



## Pre-breeding efforts for widening the genetic base of cool-season food legumes

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Globally, cool-season food legumes (CSFL) mainly chickpea, field pea, lentil, faba bean, and grass pea are grown on ~33 million ha with a production of ~46 million tonnes, contributing 47% to the global pulse output. These crops hold important position in sustainable diversified agri-food systems. Average productivity of CSFL is 1380 kg/ha which is far below their genetic potential. The average annual genetic gain reported in various legume crops is estimated 0.7% which is not enough to meet the growing demand of pulses. Despite many improved varieties, narrow genetic base has been identified as one of the major constraints for limited genetic gain in CSFL crops. This is basically due to the bottlenecks during the process of evolution and domestication, which has further been compounded using limited germplasm in national and international breeding programs. Consequently, many useful alleles/variants have been left behind in landraces and crop wild relatives (CWR). This scenario is changeable, provided we conserve, evaluate and utilize CWR in breeding these crops. Sizeable number of CWR accessions are available in the global collections held at ICARDA and ICRISAT, screening of them has resulted in identification of useful donors not only for resistance to major biotic and abiotic stresses but also for phenology, plant type and yield traits. Breeders have traditionally been reluctant about the use of CWR in breeding programs due to complex and unpredictable outcomes because of crossing barriers, linkage drag, and long breeding cycle. There are now modern tools and techniques available to identify CWR with useful alleles that will enable breeders to introgress into desired agronomic back grounds more precisely and efficiently.

### Introduction

Chickpea (*Cicer arietinum* L.), field pea (*Pisum sativum* L.), lentil (*Lens culinaris* Medikus subsp. *culinaris*), faba bean (*Vicia faba* L.), and grass pea (*Lathyrus sativus* L.) are the important cool-season food legume (CSFL) crops for sustainable diversified agri-food systems. These crops are currently grown on 33 million ha area with a production of 46 million tonnes. During the last five decades, CSFL crops, especially chickpea and lentil have experienced spectacular growth in global production, mainly because of their expansion in new areas along with enhanced productivity. However, the current average productivity of CSFL crops is estimated at 1380 kg/ha which is far below their genetic potential. The annual genetic gain reported in legume crops is estimated at 0.7% (Bogale *et al.* 2015, Belete *et al.* 2017, Idrissi *et al.* 2019). Despite many improved varieties, narrow genetic base has been identified as one of the major constraints for limited genetic gains in CSFL crops. This is basically due to the bottlenecks during the process of evolution and domestication, which was further compounded during the directed breeding programs. Pedigree analysis of chickpea and lentil varieties released in India confirmed the extensive and repetitive use of a handful of germplasm as parents in hybridization (Kumar *et al.* 2004). In lentil, for example, an early flowering exotic line Precoz (ILL4605) has been utilized extensively to tailor plant architecture having vigorous growth, early maturity, and large seeds, particularly for Indo-Gangetic plains (Kumar *et al.* 2014). One option to widen the genetic base of CSFL crops is to mainstream a more systematic and targeted use of crop wild relatives (CWR) in breeding programs (Dempewolf *et al.* 2017). CWR contains a wealth of genetically important traits/alleles due to their adaptation to a diverse range of habitats. Plant breeders have long recognized the potential value of CWR, but the absence of a simple means to determine which CWR might hold valuable genetic variation has severely limited their use. Now, with the advent of modern tools and techniques combined with systematic phenotyping, it is feasible to mainstream CWRs and prioritize their use for crop improvement.





## Present Status

ICARDA genebank holds 50,968 accessions of legume crops including 15,749 of chickpea, 14,597 of lentil, 10,034 of faba bean, 6,131 of peas and 4,457 of grasspea. The current holdings also include CWR of lentil (619), chickpea (547) and grasspea (1555). Despite having *Narbonensis* as a rich CWR, it is not accessible to faba bean improvement due to strong crossing barriers (Caracuta *et al.* 2016). However, four subspecies, namely *major*, *equina*, *minor* and *paucijuga* have been identified within *Vicia faba* for faba bean improvement. Similarly, ICRISAT genebank holds one of the largest collections of *Cicer* species wherein 20,764 accessions of cultivated and wild *Cicer* species are conserved for use in chickpea breeding programs. Despite large collections of these crops, there are major germplasm gaps at species and genotype levels, and a continuum is very much required to fill these gaps in wild genepool from the unrepresented areas of diversity in genebanks. For example, vast majority of *Cicer* collections are cultivated germplasm with many duplicates. A recent collection mission has expanded the available CWR collections of both *C. reticulatum* and *C. echinospermum* by over 10-fold (von Wettberg *et al.* 2018). Similarly, out of 187 species in the genus *Lathyrus*, global collection at ICARDA represents only 45 species.

## Screening of CWR

Wild species are valuable sources of novel variation for yield traits, phenology, and resistance to biotic and abiotic stresses. Previous screening shows marked genetic variability for desired traits among landraces and CWR in lentil, chickpea, faba bean and grass pea. Wild *Lens* has shown to carry resistance/tolerance for key stresses including drought, cold, salinity, Ascochyta blight, Stemphylium blight, rust, Fusarium wilt, Sitona weevil, Orobanche, powdery mildew and Anthracnose. The highest frequency of resistance was observed for Stemphylium blight in *L. lamottei* followed by *L. ervoides* (Podder *et al.* 2013) and Sitona weevil in *L. odemensis* followed by *L. ervoides* (El-Bouhssini *et al.* 2008). Some wild accessions showing combined resistance to Ascochyta blight and Fusarium wilt or anthracnose diseases have also been identified (Bayya *et al.* 1995, Tullu *et al.* 2006). Wild relatives of lentil also offer drought tolerance in *L. nigricans*, *L. odemensis* and *L. ervoides* (Gupta and Sharma 2006) and cold tolerance in *L. culinaris* ssp. *orientalis* (Hamdi *et al.* 1996). Donors for yield traits like 100-seed weight and pods/plant have been observed in *L. lamottei* and *L. culinaris* ssp. *orientalis* (Gupta and Sharma 2006, Singh *et al.* 2014). *L. ervoides* has been identified as a good source of growth habit, phenology, biomass and seed traits (Tullu *et al.* 2011, Tullu *et al.* 2013, Singh *et al.* 2013, Kumar *et al.* 2014). Singh *et al.* (2014) evaluated the global wild *Lens* taxa originating from 27 countries under diverse agro-climatic conditions in India for three cropping seasons, resulting in wide variations for many yield attributing traits including multiple disease resistance in *L. nigricans* and *L. ervoides* accessions. Nutritional quality traits have been measured in wild *Lens* with significant diversity for RFO, raffinose, and verbascose (Tahir *et al.* 2012) and mineral (Sengupta *et al.* 2016, Kumar *et al.* 2018) concentrations.

Annual wild *Cicer* species have great potential for chickpea improvement through genetic base broadening (von Wettberg *et al.* 2018) and by providing adaptive traits lost in the cultigen. These species have been screened for reaction to Ascochyta blight, Botrytis grey mold, dry root rot, Fusarium wilt, cyst nematode, leaf miner, seed beetle, and cold tolerance both at ICRISAT and ICARDA, and a high level of resistance to each stress has been identified. *Cicer judaicum* is reported to have resistance genes for Ascochyta blight, Fusarium wilt and Botrytis grey mould and *C. bijugum*, *C. pinnatifidum* and *C. reticulatum* for resistance to cyst nematode. For example, ILWC 7-1 of *C. bijugum* showed resistance to Ascochyta blight, Fusarium wilt, leaf miner, cyst nematode and cold, and ILWC 33/S-4 of *C. pinnatifidum* to Ascochyta blight, Fusarium wilt, seed beetle, and cyst nematode.

Screening of faba bean landraces has resulted in identification of germplasm with multiple disease resistance and heat, and herbicide tolerance in *minor*, *major* and *equina* subspecies with successful examples of introgression of Orobanche resistance to major types. Screening of 2000 landraces against Ascochyta blight, chocolate spot, and rust has resulted in identification of 424 accessions as resistant to chocolate spot, 136 to Ascochyta blight and 52 to rust (Maalouf *et al.* 2016). Similarly, screening of faba bean landraces have resulted in identification of tolerance to Orobanche (Maalouf *et al.* 2011) and herbicide (Maalouf *et al.* 2018).

Wild gene pool is a rich reservoir of rare alleles for grass pea improvement which have been evaluated for ODAP content and Orobanche tolerance. Evaluation of 142 accessions of *L. cicera* showed a range of 0.073–0.513% for



ODAP content which is much lower than that in the cultivated species (Kumar *et al.* 2013). Accessions of *L. cicera* are also good source of earliness, Orobanche tolerance and cold tolerance. Evaluation of 490 CWR accessions of *Lathyrus* revealed significant variation for ODAP content ranging from 0.024 to 0.456%. On an average, *Lathyrus annuus*, *L. cicera*, and *L. gorgoni* showed low ODAP content as compared to the cultivated species. Results of 286 accessions representing 13 species showed good sources of resistance to Orobanche and low ODAP content in CWR (Fig. 1).

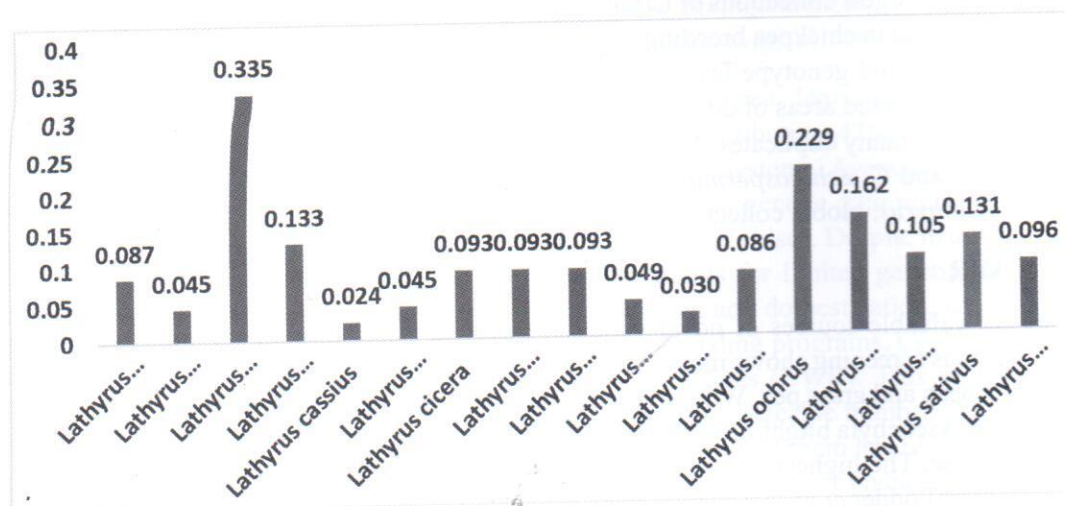


Fig 1. Screening of Lathyrus CWR for low ODAP content during 2017

## Use of CWR in breeding programs

Generally, high yielding lines are crossed to secure a high mean performance of the resulting progenies. To identify superior progenies, to ensure genetic gain in the next selection cycle, and to maintain long-term selection gain, it is important that the cross also generates high genetic variance. Mainstreaming CWR in breeding programs help to ensure high genetic variance among resultant progenies and maintain long term selection gains in a breeding program. However, the use of wild germplasm in breeding programs has been restricted mainly due to difficulties in access to exotic germplasm, extreme regional specificity of adaptation, large number of uncharacterized accessions, crossing barriers, linkage drags, and perception that wide crosses would disturb favorable combinations fixed over a period in cultivated germplasm and result in inferior recombinants. For example, interspecific crosses among *Lathyrus* species are extremely difficult with limited success. Similarly, transferring Orobanche resistance from faba bean minor to fababean major is difficult due to strong negative association between large type and Orobanche tolerance. Within the cultivated lentils, extreme specificity of adaptation limits the scope of direct introduction of exotic landraces in lentil breeding program. South Asian landraces are generally early maturing small seeded red lentils and West Asian landraces are late maturing large seeded mostly yellow lentils. Similarly, chickpea has a narrow genetic base, and this reduces the range of adaptive alleles, therefore it limits the potential solutions sought for by breeders to make adaptation selection for a wide range of agro-ecological zones. To widen the genetic base, pre-breeding efforts at ICRISAT and ICARDA breeding programs include parents of diverse origins to combine traits contributing to yield, appropriate phenology, adaptation to major biotic and abiotic stresses and market preferred traits. Derivatives from crosses between South and West Asian parents in lentil have generally shown higher yields mainly due to larger seed size introduced from the West Asian parents in the typical short duration background of South Asian genotypes (Shrestha *et al.* 2005).

Sources of extra earliness in lentil CWR, ILWL118 having less than 90 days maturity and high iron and zinc content in ILWL74 and ILWL80 have encouraged breeders to biofortify short-duration lentil through pre-breeding (Kumar *et al.* 2017). ICARDA in collaboration with NARS partners in India has implemented a pre breeding project in lentil, which has been instrumental in introgressing useful genes in mainstream breeding. Transgressive segregants





for agronomically important traits have been mined from wide crosses (Kumar *et al.* 2011, Singh *et al.* 2013, Kumar *et al.* 2014). Recent development in lentil improvement has been the successful hybridization of the cultivated lentil with *L. ervoides* using embryo rescue (Tullu *et al.* 2013) and introgression of resistance to *Orobanche crenata* and anthracnose (Fiala *et al.* 2009, Tullu *et al.* 2011). Recently, successful use of *Lens orientalis* and *L. ervoides* as a source of genes for resistance to key diseases, phenology, micro-nutrients and possibly for plant habit, and other important agronomic traits has been demonstrated with the development of elite prebred lines of lentil at ICARDA. These pre-bred lines not only performed well with >40% yield advantage over the best check (Bakaria) but also rich in micronutrients and fit in short season windows of 80-100 days (Fig. 2).

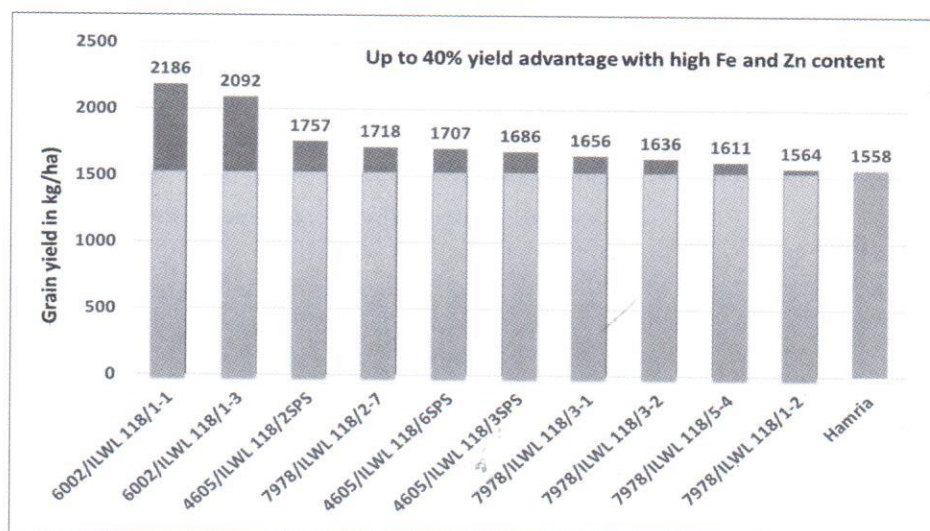


Fig. 2. Yield performance of prebred lines of lentil (*Lens culinaris* x *L. orientalis*) at Marchouch in Morocco during 2017.

Development of improved chickpea germplasm with high and stable yields and market-acceptability adapted to the Mediterranean production systems is a major goal of ICARDA Kabuli-chickpea program. Recently, good progress has been made in terms of desirable trait introgression from wild species. During the last seven years, about 10% of 996 crosses have *C. reticulatum* as one of the parents to introgress resistance to cold, drought, heat and fusarium wilt into cultivated chickpea. This has resulted in the development of improved lines with high potential yield and resistance/tolerance to major diseases and abiotic stresses (FLIP06-165C, FLIP06-166C, FLIP06-167C, FLIP06-168C, FLIP06-170C, FLIP06-173C). Grain yield of 20 breeding lines developed from interspecific crosses indicated the potential of wild *Cicer* in yield increase (Fig. 3).

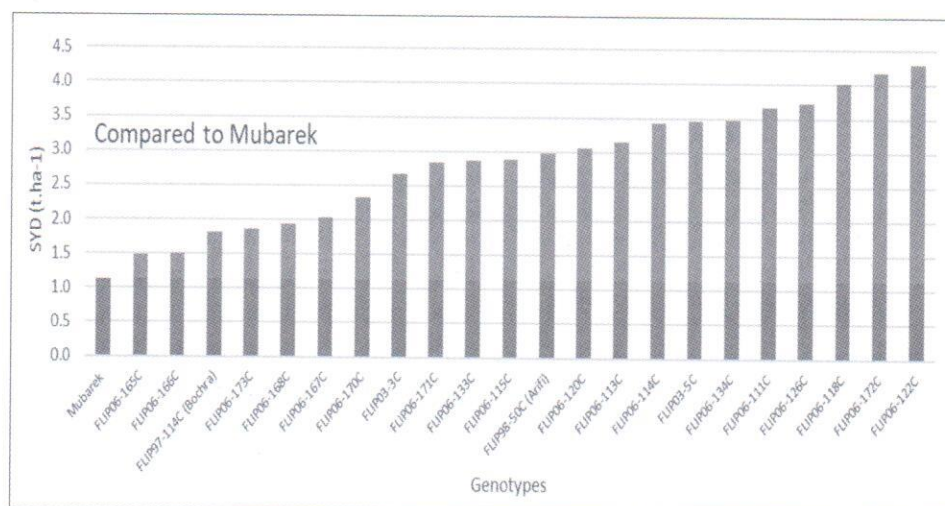


Fig 3. Performance of prebred lines of chickpea in yield trials at Terbol, Lebanon



At ICRISAT, precise evaluation of wild *Cicer* species has resulted in identification of accessions having high levels of resistance for *Ascochyta* blight, *Botrytis* grey mould and dry root rot. These accessions have been utilized in transferring resistance genes into popular chickpea cultivars following interspecific hybridization. Major focus of these activities is to combine heat tolerance with dry root rot resistance and short duration with *botrytis* grey mold resistance. To meet these objectives, simple (bi-parental) and complex (3-way and 4-way cross crosses) cross approaches are followed for creating new variability in the primary gene pool. Using complex 3-way crosses [*C. arietinum* × (*C. reticulatum* × *C. echinospermum*)], advanced backcross populations were developed in *desi* and *kabuli* chickpea genetic backgrounds (Sharma *et al.* 2016). Considerable variability for morpho-agronomic traits was observed in these populations (Saran 2019). Promising introgress lines having high-yield, early maturity, erect growth habit, high 100-seed weight, and better agronomic performance under rainfed environments (Verma 2019) have been identified for use in chickpea breeding programs (Fig. 4). Similarly, *Cicer echinospermum* has been used as a source for *Ascochyta* resistance, particularly in the Australian breeding program (Knights *et al.* 2008).

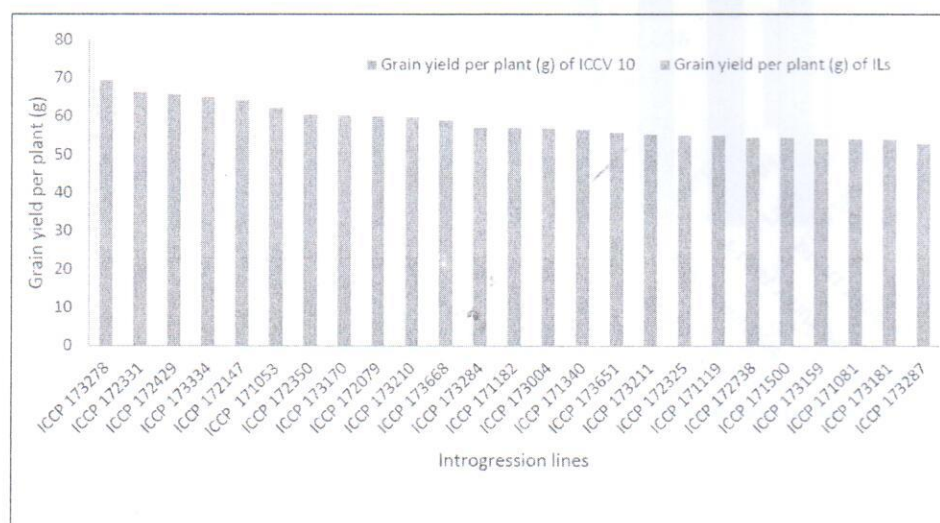


Fig 4. Performance of chickpea introgression lines derived from complex cross in *desi* background during 2018-19 post-rainy season at ICRISAT, Patancheru, India

A set of introgression lines in *desi* background were screened for *botrytis* grey mold resistance at hot spot (GBPUA&T, Pantnagar). After two years of evaluation (2017-18 and 2018-19), three introgression lines, ICCP171033, ICCP171418, and ICCP172606 were found resistant (score ≤3.0) and five introgression lines, ICCP171312, ICCP171452, ICCP171454, ICCP171711, ICCP172468, ICCP172527 moderately resistant (score 4.0-5.0) to BGM. These pre-bred lines derived from wild *Cicer* species will provide new and diverse variability for important traits for further use in chickpea improvement programs to develop new cultivars with a broad genetic base.

In faba bean, several varieties with high level of resistance to chocolate spot using landraces have been released in Ethiopia. Prominent among them are Moti, Gebelcho, Obsie and Walki (Temesgen *et al.* 2015). Recently, variety named Gora (ILB2717- 1 × R878-1) has been released in Ethiopia with higher degree of resistance to chocolate spot and larger seed size.

## New Approaches for mainstreaming CWR

During domestication and directed breeding, many alleles are inadvertently left behind in landraces and wild species. Introgression of the unattended alleles using innovative breeding tools could provide prebred lines for further use in main breeding programs. There are now genetic procedures to identify CWR with adaptation to local biotic and abiotic stresses and introgress them with ease. For example, application of population genomic scans can detect loci with exceptionally high population *Fst* values, possibly indicating loci with divergent selection for local adaptation (Baute *et al.* 2015). Conventionally, breeders have used CWR in their breeding schemes typically as sources of





resistance to various biotic and abiotic stresses with limited success. Because of linkage drag, many undesired traits persist in segregating generations which need to be removed at an early stage via repeated backcrossing with recipient parent accompanied by testing at every stage to recover the recurrent genotype with desired traits only. With the availability of high-density genome-wide molecular markers and linkage maps, we can use molecular markers as foreground and background selection for elimination of unwanted plants and recovery of recipient genotype at an early stage of the breeding procedure. Over 90% of the recurrent parental genotype can be recovered within two generations when a suitable number of markers and an adequate number of progenies are used for background selection (Tanksley *et al.* 1989). This represents a substantial saving in time compared to conventional backcross breeding. The procedure of introgression can further be fastened by integrating rapid generation advancement to advance introgression lines quickly as shown in an  $F_2$  population derived from a cross between *Lens culinaris* Medik. and *Lens ervoides* (Lulsdorf and Banniza 2018). A recent international collaboration has built a large introgression resource from the newly expanded collection of wild diversity in chickpea (von Wettberg *et al.* 2018). Advanced introgression lines, currently in  $F_4$  to  $F_6$  stages, have been developed, using 20 *C. reticulatum* and 6 *C. echinospermum* parents into five different cultivated accessions (von Wettberg *et al.* 2018, Shin *et al.* 2018).

## Conclusion

The successful use of crop wild relatives holds promise as a source of genes for resistance to diseases, phenology, nutritional quality, and possibly for plant habit, biomass production, and other important agronomic and market traits. With rapid advances in embryo rescue techniques, speed breeding, genomic tools and high throughput phenotyping techniques, the prospect of transferring useful traits from wild gene pools in CSFL crops has brightened. Wide genetic base of cultivated varieties provides a type of insurance against the epidemics of diseases and insect pests besides, of course, making the cultivated germplasm more amenable to breeding advances.

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