

How wide is the Genetic Base of Pulse Crops?

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Abstract

Genetic base as revealed by the pedigree records of released varieties appears to be narrow in major pulse crops because of the frequent use of the same parents and their derivatives in breeding programmes. Pedigree analyses of 86 chickpea cultivars that have been released through hybridization in the country traced back to 95 ancestors. This appears to be insignificant part of the germplasm accessions conserved in various gene banks around the world. Rate of infusion of new germplasm in pulse breeding programmes has also been slow due to repeated use of the same germplasm for development of cultivars. This shows that a vast majority of accessions held in gene banks have made limited contribution in development of modern cultivars. In chickpea, 41% varieties developed through hybridization have Pb 7 as one of the ancestors in their pedigree. Ancestors T 1 and T 190 appeared in 34% varieties of pigeonpea. Similarly, T 9 and T 1 appeared in 64% and 35% varieties of urdbean and mungbean, respectively. This analysis revealed that genetic base of pulse varieties is narrow and needs immediate corrective measures by involving unadapted germplasm accessions, exotics and wild relatives in breeding programmes. There are indications that novel and useful traits can be successfully combined from related species. Pre-breeding or germplasm enhancement and incorporation need emphasis for introgression of genes and gene combinations from unadapted sources into more usable breeding material. The present paper describes the results of pedigree analyses to determine the genetic base of the released cultivars, status of utilization of exotics and wild relatives, reasons for narrow genetic base and possible approaches and strategies for its widening in pulse crops.

1. Introduction

Pulses are the mainstay of Indian agriculture as India accounts for 32% global area under pulses with 25% share in the global kitty. The major pulse crops grown in the country are chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* L.), pigeonpea (*Cajanus cajan* Millsp.), urdbean (*Vigna mungo* L. Hepper), and mungbean (*Vigna radiata* L. Wilczek). India holds the first position in area as well as production of these crops (Table 1). However, the present productivity of these pulse crops is not only low as compared to potential yield realized in the experimental plots mainly because of prevalence of biotic and abiotic stresses but also static for the past several years. In order to combat these biotic stresses mainly diseases and of course to attain higher yield levels, a large number of improved varieties have been developed through different breeding methods mainly pure line selection and hybridization utilizing intra-specific diversity. After thorough evaluation across the country under All India

Coordinated Research Projects (AICRP), these varieties were released for general cultivation in the suitable zone(s) based on the yield superiority over the checks. While releasing the varieties, their pedigrees have never been taken into account. This has inadvertently led to narrow genetic base of the released varieties, which can lead to crop vulnerability due to pest and disease epidemics or unpredictable climatic factors. Notwithstanding the number of cultivars released, there has been limited progress for pulses in almost all the environments. For example, yield of lentil, urdbean and mungbean have remained on a plateau for the past ten years, while chickpea and pigeonpea observed marginal increase in yield during the period. This indicates that immediate steps should be taken up for broadening the genetic base of pulse crops.

Table 1: India's position in area and production of different pulses and present yield level

Crop	Global position		Yield (kg ha ⁻¹)	
	Area	Production	1990-92	1999-2001
Chickpea	1	1	700	797
Lentil	1	2	678	681
Pigeonpea	1	1	674	729
Urdbean	1	1	460	439
Mungbean	1	1	390	361

2. Genetic Bottlenecks

During the evolution process, all crops have been subjected to genetic bottlenecks and subsequent founder effects. As a result, a number of pulse crops have inherently narrow genetic base caused by bottlenecks at domestication, by migration or by epistatic and disease effects. During domestication, it is likely that only a small proportion of the total diversity of the wild population is sampled (Ladizinsky, 1985). Subsequent gene flow between the new cultivars and its wild progenitors might be restricted by breeding barriers, depending on the breeding system of the crop concerned and the nature of the domestication event (Cooper *et al.*, 2001). The pulse crops, which might have narrow genetic base because of bottlenecks at domestication, include *Phaseolus* beans, pigeonpea and chickpea.

Crop migration followed by natural selection over time in the new environment can lead to ecogeographic differentiation of the genetic variation. Chickpea presents an example of ecogeographic differentiation in the form of *desi* and *kabuli* types. Major crop movements occurred in the pre-Columbian times, for example, the movement of cowpea from Africa to semi-arid areas of India. The dissemination of mungbean

from its centre of origin in western Asia to Southeast Asia led to a reduction in seed protein diversity (Tomooka *et al.*, 1992). Genetic bottlenecks due to geographic movements may also have occurred very recently. For example, soybean originated in China at least 3000 years ago was not widely grown outside Asia until 1900s and its diffusion was subject to major genetic bottlenecks. The genetic base of cultivated soybean outside Asia is, therefore, extremely narrow (Shoemaker, 1986). In some regions, it is thought that initial crop introductions were based upon extremely narrow genetic bases. However, it is likely that crops in most locations have been subjected to recurrent introductions of new germplasm following the initial introductions (Simmonds, 1995).

A genetic bottleneck also occurs when the genetic diversity of a population is reduced due to decrease in its population size. Genetic diversity is likely to have been lost over time from what was domesticated due to random genetic drift caused by small population sizes resulting from either new crop introductions or crop failures. Such founder event has been an extremely important factor in narrowing the genetic base of many crops especially where their cultivation has moved beyond their centre of diversity/origin. Examples of crops with narrow genetic base arising during migration include soybean and lentil.

Alterations in a small number of key genes could potentially have major effects on the genotype and morphology as a whole. This may have been the case in the domestication of some of the cultivated legumes. For example, *kabuli* chickpea has been evolved on the basis of single point mutation and thus is based on a very narrow genetic base.

Narrowing of the genetic base may result from specialization within the crop. For example, breeding of macrosperma varieties has been mainly confined to macrosperma germplasm and breeding of microsperma to microsperma germplasm in lentil. Similarly, breeding of *kabuli* type chickpea is largely based on *kabuli* germplasm and *desi* type on *desi* germplasm except a few instances where varieties like L104, L144, L550, Pusa 267 Pusa 244, Pusa 256 and Pusa 261 have been released through *desi* x *kabuli* introgression.

Some of pulse crops have narrow genetic base arising from the lack of diversity in previous breeding practices. Pedigree analyses of the advanced breeding lines of lentil included in the initial variety trials of AICRP during the past ten years show that 131 ancestors are involved in breeding for 308 lines. The mean genetic contribution of the ancestors showed that as high as 47% of the genetic base is accounted to six ancestors. Bottlenecks during modern plant breeding might be indicated, for example, by a small number of parents or high degree of relatedness between parents as elucidated from pedigree analyses of the released varieties.

2.1 Pedigree Analyses of the Released Varieties

A total of 431 varieties of different pulse crops are released in the country. Of them, 126 varieties in chickpea, 100 in pigeonpea, 72 in urdbean, 98 in mungbean, and 35 in lentil are developed through different breeding methods. About 229 varieties are developed through hybridization following pedigree selection. Pedigree analyses of these varieties revealed narrow genetic base in most of the pulse crops (Table 2).

Table 2: Present status of genetic base of released varieties in different pulse crops

Crop	Varieties developed through hybridization	Number of ancestor: used	Relative contribution of top 10 ancestors	Name of the top five ancestors	Appearance of most frequent ancestor
Chickpea	86	95	35%	Pb 7, IP 58, F 8, Rabat, S 26	40.7% (Pb 7)
Lentil	14	22	30%	L 9-12, T 8, LG 171, JLS 2, JLS 1	17.14% (L 9-12)
Pigeonpea	47	57	48%	T1, T 190, P 4768, Sangareddy selection, Brazil 1-1	34.1% (T 1, T 190)
Urdbean	33	26	69%	T 9, D 6-7, G 31, Netimumu, AB 1-33,	63.6% (T 9)
Mungbean	49	71	79%	T 1, T 49, BR 2, G 65, Madira	34.7% (T 1)

Pedigree analyses of 86 chickpea cultivars that have been released through hybridization in the country traced back to 95 ancestors. The top ten ancestors contributed more than 35% to the genetic base of the released varieties. Most frequently used ancestors are Pb 7, IP 58, F 8, Rabat, and S 26. About 41% varieties developed through hybridization have Pb 7 as one of the ancestors in their pedigree.

The pedigrees of 35 lentil varieties released in the country were traced back to 22 ancestors. Out of these 22 ancestors, the top ten ancestors contributed 30% to the genetic base of the released varieties. L 9-12 is the most frequently used parent (17%) followed by T 8, LG 171, JLS 2 and JLS 1.

In pigeonpea, 57 ancestors were used to develop 47 varieties through hybridization. The top ten ancestors contributed 48% to the genetic base of the released varieties. Ancestors T 1 and T 190 appeared in 34% varieties. Other ancestors used very frequently are P 4768, Sangareddy selection and Brazil 1-1.

Similarly, T 9 and T 1 appeared in 64% and 35% varieties of urdbean and mungbean, respectively. In urdbean a total of 70 varieties were released involving only 26 ancestors. The relative genetic contribution of the top ten ancestors was 69% indicating very narrow genetic base of the released varieties. T 9 was the most frequently used ancestor appearing in 64% of the varieties followed by D 6-7, G 31, Netimumu and AB 1-33. In mungbean, 49 varieties were developed through hybridization involving 71 ancestors. The top ten ancestors contributed 79% of the genetic base. T 1 appeared in 35% of the varieties followed by T 49, BR 2, G 65 and Madira.

2.2 Reasons for Narrow Genetic Base of the Released Varieties

Crop improvement depends largely on the genetic variability which is available to breeders for exploitation through various methods of plant breeding. In pulses the amount of variability that is available for selection is relatively limited due to various genetic bottlenecks discussed earlier. Extensive and repetitive use of superior genotypes with common ancestors explained why the genetic base of the released varieties is narrow and the integration of new germplasm is paramount for broadening the genetic base of released varieties in major pulse crops. The number of ancestors used in developing the varieties appears to be insignificant part of the germplasm accessions conserved in various gene banks around the world (Table 3). Rate of infusion of new germplasm in breeding programmes has also been limited and slow due to repeated use of the same germplasm for developing cultivars. This shows that a vast majority of accessions held in gene banks have made little contribution in development of modern cultivars. This analysis has brought this fact out that genetic base of pulse varieties is narrow and needs immediate corrective measures by involving unadapted germplasm accessions, exotics and wild relatives in hybridization programmes.

Table 3: *Ex Situ* germplasm collections of different pulse crops

Crop	Base collection at NBPGR	World collection
Chickpea	14566	69736
Lentil	2212	27424
Pigeonpea	5454	24938
Urdbean	610	-
Mungbean	2789	-

Moreover, traits other than high yield are rarely considered as selection criteria. However, examples can be cited where selection for other traits has also been taken into account in addition to yield. In chickpea and pigeonpea, a variety must have a specified level of fusarium wilt resistance for its release in addition to high yield. In

mungbean and urdbean, a variety must have mungbean yellow mosaic virus resistance in addition to superior yield. Promotion and release are largely dependent on a comparison of the yield of new entries with checks or trial means. Data on other important traits are also collected but rarely used in promotion and release decisions.

3. Approaches for Broadening the Genetic Base

There is concern that genetic diversity is limited both within production systems and in breeding programmes and that there is need for concerted efforts to broaden the genetic base of pulse crops. Genetic base of a crop includes all the genetic diversity that is readily available to breeders and growers for adaptation to any particular environment of interest. It includes the genetic diversity of varieties already in use in any one production system, as well as the materials currently being used to develop new varieties for that production environment. Less readily available material exists *in situ* and *ex situ* and wild gene pools. This exotic unadapted material is generally not considered as part of the genetic base but can be brought into it through introgression or a less commonly used approach called incorporation. Incorporation is more powerful than introgression for broadening the genetic base. Broadening the genetic base of crops can be considered at three levels:

1. Increasing the extent of useful diversity available to breeders that is broadening the genetic base of breeders' material through genetic enhancement or pre breeding.
2. Increasing the range of useful diversity available to farmers as planting material.
3. Increasing the diversity of crops and varieties grown in the field.

3.1 Pre-breeding Approach

Pre-breeding is the transfer of genes and gene combinations from unadapted sources into more usable breeding material. It is often considered to be an activity at the interface between germplasm conservation and utilization. Dedicated programmes of pre-breeding or genetic enhancement are required immediately for broadening the genetic base of pulse crops. Two distinct pre-breeding approaches can be used. The most common approach is when a single gene is transferred into the elite gene pool by introgression either through backcrossing or by using biotechnological tools. Panmictic populations established on a very broad base such as the barley composite cross can also constitute pre-breeding approach.

3.1.1 Wide hybridization: Wild species are generally more variable than the cultivated species and form a rich reservoir of useful genes. This has been shown for soybean (Doyle, 1988) and lentil (Havey and Muehlbauer, 1989). However, there are significant

technical barriers to obtain viable hybrids between primary gene pool and wild relatives in the secondary and tertiary gene pools (Table 4). A significant element of plant breeding to broaden the genetic base of crops is concerned with finding ways to overcome such isolating barriers so as to gain access to novel and useful genetic variation. Reproductive isolation, incompatibility barriers, embryo or endosperm abortion, hybrid sterility and limited levels of genetic recombination are significant obstacles to the greater use of wild germplasm. These obstacles are in addition to those of undesirable linkages to non-agronomic traits once gene flow has been achieved.

Table 4: Different gene pools of selected pulse crops

Crop	Primary gene pool	Secondary gene pool	Tertiary gene pool
Common bean	<i>Phaseolus vulgaris</i>	<i>P. coccineus</i> , <i>P. costaricensis</i> <i>P. polyanthus</i>	<i>P. acutifolius</i> <i>P. lunatus</i> and other <i>Phaseolus</i> spp.
Chickpea	<i>Cicer arietinum</i> <i>C. reticulatum</i> <i>C. echinospermum</i>	None	<i>C. bijugum</i> , <i>C. pinnatifidum</i> , <i>C. judaicum</i> , <i>C. chorassanicum</i> , <i>C. montbretii</i>
Lentil	<i>Lens culinaris</i>	None	<i>L. nigricans</i>
Pigeonpea	<i>Cajanus cajan</i>	<i>C. acutifolius</i> , <i>C. albicans</i> , <i>C. cajanifolius</i> , <i>C.</i> <i>lanceolatus</i> , <i>C. latisepalus</i> , <i>C. lineatus</i> , <i>C. reticulatus</i> , <i>C. scarabaeoides</i> var. <i>scarabaeoides</i> , <i>C. sericeus</i> , <i>C. trinervius</i>	<i>C. goensis</i> , <i>C. heynei</i> , <i>C. kerstingii</i> , <i>C. mollis</i> , <i>C. platycarpus</i> , <i>C. rugosus</i> , <i>C. volubilis</i> and other species
Mungbean	<i>Vigna radiata</i> var. <i>radiata</i> <i>V. radiata</i> var. <i>sublobata</i> <i>V. radiata</i> var. <i>setulosa</i>	<i>V. mungo</i> var. <i>mungo</i> <i>V. mungo</i> var. <i>var silvestris</i> <i>V. aconitifolia</i> <i>V. trilobata</i>	<i>V. angularis</i> <i>V dalzelliana</i> <i>V. glabrescens</i> <i>V. grandis</i> <i>V. umbellata</i> <i>V. vexillata</i>
Urdbean	<i>V. mungo</i> var. <i>mungo</i> <i>V. mungo</i> var. <i>silvestris</i>	<i>Vigna radiata</i> var. <i>radiata</i> <i>V. radiata</i> var. <i>sublobata</i> <i>V. radiata</i> var. <i>setulosa</i> <i>V. aconitifolia</i> <i>V. trilobata</i>	<i>V. angularis</i> <i>V dalzelliana</i> <i>V. glabrescens</i> <i>V. grandis</i> <i>V. umbellata</i> <i>V. vexillata</i>

Continuing advances in wide crossing techniques such as embryo culture and the development of novel crossing strategies (bridge crosses) are making the wild gene pools of many crops ever more accessible. The success rate of gene transfer in such wide crosses can be increased by knowledge of the chromosome pairing mechanisms and their genetic control. It is likely that continuing advances in structural genomics and genetic engineering will result in new strategies for wide hybridization.

Some of the recent examples of the introgression of genes from landraces, exotics and wild relatives are given in Table 5. Introgression of resistance to cyst nematode from *C. reticulatum* is an example in chickpea (Di Vito *et al.*, 1996). The resistant selections are under evaluation in yield trials at ICARDA (Ocampo *et al.*, 2000). Similarly, lines with high levels of cold tolerance derived from *C. reticulatum* and *C. echinospermum* are now included in the yield trials. Experimental hybrids based on cytoplasmic male sterility derived from *Cajanus scarabaeoides* and *C. sericeus* in pigeonpea are being evaluated in the AICRP trials. One hybrid, GTH 1 has been released recently, which has male sterile cytoplasm from *Cajanus scarabaeoides*. India has indigenous lentils of a specific ecotype (*pilosae*), which exhibits a marked lack of genetic diversity. Erskine *et al.* (2001) used several approaches to overcome this ancient bottleneck in lentil. To broaden the genetic base of lentil, Precoz (ILL 4605), an extra large seeded and extra early germplasm line, was introduced in India besides other pre-adapted germplasm from West Asia. The intensive use of Precoz and its derivatives in hybridization has resulted in the widening of genetic base of lentil in Indian subcontinent. Pedigree analysis of the 308 IVT entries included in the last ten years showed involvement of Precoz in 23% of the entries. In mungbean, three varieties namely, IPM 99-125, Pant Mung 4 and HUM 1 have been the outcome of mungbean x urdbean crosses. These cultivars have improved plant types in addition to higher MYMV resistance and synchronous maturity.

Table 5: Successful introgression in pulse crops

Crop	Exotics/wild relatives	Character	References
Chickpea	<i>C. reticulatum</i>	Cyst nematode	Di Vito <i>et al.</i> (1996)
	<i>C. reticulatum</i> and <i>C. echinospermum</i>	Cold tolerance	ICARDA (1995)
Lentil	<i>Lens orientalis</i>	Cold tolerance Agronomic traits	Hamdi <i>et al.</i> (1996) Abbo <i>et al.</i> (1992); ICARDA (1995)
	Precoz	Extra large seed and extra earliness	Erskine <i>et al.</i> (2001)
Pigeonpea	<i>Cajanus sericeus</i>	Male sterility	Ariyanayagam <i>et al.</i> (1995)
	<i>C. scarabaeoides</i>	Male sterility	Tikka <i>et al.</i> (1997)
Mungbean	<i>Vigna mungo</i>	YMV resistance, plant type traits	Singh and Dikshit (2003)

3.1.2 Transgenic: Transgenic approaches provide new options for broadening the genetic base in those cases where current options are lacking in their efficacy or existence. Plant genetic transformation techniques such as *Agrobacterium* mediated transformation and direct gene delivery system (biolistics) allow the precise transfer of genes from any organism into either plant nuclear or chloroplast genomes. Many isolated plant genes are now being transferred between sexually incompatible plant species. In chickpea and pigeonpea, *Helicoverpa* pod borer is a major insect pest for which no genetic solution exists. Screening of more than 5000 germplasm accessions revealed that there are no resistant sources against this insect pest in the germplasm pool. This requires development of transgenics having *Cry IAb* and *Cry IAa* genes from the soil bacterium, *Bacillus thuringiensis* to combat menace of *Helicoverpa* pod borer. Similar is the case for botrytis gray mold in chickpea.

In addition, most traits important to pulse production, such as yield and quality, are polygenic. Many beneficial genes may have been left behind due to the genetic bottleneck imposed on different pulse crops during early domestication and through modern breeding practices. It is likely that useful genes remain undetected among thousands of germplasm accessions. Future challenges for pulse improvement will require integrating tools developed through contemporary technologies with those of classical breeding. Recent genome mapping applications confirm that superior genes may be derived from a phenotypically inferior germplasm. DNA markers provide new tools that can be used effectively in germplasm characterization. This approach looks directly for genes or genotypes of interest, while eliminating undesirable factors from other parts of the genome. With emerging genomic tools, it is likely that most beneficial genes from germplasm will be discovered and tagged for deployment by the new approach.

3.2 Incorporation

While introgression is a useful method of introducing specific traits into a breeding population, sometimes a comprehensive broadening of the genetic base through incorporation is warranted when new genetic variability for polygenic traits is needed (Simmonds, 1993). The objective of incorporation is to broaden the genetic base of a crop by adding a wide range of new parents. This involves crossing diverse genotypes and then selecting the resulting population over a large number of generations in the target environment of the breeding programmes. The resultant population may be used directly in the breeding programme or first crossed with other locally adapted material. The simplest incorporation programme uses natural selection to adapt diverse germplasm to local conditions. This was the method used to produce Harlan's Composite Cross II of barley which was made by crossing 28 cultivars from the world collection in all

combinations followed by 60 generations of selection in California, USA. However, incorporation approach in pulse crops is yet to start for broadening the genetic base.

New plant breeding approaches such as participatory variety selection, participatory plant breeding and on-farm genetic resources conservation can contribute to these base-broadening goals. Varietal diversity in the field can contribute to the conservation of plant genetic resources besides continuous crop evolution. This process can be accelerated through farmer participatory plant breeding.

A range of factors may in fact considerably limit access to suitable new varieties at the farm level. These can include poor seed distribution systems, breakdown in informal seed supply networks, an inappropriate regulatory framework and the lack of suitable varieties. Because of these factors, improved varieties can often show low adoption rates in pulse crops. Newly released varieties find it difficult to get wide acceptance by the farmers due to non-availability of quality seed. At present, there is less than 3% seed replacement rate in pulse crops, which need to be taken up to 10-15% for achieving the desired results.

4. Future Strategies

- Making use of the broadest starting materials: Core collection may be useful tools to select genetically diverse starting material. Development of core collection for important pulse crops is a step in right direction
- A need for extensive recombination
- Weak and progressively decentralized selection: Whenever possible, selection should be based on multiple large populations and carried out over several generations in target environments in order to exploit genotype by environment interaction.

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