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Use made of wild legume relatives in breeding

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Abstract

Presently vast genetic resources are available for improvement of the main crops used by humans and animals. The *ex-situ* collections safeguard those resources collected in the past, although not all collections are safe even today as far as personnel and facilities are concerned. Use of *in-situ* collections is feasible but meeting with obstacles. Free accessibility is not as straightforward as has been in the past. Apart from the cultivated accessions of crops, wild relatives have always attracted breeders, for these contribute many useful traits. Their genetic background, particularly of species in the secondary or tertiary gene pool, makes transfer difficult requiring new techniques to effectuate gene transfers. There have been many attempts and evaluation and conservation of wild relatives is usually a task taken up by most genebanks. Genetic modification, the modern way of transferring wanted genes, has barely begun for the legume food crops. This paper presents some examples of successful use made of wild relatives of chickpea, pigeonpea, fababean and lentil for breeding during the past decennia.

Introduction

When the senior author was invited to join ICRISAT, the International Crops Research Institute for the Semi-Arid Tropics, in 1974 as a Pulse Germplasm Botanist, it was with the specific background he had acquired writing a taxonomic revision of the genus *Cicer*,

chickpea and its wild relatives. The other aspects of his thesis monographing the chickpea were soon taken over by further developments, but apart from some newly described species and additions to the taxa of *Cicer*, that part is still common reference (Van der Maesen, 1972). Duties involved the collection of wild relatives in place, and of other mandate crops of the CGIAR (of the Consultative Group of International Agricultural Research) institutes.

The use of “wild” genes in plant breeding is fraught with many obstacles. Growing the species produces difficulty, and the production of pollen at the right time is no sinecure to enable cross-pollination. Nevertheless, there have been many attempts and evaluation and maintenance of wild relatives is usually a task taken up by most genebanks (Table 1 and 2).

Pre-breeding could be and is often a task for genebank personnel, who usually have the required breeding skills. Genetic resources of tropical legumes are variously available *ex-situ* (van der Maesen, 1988) and gaps still exist. Some species are endangered or even presumed to be extinct, such as *Cicer subaphyllum* Boiss. (collected only in 1841) and the close relative *C. stapfianum* Rech.f. (only found in 1885). *Cajanus grandiflorus* (Benth. ex Baker) Maesen was found in India for the last time in 1948, *C. villosus* (Benth. ex Baker) Maesen as long ago as 1895. *C. subaphyllum* and *C. stapfianum* were recently (1997) rediscovered in Persia (Javadi pers. comm. 2007).

Even though the availability in genebanks is incomplete, even poor (Berger *et al.*, 2003, Table 2), their conservation should be given sufficient attention, seen the difficulties met when the rules of the CBD (Convention of Biological Diversity) are strictly followed (van der Maesen *et al.*, 2005). For detailed discussions of protection and farmers’ rights with particular reference to India, see Singh, (2005).

Chickpea

The genepool of chickpea includes 8 annual and 34 perennial wild species and constitutes valuable genetic resources for the improvement of cultivated *Cicer arietinum* L. Most studies on wild *Cicer* species have concentrated on annual species because of various difficulties associated with propagation of perennial species. In almost all studies, *Cicer reticulatum* Ladiz., the purported wild progenitor of chickpea, has been found to be the most closely related wild species, followed by *C. echinospermum* P.H.Davis. Several economic traits have been identified in wild *Cicer* species, such as resistance to diseases and nematodes, tolerance to cold, and higher seed protein content and plant biomass (Singh *et al.*, 1997, Table 3). At the International Center for Agricultural Research in Dry Areas (ICARDA) in Syria, resistance to cyst nematodes has been introgressed from *C. reticulatum* into the cultigen. Two lines with high level of resistance to cyst nematode and relatively good agronomic traits (ILC 10765 and ILC 10766) have been registered (Malhotra *et al.*, 2002).

Table 1. Wild relatives of grain legumes available in *ex-situ* collections

Country	Institute	Town	<i>Cajanus</i>	<i>Cicer</i>	<i>Lens</i>	<i>Pisum</i>	<i>Vicia</i>
Australia	SARDI	Adelaide SA		1		8	592
	Austr. Tropical Crops & Forages	Biloela Qld	195				
	Austr. Temp. Field Crops Coll.	Horsham Victoria		144	16	30	150
	CSIRO	Townsville					111
Canada	Univ. Saskatchewan	Saskatoon			218		
China	CAAS	Beijing					220
Colombia	CIAT	Cali	83				3
Czech Rep.	AGRITEC Res. Inst. for Field Crops	Sumperk Troubsko				1	21 49
Ethiopia	ILRI	Addis Ababa		3		18	255
Germany	BAZ IPK	Braunschweig Gatersleben					53 53
Hungary	Inst. Agrobotany	Tapioszele					624
India	ICRISAT IGFRI	Patancheru Jhansi	209 10	135			16
Israel	Hebrew Univ.	Jerusalem		n.d.	n.d.	n.d.	n.d.
Pakistan	NARC	Islamabad		90			90
Poland	Inst. Pl. Genetics	Poznan				80	
Portugal	Est. Agronomia Nacional	Oeiras					175
Russia	VIR	St. Petersburg					419
Spain	INIA	Alcala de Henares			97		2264
	Centro de Investigac.	Cordoba					700
	Centr. Inv. Agr. de Albaladejito	Cuenca			25		
Syria	ICARDA	Aleppo		241	467	50	
Turkey	Aegean Agr. Res. Inst.	Izmir-Menemen		32	25		821
Ukraine	Inst. Plant Prod. V.J. Yurjev	Kharkiv			25		
UK	RBG Kew John Innes Cent.	Wakehurst Norwich		7		1 117	41
	ICUC, Univ.	Southampton			136	16	1834
USA	USDA-ARS Desert Legume Progr.	Pullman WA Tucson AR		145	124	90	552 12

Source: in part from IPGRI database, consulted 3-10-2005, data for various dates, some up to 2002; n.d. = no data provided.

Table 2. Number of annual wild *Cicer* (and *C. anatolicum*) accessions held in the world collection, based on data from the genebanks of the CGIAR system, as well as those registered with the International Plant Genetic Resources Institute (Berger *et al.*, 2003)

Species	ICARDA	ATC	USDA-ARS	ICRISAT	AARI	ICUC	ILRI	IPK	VIR	Total
<i>C. anatolicum</i>	1	2	5	3	3					14
<i>C. bijugum</i>	41	35	18	8	2					104
<i>C. chorassanicum</i>	5	3	2	3						13
<i>C. cuneatum</i>	5	3	1	1			2			12
<i>C. echinospermum</i>	13	10	11	4	5					43
<i>C. judaicum</i>	73	4	35	23		5				135
<i>C. pinnatifidum</i>	52	3	21	11	7			3	1	98
<i>C. reticulatum</i>	60	50	17	6	6					139
<i>C. yamashitae</i>	5	3	3	3						14
Total	255	111	112	62	23	5	2	3	1	572

ICARDA: International Centre for Agricultural Research in the Dry Areas, Aleppo, Syria.

ATC: Australian Temperate Field Crops Collection, Horsham, Victoria.

GRIN: USDA-ARS, National Genetic Resources Program, Pullman, WA, USA.

ICRISAT: International Crops Research Institute for Semi-Arid Tropics, Patancheru, India.

AARI: Aegean Agricultural Research Institute, Izmir, Turkey.

ICUC: International Centre for Underutilised Crops, Southampton, UK.

ILRI: International Livestock Research Institute, Addis Ababa, Ethiopia.

IPK: Institute for Plant Genetics and Crop Plant Research, Gatersleben, Germany.

VIR: N.I. Vavilov Research Institute of Plant Industry, St. Petersburg, Russia.

Note that the sum total of accessions held across the various genebanks considerably overestimates the actual number in the world collection because of duplication.

Table 3. Useful characters in wild relatives of grain legumes

<i>Cajanus</i>	cytoplasmic male sterility, higher seed protein content, resistance to podborer
<i>Cicer</i>	resistance to diseases, e.g. Phytophthora root rot, Ascochyta blight and (cyst) nematodes, tolerance to cold, higher seed protein content and plant biomass
<i>Lens</i>	winter hardiness, yield potential
<i>Pisum</i>	variation per se, obviating blanching treatment
<i>Vicia</i>	resistance to black bean aphid, cold resistance

Promising high yielding lines with good agronomic and seed traits have been obtained from crosses of chickpea with *C. reticulatum* and *C. echinospermum*. The yield level of the top 10 lines ranged between 4.5 to 5.7 t ha⁻¹ (Malhotra *et al.*, 2003). Transgressive segregates for early flowering were obtained from *C. arietinum* x *C. reticulatum* crosses. Some of these flowered in 37 to 39 days as compared to 70 days for chickpea and 92 days for wild species (Singh *et al.*, 1984). These lines were cold tolerant and useful for development of early and cold tolerant cultivars (Malhotra *et al.*, 2003). Lines with high level of cold-tolerance, high yield and high biomass have been obtained from crosses of chickpea with *C. echinospermum* (ICARDA 1995). *Phytophthora* root rot is a serious disease of chickpea in Australia. Resistant sources to this disease are lacking in cultivated chickpea, however, a

good level of resistance has been found in *C. echinospermum*. Efforts were initiated to transfer resistance from this wild relative to the cultivated chickpea (Knights *et al.*, 2002) and Croser *et al.* (2003) reviewed progress and prospects for introducing several characters from wild *Cicer* spp. Now an efficient protocol for embryo rescue is also available (Coyne *et al.*, 2005). Various aspects and further developments are described in the chickpea handbook edited by Yadav *et al.* (2007).

Non-monophyly of even the annual species, suggesting shifts in the traditional infrageneric classification of *Cicer* (Javadi and Yamaguchi 2004a & b, 2005), perhaps explain the widely different results in crossing those with the chickpea.

Faba bean

So far, all attempts to obtain interspecific hybrids between faba bean, *Vicia faba* L. and related *Vicia* species (vetch) have been unsuccessful. It was concluded that postzygotic barriers prevent the development of interspecific hybrid embryos (Wijaya *et al.*, 2004). After selecting six out of eleven wild vetch species, morphologically similar to faba bean, three of these were crossed to faba bean. Despite thousands of pollinations in the most recent attempts no seeds were obtained, but *V. galilaea* Plitmann & Zohary produced slightly better pod and seed development than combinations of *V. faba* with *V. narbonensis* L. and *V. peregrina* L. The disparate chromosome numbers, size and DNA content are obvious obstacles (Bond, 1995). *Vicia narbonensis* is a secondary or forage crop in itself, some few thousands of ha are reported from Spain, Turkey and Iraq (Erskine, see Enneling and Maxted, 1995)

Lentil

Lentil has four wild relatives: i) *Lens culinaris* Medik. subsp. *orientalis* (Boiss.) Ponert, ii) *L. odemensis* Ladiz., iii) *L. ervoides* (Brign.) Grande, and iv) *L. nigricans* (M. Bieb.) Godr. *Lens culinaris* subsp. *orientalis* is normally crossable with cultivated lentils and is considered to be its progenitor (Ladizinsky, 1979). In lentil, wild species have been used to improve the yield potential of the cultigen (Table 3). After crossing lentil with its wild progenitor *L. culinaris* subsp. *orientalis* at ICARDA, 10 lines were selected from bulk segregating populations for distribution worldwide in the Lentil International Nursery Program in 1984. Tests were done in 13-15 countries from 1985-1988. Among these selections, the small seeded ILL 5700 ranked 3rd, 1st, 7th and 2nd for average yield in set of 24 entries tested in 13-15 countries from 1985-1988. This selection with wild parentage has since been widely used in crossing to introgress wild gene into the cultivated plant. The other major use of wild germplasm in lentil breeding has been to introgress winter-hardiness genes from *L. culinaris* subsp. *orientalis* (Robertson & Erskine, 1997).

Pea

As most taxa in the genus *Pisum* interbreed, there are apparently just two true species: *Pisum sativum* L. and *P. fulvum* Sibth. & Sm. The other wild taxa are better considered subspecific entities: *P. sativum* subsp. *elatius* (M. Bieb.) and subsp. *humile* (Holmboe)

Greuter, Matthäs & H.Risse. Under the name of wild peas many other Leguminosae are known, such as *Lathyrus* and *Vigna* spp.

Pigeonpea

Taxonomically, the genera of *Atylosia*, *Dunbaria*, and *Rhynchosia* are very close to pigeonpea (subtribe *Cajaninae* in the tribe of the beans, *Phaseoleae*). Biosystematic studies including morphology, karyotype, cross-compatibility, fertility and finger printing revealed that *Atylosia cajanifolia* Haines is the closest relative of pigeonpea. Next of kin are *A. lineata* Benth. ex Baker, *A. scarabaeoides* (L.) Benth., *A. sericea* Benth. ex Baker, *A. albicans* Wight and Arn., *A. volubilis* (Blanco) Gamble, *A. platycarpa* Benth. and *R. rothii* Benth. ex Aitch., in that order (Pundir and Singh, 1985). Based on the morphological, cytological, and chemotaxonomical data, two of the above three genera were considered to be congeneric and consequently all species of *Atylosia* (28) have been merged in *Cajanus*. Some other species in related genera have also been merged into *Cajanus*. Thus the genus *Cajanus* now includes 32 species including the cultigen *C. cajan* (L.) Millsp. (van der Maesen, 1986). Further *Cajaninae* are listed by van der Maesen et al. (1986).

Hybrid vigor has been utilized in a number of agricultural and horticultural crops. However, this has not been possible in food legume crops until the last 10-15 years mainly due to the cleistogamous nature of flower that does not permit economical mass pollen transfer, necessary for large-scale hybrid seed production. Pigeonpea, however, is an exception where insect-mediated natural out-crossing up to 70% has been reported (Saxena et al., 1990). The availability of cytoplasmic-genic male sterility (CMS) in this crop has opened up the possibilities of developing commercial hybrids in this legume crop. Some wild relatives of pigeonpea have been utilized to develop the CMS system in this crop. Cultivated pigeonpea crosses with *Cajanus sericeus* (Benth. ex Baker) Maesen (Arinayagam et al., 1995), *C. scarabaeoides* (L.) Thouars (Tikka et al., 1997; Saxena and Kumar, 2003), *C. volubilis* (Blanco) Blanco (Wanjari et al., 1999), and *C. acutifolius* (F.v.Muell.) Maesen (Mallikarjuna and Saxena, 2005) were useful to obtain a CMS system. Experimental hybrids developed by using CMS lines have demonstrated a yield advantage of over 25% (Saxena 2004).

Some wild relatives of pigeonpea, namely *C. scarabaeoides* (L.) Thouars, *C. sericeus* (Benth. ex Baker) Maesen, *C. albicans* (Wight and Arn.) Maesen, *C. crassus* (Prain ex King) Maesen, *C. platycarpus* (Benth.) Maesen, and *C. cajanifolius* (Haines) Maesen have higher seed protein content (mean 28.3%) compared to pigeonpea cultivars (24.6%) (Singh & Jambunathan, 1981). The high seed protein content of *C. scarabaeoides* was successfully transferred to a good agronomic background in pigeonpea. The line developed (ICPL 87162) had consistently higher seed protein content (29.3 to 33.8% in split cotyledons, dhal) compared with a range from 22.6 to 25.3% in BDN-1, a much-used cultivar of pigeonpea (Reddy et al., 1997). Investigations at ICRISAT have revealed higher level of resistance to podborer (*Helicoverpa armigera*) in *C. scarabaeoides* compared to *C. cajan*. Research to transfer this resistance to good agronomic background of pigeonpea is in progress. The

reported results are an improvement over what had been possible to report in 1998 (van der Maesen, 1998).

Conservation of Wild Species

As referred to before, the conservation of wild species is difficult for a number of species. Particularly the wild perennial species of *Cicer* cannot be grown at the major places of breeding, in India below 2000 m.a.s.l., in the Middle East, etc., where cool seasons have short day length, where no winter rest can be given, and/or where the moisture regime is totally different from the high-altitude, long-day length, and high insolation conditions. Only in some field stations near Pullman, Washington state, USA, these conditions can be met to a reasonable degree, and it is there that the perennials can be grown with success. If a garden could be created along the stretch Khyelang to Leh, where snow is common in winter, perennial chickpea species may thrive in the Indian subcontinent. It is the only area where a perennial *Cicer*, *C. microphyllum* Benth., is native. Similar climatic conditions exist in high-altitude Pakistan and Afghanistan, but as in India, political inaccessibility is even more severe in these border areas than physical inaccessibility. Many wild perennials grow in Turkey, Iran and Central Asian republics (perhaps including unknown species new to science), but the possibility of high-altitude maintenance gardens there is a bit far-fetched. Some are in need of (re)collection as their taxonomical status should be reconsidered, and even some may have to be considered as extinct (see Introduction).

Annual *Cicer* spp. are less difficult to maintain, but some are only available as a single accession (*C. cuneatum* Hochst. ex Rich., *C. canariense* A.Santos Guerra & G. P. Lewis). Several species have by no means a good geographical representation in genebanks

Seen the utility of wild species in supplying useful genes to the pulses mentioned, new attention in expanding the wild germplasm should be given, also because human occupation of certain areas often goes at the detriment of all but the strongest wild weeds.

Conclusions

Useful results have been obtained by crossing wild relatives of grain legumes, but at the cost of much labor. With the new tools of genetic engineering becoming available, gene transfer from wild relatives should be feasible more rapidly. In the meantime the wild gene pool should remain accessible *ex situ* (see Table 1) and preferably be expanded for use in breeding and taxonomic verification. The availability of wild *Cicer* species is very uneven (Berger *et al.* 2003, see Table 1 & 2) and repetitive, and this is not the only genus in that situation.

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