Contribution of wild *Crucifers* in *Brassica* improvement : past accomplishment and future perspectives

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Abstract

Brassica related wild germplasm "*Brassica* coenospecies" is a potential source of many useful nuclear genes for enriching conventional germplasm, and cytoplasmic genes for inducing male sterility. Intensive hybridization between wild and crop species representing interspecific, intergeneric and intertribal combinations have accumulated vast information on genome homoeology. Biotechnological tools have made possible to circumvent even the tribal boundaries. Genes of interest have been introgressed frm wild germplasm to crop species which include resistance to beet cyst nematode, alternaria blight and black leg. A number of alloplasmics of crop species have been synthesized combining wild cytoplasms for expression of male sterility. Wild germplasm is to be systematically characterized for various traits.

Key words: Wild germplasm, *Brassica* coenospecies, cytodemes, sexual and somatic hybrids, alloplasmics

Introduction

Crop *Brassica*s lack many useful traits which cause considerable economic losses. Fungal diseases viz. alternaria blight, black leg; and aphids,salinity and drought are the major biotic and abiotic stresses. Enriching conventional germplasm with alien genes from the related germplasm and widening the genetic base is a desirable approach. Majority of species in this germplasm are wild and weedy, and carry genes which can confer agronomic advantages to conventional cultivars (Table 1). This germplasm has the potential to exchange genetic material with crop species as evidenced by chromosome pairing in the hybrids between them. In view of advances made in molecular biology, identification of QTL for productivity traits and pyramiding them into crop cultivars assume significance.

Table 1. Some desirable traits in wild germplasm

Alternaria blight resistance	Clubroot resistance	High linoleic acid content
Coincya spp.	Armoracia rusticana	Orychophragmus violaceus
Camelina sativa	Pod shatter resistance	*Mathiola incana*
Capsella bursa-pastoris	Enarthrocarpus lyratus	High erucic acid content
Diplotaxis erucoides	*Hirschfeldia incana*	Crambe abyssinica
Diplotaxis catholica	*Raphanus* spp.	*Erucastrum cardaminoides*
Black leg resistance	*Trachstoma balii*	*Sinapidendron angustifolia*
Arabidopsis thaliana	Zinc tolerance	High linolenic acid content
Sinapis arvensis	*Thlaspi caerulescens*	*Alyssum* spp.
Diplotaxis muralis	Cold tolerance	*Barbarea* spp.
Diplotaxis tennuifola	Coincya richeri	*Cardamine*
White rust resistance	*Erucastrum abyssinicum*	*Conringia*
Eruca vesicaria	Drought tolerance	*Lepidium sativum*
Raphanus sativus	*Brassica* tournefortii	C3-C4 intermediate photosynthesis
Flea beatle resistance	*Diplotaxis acris*	Diplotaxis muralis
Arabidopsis thaliana	*Diplotaxis harra*	*Diplotaxis tennuifola*
Capsella bursa-pastoris	*Eruca sativa*	*Moricandia arvensis*
Crambe abyssinica	*Lesquerella* spp.	*Moricandia nitens*

Cytogenetical architecture of *Brassica* coenospecies

Initial investigations on wild germplasm were confined to mainly determine the chromosome numbers. Manton (1932) in her pioneer work reviewed the substantial portion of *Cruciferae* family for somatic chromosome numbers. Mizushima (1950 onwards) studied cytology in a number of interspecific and intergeneric hybrids and arrived at genome homoeology. However, a major step was by Harberd (1972) who classified *Brassica* related wild germplasm which he referred to as *Brassica* coenospecies into cytodemes based on chromosome pairing and fertility in a large number of interspecific and intergeneric hybrids. Initially 11 genera comprising *Brassica, Coincya* (*Hutera*), *Diplotaxis, Eruca, Erucastrum, Hirschfeldia, Sinapis, Sinapidendron* and *Trachystoma* of subtribe *Brassicinae*, and 2 genera from subtribe *Raphaninae* viz. *Enarthrocarpus* and *Raphanus* were included. Recent investigations involving cytogenetics and cp DNA homologies strongly suggested the

inclusion of 3 more genera viz. *Moricandia*, *Pseuderucaria* and *Rytidocarpus* of subtribe *Moricandiinae* (see Warwick and Sauder 2005). At present 63 cytodemes are recognized including those of the crop species. Majority of cytodemes are diploids (around 60%). Auto- and allopolyploidy have also participated in the evolution of a number of cytodemes. The lowest chromosome number in coenospecies is 2*n*=14,while 2 species *Moricandia spinosa* (2*n*=84,*x*=6) and *Brassica repanda* (2*n*=160,*x*=8), both autopolyploids, have the highest chromosome numbers. The genus *Moricandia* is exclusively polyploid. The distribution of wild germplasm is mostly centered in 3 phytochoria viz. Mediterranean, Irano-Turanian and Saharo-Sindian extending from Iberian peninsula to north-west of India. However, most variability occur in Algeria, Morocco and Spain. There were several expeditions to collect wild germplasm during 1970-75 notably by Prof. C.Gómez-Campo of Spain, Profs. U.Mizushima, S.Tsunoda and K.Hinata of Japan. Large collections were made and deposited in Crucifer Gene Banks at Universidad Politecnica, Madrid, Spain and Tohoku University, Sendai, Japan.

Table 2. Intertribal somatic hybrids in *Brassiceae*

Somatic hybrid	Desirable trait	Reference
Tribe *Arabideae*		
Armoracia rusticana(*n=16*) + *B.oleracea*	Club root resistance	Navatilova et al.1997
Barbarea vulgaris (*n=8*) + *B.oleracea*	Cold tolerance	Ryschka et all 1999
Barbarea vulgaris (*n=8*) + *B.rapa*	Cold tolerance	Oikarinen & Ryoppy 1992
Barbarea vulgaris (*n=8*) + *B.napus*	Cold tolerance	Fahleson et al 1994
Barbarea stricta (*n=8*) + *B.rapa*	Cold tolerance	Oikarinen & Ryoppy 1992
Tribe Drabeae		
Lesqurrella fendleri (*n=6*) + *B.napus*	Drought tolerance, lesquerolic oil	Skarzhinskaya et al 1996,1998; Schroder-Ponttoppidan 1999
Tribe *Lepideae*		
Capsella bursa-pastoris(*n=16*) + *B.oleracea*	Flea beetle and Alternaria resistance	Nitovskaya et al 1998: Sigareva & Earle 1999
Lepidium meyenii (*n=32*) + *B.oleracea*	Glucosinolate content	Ryschka et al 2003
Thlaspi perfoliatum (*n=21*) + *B.napus*	Nervonic acid content	Fahleson et al 1994
Thlaspi caerulescens (*n=7*) + *B.napus*	Zinc and cadmium tolerance	Brewer et al 1999
Tribe Lunarieae		
Lunaria anuua (*n=14*) + *B.napus*	Nervonic acid content	Craig & Millam 1995
Tribe *Hesperideae*		
Matthiola incana (*n=7*) + *B.oleracea*	Oil quality	Ryschka et al 1999
Tribe *Sisymbrieae*		
Arabidopsis thaliana (*n=5*) + *B.nigra*	Flea beetle resistance	Siemens & Sacristan 1995
Arabidopsis thaliana (*n=5*) + *B.oleracea*		Nitovskaya & Shahkhovskii 1998 Yamagishi & Nakagawa 2004
Arabidopsis thaliana (*n=5*) + *B.rapa*		Gleba & Hoffmann 1979,1980
Arabidopsis thaliana (*n=5*) + *B.napus*	Herbicide resistance, Black leg resistance	Bauer-Weston et al 1993 Forsberg et al 1994,1998 Yamagishi et al 2002
Camelina sativa (*n=20*) + *B.oleracea*	Alternaria resistance	Hansen 1998: Sigareva & Earle 1999
Camelina sativa (*n=20*) + *B.carinata*	Alternaria resistance	Narasimhulu et al 1994
Camelina sativa (*n=20*) + *B.oleracea*	Alternaria resistance	Hansen 1998: Sigareva & Earle 1999
Camelina sativa (*n=20*) + *B.carinata*	Alternaria resistance	Narasimhulu et al 1994

Utilization

A vast majority of taxa in the coenospecies belong to secondary and tertiary gene pool, thus rendering their genes inaccessible to breeding programs. Several types of barriers restricting wide hybridizations operate which include unilateral incompatibility, and pre-and post fertilization barriers These are overcome through hybrid ovary and embryo cultures. However, the spectacular advances in protoplast fusion technology during last 25 years have circumvented even the tribal boundaries. As a consequence, a vast number of sexual and somatic hybrids have been obtained. Sexual hybrids have been studied for their cytology to interpret genome relationships. High genome homeologies have been proposed among different genera even across subtribal boundaries. In several instances, synthetic alloploids have been obtained to restore fertility following chromosome doubling. Although the successful cell fusion in Cruciferae was attempted by Kartha et al (1974), the first somatic hybrid *B.rapa* + *Arabidopsis thaliana* was regenerated by Gleba & Hoffman (1979). Subsequently, a large number of somatic hybrids have been obtained combining crop species with taxonomically divergent wild species. These include interspecific, intergeneric and a substantial number of intertribal combinations from six different tribes (Table 2). In many instances, the desirable trait is expressed in the hybrids, however, the introgression remains a probelem due to lack of sufficient intergenomic chromosome homoeology and also high degree of sterility. Nevertheless, in some instances, genes have been introgressed and characters expressed e.g. resistance to beetcyst nematode from *Raphanus*, alternaria leafspot from *Diplotaxis erucoides* and black leg from *Brassica tournefortii* and *Sinapis arvensis*. In somatic hybrids, desirable characters have also expressed e.g. high erucic and nervonic acid content, high level of zink accumulation, C3-C4 intermediate photosynthesis etc. However, their introgression has not largely been possible. The most rewarding utilization is in developing

alloplasmics combining cytoplasm of wild species with the crop nuclei for expressing male sterility. Stable cytoplasmic male sterile lines have been obtained in *B.rapa*, *B.juncea* and *B.napus*. These are based on *Arabidopsis thaliana, Brassica tournefortii, Diplotaxis catholica, Eruca sativa, Erucastrum canariense, Moricandia arvensis, Orychophragmus violaceus, Raphanus, Sinapis arvensis* and *Trachystoma ballii* cytoplsms. Fertility restoration genes have been introgressed from respective cytoplasmic donors for some of the systems. Heterotic *B.napus* hybrids based on *Raphanus/ogu* system have been marketed in Canada and Europe. *B.juncea* hybrids based on *Moricandia* sustem have been developed in India and are now in advanced state of field trials.

Several wild species genomes have been dissected and chromosome addition lines have been generated. The species include *Arabidopsis thaliana, Brassica oxyrrhina, Crambe abyssinica Diplotaxis erucoides*, *Moricandia arvensis,* and *Sinapis arvensis*. Genes controlling traits of interest have been located and in some cases introgressed to crop species. Chromosome additions have been characterized through RAPD, RFLP, AFLP and FISH.

Future perspectives

Many articles have appeared in the last 75 years on various aspects of wild germplasm. Unfortunately, this germplasm has not been thoroughly characterized for various traits and available information is fragmentary. It is to be systematically investigated for proper utilization. Also more collections are needed from centers of variability before these species become extinct due to extensive cultivation. Introgression of desirable genes requires knowledge of precise location on chromosomes. Genome dissection and generating various chromosome addition lines in the background of crop genomes are desired goals. In situ hybridization techniques will be helpful to identify such additions with more precision. In the absence of intergenomic chromosome pairing, translocations can be induced through mutagenesis. Asymmetric somatic hybridizations in several instances have been carried out to achieve such introgression successfully. In view of advances made in molecular biology, identification of QTL for productivity traits and pyramiding them into crop cultivars assume significance.

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