independence and Security Act of 2007, accessible at: www.epa.gov/otaq/fuels/renewablefuels/documents/420f12078.pdf.

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