



**1<sup>st</sup>** International Experts Workshop  
on  
**Pre-breeding utilizing Crop Wild Relatives**

**April 24-26, 2019**

**Rabat, Morocco**

**PROGRAM & ABSTRACT BOOK**



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- Dr. Michael Baum, ICARDA, Rabat, Morocco
- Dr. Rachid Dahan, INRA, Rabat, Morocco
- Dr. Nils Stein, IPK, Gatersleben, Germany
- Dr. Benjamin Kilian, GCDT, Bonn, Germany
- Dr. Ahmed Amri, ICARDA, Rabat, Morocco

## LOCAL ORGANISING COMMITTEE

---

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- Dr. Abderrazek Jilal, INRA, Rabat, Morocco
- Dr. Shiv Kumar Agrawal, ICARDA, Rabat, Morocco
- Dr. Ramesh Pal Singh Verma, ICARDA, Rabat, Morocco
- Dr. Filippo M Bassi, ICARDA, Rabat, Morocco
- Ms. Oumekaltoum Essahli, ICARDA, Rabat, Morocco



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# **1<sup>st</sup> International Experts Workshop on Pre-breeding utilizing Crop Wild Relatives**



**First International Experts Workshop**  
**on**  
**Pre-breeding utilizing Crop Wild Relatives**  
**(1<sup>st</sup> PBCWR)**  
**Rabat, Morocco**  
**(April 24-26, 2019)**

**Program**

*Wednesday, 24<sup>th</sup> April 2019*

**Session 1: Welcome statements and opening lectures**

**Chair: Mahmoud El-Solh; Rapporteur: Ahmed Amri**

<b>08:30 – 09:00</b>	<b>Mohamed Sadiki (MoA), Michael Baum (ICARDA), Faouzi Bekkaoui (INRA-Morocco), Luigi Guarino (GCDT), Daniel van Gilst (NORAD)</b>	
<b>09:00 – 09:30</b>	<i>Towards a Futuristic Elite Crop-CWR Germplasm Enhancement Program</i>	<b>Bikram S Gill</b>
<b>09:30 – 10:00</b>	<i>Coffee break</i>	

**Session 2: Collecting and conserving crop wild relatives**

**Chair: Rachid Dahan; Rapporteur: Athanasios Tsivelikas**

<b>10:00 - 10:20</b>	<i>Promoting in situ/ ex situ conservation linkages of crop wild relatives of cereals and legumes</i>	<b>Mariana Yazbek</b>
<b>10:20 - 10:40</b>	<i>The role of botanic gardens in collecting and conserving crop wild relatives + CWR initiative</i>	<b>Chris Cockel</b>
<b>10:40 - 11:00</b>	<i>Targeting novel diversity in genetic resources conserved ex situ and in situ</i>	<b>Zakaria Kehel</b>
<b>11:00 - 11:20</b>	<i>Conservation of tropical dryland food legumes and cereals genetic resources</i>	<b>Vania Azevedo</b>
<b>11:20 - 11:40</b>	<i>Wheat Improvement for the Heat Prone Agro-ecologies of Sudan and Sub-Saharan Africa: Breeding and Pre-breeding Approaches for Climate Change Resilient Varieties</i>	<b>Izzat Tahir</b>
<b>11:40 - 12:00</b>	<i>Use of tree genetic resources for land restoration: Case study of <i>Dacryodes edulis</i> G. Don Lam (Burseraceae) in Cameroon.</i>	<b>Alain Tsobeng</b>
<b>12:00 - 12:30</b>	General discussion	
<b>12:30 – 13:30</b>	<i>Lunch break</i>	



### Session 3: Exploring the value of crop wild relatives

Chair: **Asmund Bjornstad**; Rapporteur: **Mariana Yazbek**

<b>13:30 - 13:50</b>	<i>Global efforts to explore the value of crop wild relatives</i>	<b>Benjamin Kilian</b>
<b>13:50 - 14:10</b>	<i>Glimpses of domestication history of cereal crops through genebank genomics</i>	<b>Mona Schreiber</b>
<b>14:10 - 14:30</b>	<i>Improving efficiency in the exploitation of genetic resources through molecular techniques</i>	<b>Antonio Costa de Oliveira</b>
<b>14:30 - 14:50</b>	<i>Evaluation of wild relatives and pre-breeding germplasm for resistance to major insects and diseases</i>	<b>Mustapha El-Bouhssini</b>
<b>14:50 - 15:10</b>	<i>Can we find abiotic stress tolerance in wheat related-wild species? - Experiences and lessons learned in pre-breeding of wheat with drought and heat stress tolerance</i>	<b>Hisashi Tsujimoto</b>
<b>15:10 - 15:30</b>	<i>Chromosome engineering to leverage alien genetic variation and make a better wheat in the face of current and future challenges</i>	<b>Carla Ceoloni</b>
<b>15:30 - 15:40</b>	General discussion	

**15:40 – 16:00** *Coffee break*

**16:00 – 17:30** **Poster session and Visits to laboratories and genebank**

**17:30 – 19:30** **Sightseeing in Rabat**

*Thursday, 25<sup>th</sup> April 2019*

### Session 4: Pre-breeding efforts part 1

Chair: **Peter Werner**; Rapporteur: **Sajid Rehman**

<b>08:30 – 08:50</b>	<i>Use of synthetics in bread wheat breeding programs</i>	<b>Degu Wuletaw Tadesse</b>
<b>08:50 – 09:10</b>	<i>Use of wild relatives in oat pre-breeding</i>	<b>Asmund Bjornstad</b>
<b>09:10 – 09:30</b>	<i>Mobilizing useful genes from distant species of wheat</i>	<b>Ian King</b>
<b>09:30 – 09:50</b>	<i>Use of wheat wild ancestors for pre-breeding at CIMMYT</i>	<b>Masahiro Kishii</b>
<b>09:50 – 10:10</b>	<i>The magic of CWR in durum wheat breeding</i>	<b>Filippo Bassi</b>
<b>10:10 – 10:20</b>	General discussion	
<b>10:20 -10:40</b>	<i>Coffee break</i>	



## Session 5: Pre-breeding efforts part 2

Chair: **Michael Baum**; Rapporteur: **Moez Amri**

10:40 - 11:00	<i>Excellence in pre-breeding efforts in barley</i>	<b>Ramesh Verma</b>
11:00 - 11:20	Spring wheat pre-breeding in CIMMYT from exotics: Synthetics and Landraces	<b>Sivakumar Sukumaran</b>
11:20 - 11:40	Pre-breeding to enhance genetic gains in lentil, chickpea and grass pea	<b>Shiv Kumar Agrawal</b>
11:40 - 12:00	<i>Utilizing the underutilized CWR - Pigeonpea pre-breeding from discovery to delivery</i>	<b>Shivali Sharma</b>
12:00 - 12:20	<i>Utilizing the underutilized CWR - cowpea</i>	<b>Christian Fatokun</b>
12:20 – 12:30	General discussion	
12:30 – 13:00	<b>Poster session</b>	
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13:00 – 14:00	<i>Lunch break</i>	

## Session 6: From pre-breeding to impact in farmer's fields

Chair: **Geoff Hawtin**; Rapporteur: **Shiv Kumar Agrawal**

14:00 - 14:20	<i>Impacts of use of crops wild relatives towards food security</i>	<b>Perry Gustafson</b>
14:20 - 14:40	<i>Successful Multipartite Collaboration on Pre-breeding and Breeding for Hessian fly Resistance: INRA- Kansas State University-ICARDA-CIMMYT</i>	<b>Ahmed Amri</b>
14:40 - 15:00	<i>Pre-breeding and breeding efforts of cereals in Algeria</i>	<b>Abdelkader Benbelkacem</b>
15:00 - 15:20	<i>Farmer-breeder collaboration in plant breeding: current approaches and perspectives and implications for CWR prebreeding efforts</i>	<b>Ola Westengen</b>
15:20 - 15:40	<i>Engaging farmers in sorghum improvement</i>	<b>Eva Weltzien</b>
15:40 - 16:00	<i>Sharing data through Germinate</i>	<b>Paul Shaw</b>
16:00 - 16:10	General discussion	
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16:10 - 16:30	<i>Coffee break</i>	



## Session 7: Sharing pre-breeding resources globally for maximum impact

Chair: **Luigi Guarino**; Rapporteur: **Ramesh Verma**

<b>16:30 – 16:50</b>	<i>Importance of the policy framework of the ITPGRFA</i>	<b>Alvaro Toledo</b>
<b>16:50 – 17:50</b>	<i>Round table how to strengthen pre-breeding efforts to meet the future challenges and on conservation and access to pre-breeding germplasm</i>	<b>Shivali Sharma, Mahmoud El Solh, Paul Shaw, Ahmed Amri, Peter Werner, Eva Weltzien</b>
<b>17:50 – 18:00</b>	Closing session	<b>Michael Baum &amp; Hannes Dempewolf</b>
<b>20:00 – 22:00 Official dinner</b>		

### *Friday, 26<sup>th</sup> April 2019*

07:30 – 12:30 Field visit to Marchouch

**13:00 – 14:00 Lunch**





# ABSTRACTS



## AB01: Towards a futuristic elite crop-CWR germplasm enhancement program

*Bikram S Gill<sup>1</sup>, W John Raupp, Duane L Wilson<sup>1</sup>, Hannah Shult<sup>1</sup>, Dal-Hoe Koo<sup>1</sup>, Narinder Singh<sup>1</sup>, Allan Fritz<sup>2</sup>, Mary Guttieri<sup>3</sup>, Jesse Poland<sup>1</sup> and Bernd Friebe<sup>1</sup>*

<sup>1</sup>Wheat Genetics Resource Center, Department of Plant Pathology and <sup>2</sup>Department of Agronomy, Kansas State University, Manhattan, KS, USA ; <sup>3</sup>USDA Agricultural Research Service, Center for Grain and Animal Health Research, Manhattan, KS, USA

Corresponding Author: BS Gill ([bsgill@ksu.edu](mailto:bsgill@ksu.edu))

### Introduction

Crop wild relatives (CWRs) will play a major role as we face the challenge of feeding 9 billion people by 2050 under reduced water and fertilizer inputs, soil degradation and a warming planet producing episodes of extreme weather. Anecdotal evidence suggests that in the last few years breeder's nurseries have suffered a massive loss of breeding material because extreme weather including cold, heat and drought disrupts crop adaptation or spawn new pest epidemics. Over millennia, CWRs, having been exposed to climate extremes and disease epidemics and evolved adaptive traits, will be one important source of novel traits. However, in the Anthropocene era, we are also witnessing a massive genetic erosion of CWRs in native agroecosystems and, thus, we also must deal with challenge of conserving CWRs in nature as genetic reservoirs for future agriculture (Gill et al. 2014). Here we present a framework for a comprehensive program of CWR management, conservation and utilization for germplasm enhancement for wheat.

### CWR diversity analysis, unique and core sets

Our first urgent task is a diversity analysis of CWR collections in gene banks for more efficient curation and identification of unique (non- redundant) sets of georeferenced accessions for each CWR species of the primary, secondary and tertiary gene pools (Amri et al. 2016; Singh et al. 2019a). The second task is to develop core sets of accessions capturing greater than 90% of the diversity of each CWR species. Recently, using genotyping-by-sequencing (GBS), we identified unique and core sets of accessions for *Triticum turgidum* and *timopheevii* and *Aegilops tauschii*, the primary gene pool of *T. aestivum* (Singh et al. 2019a, b; and unpublished results). This data also has been used to pinpoint the center of genetic diversity for each species as a guide for future conservation and utilization in prebreeding.

As an example, we analyzed our WGRC working collection of 549 *Ae. tauschii* accessions and found 26% duplicates and identified 421 unique accessions. At CIMMYT, 43% of accessions were duplicated and the collection at Punjab Agricultural University (PAU) had 54% duplication. Because substantial portions of PAU and CIMMYT collections came from the WGRC, we were able to cross reference passport data. Overall, we identified 564 unique accessions among the three gene banks. We were able to impute passport information from genotypic data, including geographic origin for collections of unknown origin. In addition, we were able to evaluate genetic diversity of a set of newly collected *Ae. tauschii*; 36 of 44 new collections were unique, seven were duplicated and one was identical to a previously collected unique set. This foundation data set and methodology (see Singh et al. 2019a) can be extended to *Ae. tauschii* holdings in other gene banks to identify a truly unique world collection of *Ae. tauschii* for efficient curation, conservation and prebreeding.



The GBS data set (Singh et al. 2019b) also confirmed previous reports (Lubbers et al. 1991; Wang et al. 2013) on the incipient speciation of *Ae. tauschii* into lineages L1 (form *tauschii*) and L2 (form *strangulata*). Caspian Iran is the center of diversity and origin of *Ae. tauschii* where both lineages coexist: L2 at lower elevations and L1 at higher elevations. Afghanistan is the center of diversity for L1. GBS data along with phenotypic data was used to identify a core set of 40 *Ae. tauschii* accessions, 11 from L2 and 29 from L1, capturing 84% of genetic diversity of the species (Singh et al. 2019b). Because the D-genome of *T. aestivum* originated from L2 lineage, the 29 L1 accessions represent new genetic diversity for wheat improvement.

### **Evaluation of unique CWR set for hotspots of genetic diversity for each trait**

Climatic, edaphic and many other factors have shaped the adaptive evolution of each of the CWR species. The FIGS (Focused Identification of Germplasm Strategy) (Bari et al. 2012) and experimental evaluation of CWRs, among others, should be used to identify hotspots of genetic diversity for specific traits. We have good evaluation data for *Ae. tauschii* for disease and insect resistance (our unpublished results). The L2 lineage in Caspian Iran is hotspot for genetic diversity for seedling resistance to leaf rust, whereas adult-plant resistance to leaf rust is restricted to L1 lines in Afghanistan. Lineage 2 also has resistance to the wheat curl mite. Both lineages include accessions resistant to stripe rust, Septoria, Hessian fly and greenbug.

### **Germplasm enhancement strategies using primary gene pool CWR species**

Genetic transfers from primary gene pool species *T. turgidum* and *Ae. tauschii* can be made by direct crosses and backcrosses with *T. aestivum* (McFadden 1927; Gill and Raupp 1987). The most widely used approach is the production of synthetic wheat by crossing *T. turgidum* with *Ae. tauschii* (McFadden and Sears 1946). We are producing “super-wild synthetic” wheats from hybridization of core set accession of *T. turgidum* subsp. *dicoccoides* and *Ae. tauschii*. A third approach, using a bridge-cross scheme, is through the production of octoploids (AABBDDDD) by colchicine doubling of *T. aestivum*/*Ae. tauschii* (ABDD) F1 hybrids (discussed in Singh et al. 2019b; see also Zhang et al. 2018). We are using this strategy for genetic transfers from *Ae. tauschii* via octoploid amphiploids, followed by backcrossing with elite wheat recurrent parents (Fritz A, unpublished results). We are transferring genes from the *T. turgidum* core set by direct crosses with *T. aestivum* cultivars, followed by backcrosses of F1 hybrids with elite genotypes (Gutteri M, unpublished).

### **Germplasm enhancement strategies using secondary gene pool CWR species**

The secondary gene pool CWR species of *T. aestivum* include three *Triticum* species: *T. timopheevii* (A<sup>t</sup>A<sup>t</sup>GG), *T. monococcum* subsp. *aegilopoides* (A<sup>m</sup>), and *T. urartu* (A<sup>u</sup>). Although the A-genome of polyploid *Triticum* species traces to *T. urartu*, certain species-specific translocations (T4A-5A-7B) in *T. turgidum* and *T. aestivum* and others (T6A-1G-4G) in *T. timopheevii* preclude recombination and, hence, genetic transfers involving these chromosomes. In addition, formidable hybridization barriers, including hybrid seed abortion, hybrid embryo lethality and floral defects of the ovary and stamens in F1 hybrids preclude genetic transfers from many accessions of *T. monococcum* and *T. urartu* (Cox et al. 1991).

The polyploid D-genome cluster species of *Aegilops*, such as *Ae. cylindrica* (CCDD), *Ae. crassa* (DDMM and DDMMM), *Ae. juvenalis* (DDMMUU) and *Ae. ventricosa* (DDNN) also constitute the secondary gene pool of wheat as they share the D-genome. The D-genome of *T. aestivum* has no known chromosomal rearrangements, but similar information is not available for the D-genome



chromosomes of polyploid *Aegilops* species. However, most chromosomes are accessible for genetic transfers by recombination.

### **Germplasm enhancement strategies using tertiary gene pool CWR species**

Polyploid *Aegilops* species of the secondary gene pool that also contain an additional genome(s) other than A or D, all other diploid and polyploid *Aegilops* species that carry genomes other than A, B or D, including all other genera, and species of the Triticeae tribe constitute the tertiary gene pool of wheat. The *Ph1* gene does not allow pairing among the homoeologous chromosomes of A, B or D genomes of polyploid wheats (Riley and Chapman 1958) and also those of the homoeologous chromosomes of the tertiary gene pool species. Broadly, two general methods, one interfering with the homoeologous recombination system (Riley et al. 1968, Sears 1977) and the second using irradiation (Sears 1956) have been used for accessing genes from the tertiary gene pool species (for review see Qi et al. 2007; Lukaszewski 2016).

As a rule, induced homoeologous recombination is the method of choice because genetically compensating wheat/alien segments are exchanged. However, irradiation is a back-up method in those instances where either the alien (CWR) chromosome is structurally rearranged and, hence, is no longer competent for synapsis and homoeologous recombination with a wheat chromosome or if the target gene is in a proximal (centromeric) region where recombination is highly suppressed.

Recently, we have discovered a homoeologous pairing promoter factor/s on chromosome 5M<sup>g</sup> of *Ae. geniculata* (*Hpp5M<sup>g</sup>*) that greatly enhanced homoeologous recombination in plants that are lacking *Ph1* (our unpublished results). Moreover, homoeologous recombination is also observed in proximal regions where even homologous recombination is suppressed.

### **Cryptic alien transfers**

Necdotal reports have suggested transfer of target genes in wheat/alien derivatives although the transfers could not be experimentally verified in earlier experiments. Kuraparthi et al. (2007, 2009) reported cryptic transfers of leaf rust resistance gene *Lr58* from *Ae. triuncialis* and *Lr57/Yr40* genes from *Ae. geniculata*. They were not able to verify the transfers by GISH but did detect them using molecular markers at the tip of the chromosomes, which are known to be recombination hotspots. By using a more sensitive GISH technique we could later visualise the *Ae. geniculata* segment at the telomere of the short arm of chromosome 5D (Zhang et al. 2015). We now know that the *Ph1* gene is leaky and the *Ph1* effect can be suppressed in specific hybrid combinations (Koo et al. 2017). A second source of cryptic variation may be non-crossover recombination leading to transfer of small interstitial alien segments (our unpublished results). Finally, another source of cryptic variation may be recently discovered eccDNA elements, where genes can escape from chromosomes and exist as autonomously replicating circular or episomal DNA elements, which are inherited in the progeny (Koo et al. 2018).

### ***In situ* and *Ex situ* conservation and germplasm enhancement**

Agriculture began in centers of origins of crop plants from domestication of CWRs in the so-called “Gardens of Eden” or native agroecosystems. The rich natural CWR genetic diversity broadened the crop genetic base through spontaneous hybridization and selection. An expanding human population and area under agriculture have greatly eroded CWRs. Fortunately, we have done a good job of *ex situ* conservation of CWRs. However, we must address the critical issue of *in situ*



conservation of CWRs as future reservoirs of genetic diversity molded by climate change and global warming. Wilson (2016) has argued that we need half of the earth to conserve nature for a sustainable planet. Hundreds and thousands of CWR species are native to centers of origin of crop plants. It is the *in situ* conservation of all the CWRs in these centers of origin of crop plants that should be considered a number one food security issue of the 21<sup>st</sup> century. Perhaps native habitat loss is so great that such a project is not even feasible. We must then attempt to tackle the thorny issue of rewilding of CWRs in not-native countries, such as the Great Plains of the USA and other similar regions of the world.

### **Futuristic germplasm enhancement program**

Advances in genotyping, phenotyping and sexual biology (crossability/fertilization/meiosis/recombination) open immense possibilities of accessing great genetic diversity of CWRs. WGRC research in genetics, germplasm enhancement and graduate education can perhaps serve as a great model; multidisciplinary, multinational in which university, federal, industry and more importantly wheat growers have ownership. For each CWR, we need to establish cross gene bank set of world's unique set of accessions with accurate passport data as demonstrated for *Ae. tauschii*. These unique CWR sets should be focus of *ex situ* and *in situ* conservation, phenotyping and germplasm enhancement. Core sets should be identified for each CWR species, and immortalized as amphiploids. We have accomplished this for *Ae. tauschii* by producing 11 amphiploids involving 8 of the 40 accessions of the core set. Amphiploids will be made available to the breeding community. For the species of the tertiary gene pool, amphiploids will be used to isolate a set of Robertsonian translocations for evaluation and chromosome engineering (Qi et al. 2007). Bulk populations of the core sets, amphiploids, and Robertsonian translocation (alien translocation lines) may be grown in garden plots across wheat-growing regions as genetic reservoirs for near and future germplasm enhancement.

### **Acknowledgements**

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## AB02: Complementary *ex situ* and *in situ* conservation of wild relatives of temperate cereals and legumes

Ahmed Amri<sup>1</sup>, Ali Shehadeh<sup>1</sup>, Mariana Yazbek<sup>1</sup>, Athanasios Tsivelikas<sup>1</sup>, Zakaria Kehel<sup>1</sup>, Sami Lalla<sup>2</sup>, Wateik Zair, Nora Castañeda-Álvarez, Holly Vicent<sup>3</sup> and Nigel Maxted<sup>3</sup>

<sup>1</sup>International Center for Agricultural Research in the Dry Areas (ICARDA), Rabat-Morocco; <sup>2</sup>Institut National de la Recherche Agronomique (INRA), Morocco; <sup>3</sup>School of Biosciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK;

Corresponding author: Ahmed Amri ([a.amri@cgiar.org](mailto:a.amri@cgiar.org))

### Introduction

Cereals (wheat and barley), food legumes (chickpea, faba bean and lentil), and temperate forage legumes (*Lathyrus*, *Medicago*, *Pisum*, *Trifolium* and *Vicia*) are important crops for the global food security and major components in sustaining the livelihoods of rural communities living in non-tropical drylands and beyond. In the light of the fast-growing population and the adverse effects associated with climate change, sustaining agricultural development and food security through crop improvement will require efficient conservation and use of plant genetic resources. Dryland agrobiodiversity, mainly landraces and crop wild relatives will be critical for ensuring continuous genetic gains under recurrent droughts, heat spells, increasing salinity stress, and changes in importance and virulence spectra of diseases and pests. The Mediterranean basin, West Asia, Central Asia, and Abyssinia encompass four major Vavilovian centers of diversity for species of global importance including cereals, food legumes, temperate forage and range species and dryland fruit trees. Landraces of these crops are still found in the traditional farming systems and their wild relatives in the remaining non-degraded natural habitats. However, these genetic resources are under alarming threats of over-exploitation, land reclamation, use of introduced species and newly released varieties, stressing the need for further judicious collecting and for using both *ex situ* and *in situ* conservation approaches to ensure continuous supply of needed diversity by the breeding programs and of adapted species for direct use in the rehabilitation of degraded systems. Overgrazing, fire, and land use change and fragmentation along with the adverse effects of climate change are causing significant losses of wild relative species. Both the Convention on Biological Diversity (CBD, 1992) and the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA, 2001) stressed the importance of crop wild relatives and the need for complementary efforts for their efficient *in situ* and *ex situ* conservation. *In situ* conservation approaches allow for a dynamic conservation of a large species richness along with larger genetic base. Most of the existing natural reserves are not specifically targeting the conservation and management of crop wild relatives and the existing *ex situ* collections have small holdings of accessions of these species.

### Gap analysis in *ex situ* and *in situ* conservation of crop wild relatives

Overall, crop wild relatives are under-represented in the existing *ex situ* collections as only 6% of the crop wild relative species are held in the genebanks and represent between 2% to 6% of the total holdings (Maxted and Kell, 2009). Only few genebanks have targeted the collection and conservation of crop wild relatives, including Millennium Seed Bank for large number of species, CGIAR genebanks for their mandated crops, Kansas State University genebank for wheat. Similarly, only few natural reserves have targeted specifically the *in situ* conservation of crop wild relatives. Gaps in *ex situ* and *in situ* collections are assessed using different approaches: numbers



of conserved accessions per species, diversity trees, geographic distribution and coverage using methods such as DIVA-GIS or MaxEnt, and more recently trait gaps using specific algorithms linking environmental conditions with adaptive traits (Hijmans et al, 2001). Crop Trust, in collaboration with CGIAR and other partners have developed conservation strategies for the 22 crops of the CGIAR mandate which included gaps for both cultivated and wild relative species. ICARDA participated in conservation strategies of barley, wheat, faba bean, temperate forages and collaborated with Birmingham University in conducting ecogeographic surveys and gap analysis for *Aegilops*, wild *Triticum*, wild *Hordeum*, *Lathyrus*, *Medicago*, etc. (Maxted et al., 2012; Ali Sheahdeh et al., 2013; Vincent et al., 2017; Lalla et al., 2017). These studies showed that all crops without exception and with different degrees have gaps in both *ex situ* collections and in *in situ*. For wheat and barley wild relatives, more collecting missions are needed in the countries of North Africa, southern and Central Europe, and in Eastern Asia and substantial efforts are needed for establishing natural reserves targeting these species mainly in the Fertile Crescent and Central Asia regions. For *Lens* and *Cicer* wild species, only few accessions are available in the genebanks worldwide calling for subsequent efforts of collecting mainly in the Mediterranean, West Asian and Central Asian Vavilovian centers of diversity. For temperate forage and range legumes species, although several species are represented in the genebank holdings, more accessions are needed to add novel diversity for adaptive traits such as tolerance to drought, heat, salinity, etc. For all cereal and legume wild relatives, more protected or well-managed areas are needed in all Vavilovian diversity centers, but mainly within the Fertile Crescent region where their species richness is higher. In addition, the existing protected areas should be assessed for the presence of crop wild relatives and apply appropriate management of promoting the *in situ* conservation. The countries such as China, Ethiopia, India, Iran, India, Morocco and Turkey should play an important role in ensuring the *ex situ* and *in situ* conservation of the crop wild relatives of cereals and legumes. Also, substantial collecting and conserving efforts are needed for the genus *Avena*. This contribution will highlight the results of gap analysis for *Aegilops* and wild *Triticum* (Amri et al., 2014).

### **Promoting *in situ* conservation of crop wild relatives**

ICARDA plays a key role promoting the conservation of non-tropical dryland agrobiodiversity. Its genebank holds in-trust more than 157,000 accessions including important collections of wild relatives of wheat, barley, lentil, chickpea, grass pea and temperate forage legumes. For the *in situ* conservation efforts, ICARDA has undertaken with the national partners the first study on status and threats of landraces and wild relatives of cereals, legumes and dryland fruit trees in four countries (Jordan, Lebanon, Palestine and Syria) during 1999 to 2005, within the GEF funded project on conservation and sustainable use of dryland agrobiodiversity in the Fertile Crescent (Amri et al., 2005). The highlights of this project are summarized as follows:

- Both landraces and wild relative species are subjected to extensive and alarming losses due to overuse, land reclamation and fragmentation and adverse effects associated with climate change;
- Biodiversity hotspots including high species richness identified in the four countries and recommendations were made for the establishment of natural reserves and transboundary reserves targeting crop wild relatives. So far, only Al-Lujat reserve was established in the southern part of Syria;
- A holistic approach for *in situ*/on-farm conservation was developed, which includes suggestions of management plans including technical, socio-economic, institutional and policy options;





- Regulated grazing, deferred grazing and rehabilitation using native species along with water harvesting techniques are some of the technical packages to promote *in situ* conservation of wild relatives;
- In addition, public awareness, add-value and alternative sources of income actions for the custodians of agrobiodiversity, introduction of forage species within farming systems, and more research activities related to crop wild relatives will also help in promoting *in situ* conservation;
- Above all, the conservation of crop wild relatives should be tightly linked to their use in breeding new adapted varieties through strengthening of pre-breeding activities, their direct use in domestication of new species as in case of forages and the diversification of farming systems and their use in restoration and rehabilitation of degraded ecosystems.

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## AB04: Targeting novel diversity in genetic resources conserved *ex situ* and *in situ*

Zakaria Kehel<sup>1</sup>, Julian Ramirez-Villegas<sup>2</sup>, Harold Achicanoy<sup>2</sup>, Andres Mendez<sup>2</sup>, Maria Victoria Diaz<sup>2</sup>, Chrystian Sosa<sup>2</sup>, Colin Khoury<sup>2</sup>, Kenneth Street<sup>1</sup>, Luigi Guarino<sup>3</sup>, Tsivelikas Athanasios<sup>1</sup>, Ali Shehade<sup>4</sup>, Mariana Yazbek<sup>4</sup>, Ahmed Amri<sup>1</sup>

<sup>1</sup>International Center for Agricultural Research in the Dry Areas (ICARDA), Rabat-Morocco; <sup>2</sup> CIAT, Cali, Colombia; <sup>3</sup> Global Crop Diversity Trust, Bonn, Colombia; <sup>4</sup> ICARDA, Terbol, Lebanon

Corresponding author: Zakaria Kehel ([z.kehel@cgiar.org](mailto:z.kehel@cgiar.org))

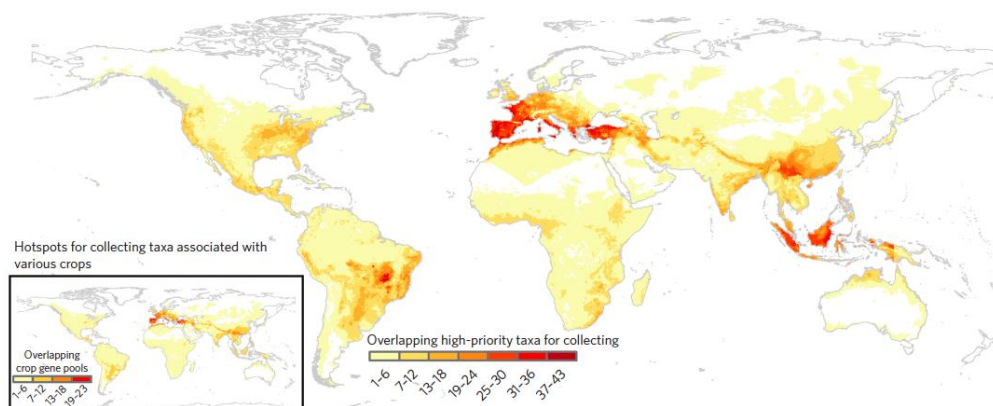
### Introduction

Sustainable development is affected by interlinked challenges: climate change, agrobiodiversity loss and land degradation, which led to three binding international conventions: The Climate Change convention (CC), the United Nations Convention to Combat Desertification UNCCD, and the Convention on Biological Diversity (CBD). Agrobiodiversity continues to support the livelihoods of rural poor in remote and mountainous areas, it also is considered as a reservoir of valuable traits for breeding programs around the world including genes for adaptation to climate change adverse effects; and can be a source of material for rehabilitation of degraded agro-ecosystems. Agrobiodiversity is subject to alarming loss due to habitat destruction, invasive species, urbanization, and the shift from traditional to industrial agricultural practices, among other factors. Additionally, climate change is projected to have a drastic effect on both natural and agricultural ecosystems (Meilleur et al. 2004; Brooks et al. 2006, Hawkins et al. 2008, Burke et al. 2009). Concerted efforts are certainly needed at national, regional and international levels to conserve and sustainably use the agrobiodiversity using both in-situ and ex-situ conservation approaches. *Ex-situ* collections still have large gaps in the collections for both wild relatives and landraces, despite the global efforts that substantially improved the conservation of plant genetic resources in genebanks. Gap analysis has been widely applied for conservation purposes (Maxted et al. 2008). Ramirez-Villegas and coworkers (2010) has developed and applied a gap analysis methodology to collect the crop gene pool of Phaseolus beans and evaluated conservation deficiencies at three different levels (taxonomic, geographic and environmental). The importance of filling gaps in genebanks of plant genetic resources was recognized through a review of global crop conservation strategies by Khoury and coworkers (2010). Predictive distribution maps for Lathyrus were produced based on climatic data, and *ex situ* conservation gaps were identified in recent study (Shehadeh et al. 2013).

Major challenge managers of *ex-situ* collections are facing is the ability to identify one or few accessions that are useful for plant improvement or that matches specific user requirements. Several methods of linking traits to a genebank accession were reviewed (Angil et al. 2018) including large phenotyping, core collections, molecular techniques, genome wide association studies (GWAS) and the focused identification of the germplasm strategies (FIGS). FIGS is a useful approach to identify subset of accessions with a high probability of containing specific target traits based on the ecogeographical information of the sites where the populations were collected (Mackay et al. 2004). FIGS has been used to identify sources of resistance to Sunn pest in wheat in Syria El Bouhssini et al. 2009) and to Russian wheat aphid in bread wheat (El Bouhssini et al. 2011). FIGS has also been used to identify traits related to abiotic stresses, such as drought adaptation in *Vicia faba* (Khazaei et al 2013).

## Targeting novel diversity in *in-situ* collections: gap analysis

Understanding the level of representation of crop diversity in conservation systems is a first and critical step towards its conservation and use. Methods to assess the current degree of representation of crop genetic diversity in genebanks and at informing further collecting actions to increase this representation have been under development for more than a decade. These methods are generally based on eco-geographic theory and modeling and have proved useful in estimating the diversity of crop wild relatives and other wild species maintained in genebanks in comparison to standing extant diversity in their natural environments. A recent global analysis for crop wild relatives reported that more than two thirds species of 81 major food and agriculture crops are in urgent need of collecting (Castañeda-Álvarez et al. 2016) (Fig. 1). Major efforts for the conservation of these species are underway (<https://www.cwrdiversity.org/project/>).



**Figure 1** Richness of gaps in crop wild relative taxa from 81 crops (top panel, taken from ref 14), and richness of gaps in landrace groups of 8 crops (bottom panel, unpublished).

The application of these methods to cultivated plants, and crop landraces (traditional varieties of cultivated plants), is, however, less straightforward than for crop wild relatives. Crop landrace spatial distributions are partly determined by anthropogenic factors as well as climate and soils, which requires additional considerations when modeling their distributions. A novel landrace gap analysis approach was developed to predict gaps and estimate coverage (and therefore track conservation progress) of *ex situ* collections. Our methods explicitly aim to include anthropogenic drivers to model the geographic distributions of recognized genetic units of landrace diversity. We evaluate our method through statistical analyses and with expert knowledge, the latter of which also informing recent patterns of land use change that are difficult to model. Results of applying this method to eight crops, namely, common bean, African rice, banana, sorghum, potato, maize, wheat, and barley, suggest significant gaps in the Fertile Crescent, the Mediterranean, and West Africa.

These methods represent an advance in tools that can be deployed to understand the potential distributions of wild and cultivated crop diversity, and to determine the comprehensiveness of conservation of this diversity *ex situ*.

## Targeting diversity in *ex-situ* collections: FIGS

Distribution of genetic resources is a key and core genebank activity aiming at responding to requests from various users including breeders, researchers, farmers, etc. Most often, when the request does not specify the germplasm and traits sought, a random sample is selected and sent.



Core collections, proposed originally by Brown (1989), were developed for major crops which include 10% of holdings representing the geographic- or characterization-based diversity. The Generation Challenge Program (GCP) developed reference sets representing 10% of the core collection selected based on molecular diversity. Mini-core collections are also used to allow for minimizing the number of accessions to be evaluated. The Focused Identification of Germplasm Strategy (FIGS) developed by ICARDA in collaboration with partners in Australia and Russia is an alternative approach for mining genebank accessions. This approach is providing a better alternative to random sampling and the use of core collections since it is specific to each trait and is selecting manageable size subsets with higher probability of finding the desired traits. It is based on finding the relationship between the environmental conditions of collection sites and the traits requested by users. FIGS uses two approaches, filtering and modeling, both of which select best-bet environments that are likely to have imposed selection pressure for specific traits on *in situ* populations over time. Developing a FIGS filtering strategy requires deep understanding of the ecology and the optimal conditions of the expression of the trait under study, how these conditions affect the crop, and how this will relate to a selection pressure on an *in situ* population. The FIGS modeling pathway explores the mathematical relationship between the adaptive trait of interest and the long-term climatic and/or soil characteristics of collection sites. The mathematical conceptual framework of FIGS is based on the paradigm that the trait as a response variable depends on the environment attributes considered as the covariates. The quantification process leads to the generation of *a priori* information, which is used in the prediction of accessions that would carry the desired trait.

The application of FIGS has allowed to send 75 subsets so far, most of which confirmed the relevance of the approach in identifying sources of sought traits in relatively small subsets, which could also reduce the efforts undertaken by the genebanks in regenerating the accessions.

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## AB5: Wide variability in the ICRISAT germplasm collections as a source for genetic enhancement of crop cultivars

Vania C R Azevedo, Mani Vetriventhan, Senthil Ramachandran, Venu G Reddy, Prabath Singh, Vinod Kumar, Ramesh Babu, D Naresh, Ganesh Pawar, Surendar R Mala, and Hari D Upadhyaya

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India

Correspondence: Vania Azevedo ([v.azevedo@cgiar.org](mailto:v.azevedo@cgiar.org))

### Introduction

Sustained gains in plant breeding rely on variation in crop gene pool. Therefore, it is essential to conserve crops' diversity *in situ* and *ex situ*. Globally over 7.4 million germplasm accessions have been conserved in about 1750 genebanks. The greater use of germplasm diversity in crop improvement programmes could potentially broaden genetic base of crop cultivars. The ICRISAT genebank conserves over 128,000 germplasm accessions of six mandate crops (sorghum, pearl millet, finger millet, chickpea, groundnut, pigeonpea) and five small millets (foxtail millet, proso millet, barnyard millet, little millet and kodo millet). The collection is dominated with cultivated germplasm (97.7%) while wild and weedy relatives represent only 2.3%. Large variability was reported in the germplasm collections conserved at ICRISAT genebank, as revealed by characterization and evaluation of small subsets of total collection. However, large-scale evaluation to uncover hidden untapped potential is required to identify and introduce novel variability in to the crop improvement program.

### ICRISAT genebank

ICRISAT genebank conserves 128155 accessions (125270 cultivated and 2885 wild) of six mandate crops (sorghum, pearl millet, finger millet, chickpea, groundnut, pigeonpea) and five small millets (foxtail millet, proso millet, barnyard millet, kodo millet, little millet), originating from 144 countries. Wild germplasm includes 461 accessions of 26 species in sorghum, 816 accessions of 28 species in pearl millet, 479 accessions of 49 species in groundnut, 308 accessions of 18 species in chickpea, 562 accessions of 68 species in pigeonpea (Table 1).

**Table 1.** Current status of germplasm accessions conserved at the ICRISAT genebank (as on March 2019)

Crop	Cultivated	Wild	Total
Sorghum	41355	461	41816
Pearl millet	23557	816	24373
Chickpea	20456	308	20764
Pigeonpea	13221	562	13783
Groundnut	15143	479	15622
Finger millet	7314	205	7519
Small millets	4224	54	4278
Total	125270	2885	128155

### Germplasm trait specific sources

Germplasm collections have been characterized for morpho-agronomic and nutritional traits following crop specific descriptors, and for biotic and abiotic stress tolerance by ICRISAT and NARS researchers. Studies revealed a large variability and identified sources for important traits



in cultivated germplasm. Summary germplasm as sources for important traits in the cultivated and wild accessions is mentioned in Table 2.

**Table 1.** Germplasm trait specific sources identified in cultivated and wild germplasm conserved at ICRISAT genebank, India

Crop	Cultivated germplasm	Wild germplasm
Groundnut	Drought, heat, salinity, low temperature stress, low P; early and late leafspot, rust, peanut bud necrosis, bacterial wilt, tomato spotted wilt virus, soil born fungal disease; seed quality traits (oil, protein, Fe and Zn); Aflatoxin (<math>4\mu\text{g kg}^{-1}</math>)	Early leaf spot, late leaf spot, rust, peanut mottle virus, Groundnut rosette virus, tobacco streak virus, tomato spotted wilt virus, peanut bud necrosis virus, root-knot nematode, <i>Helicoverpa armigera</i> and <i>Spodoptera litura</i> , leaf miner etc.
Chickpea	Early maturity, grain yield; Drought, salinity, heat; <i>Fusarium</i> wilt, dry root rot, Ascochyta blight, botrytis gray mold; legume pod borer, pulse beetle; herbicide	Fusarium wilt, botrytis gray mold, Ascochyta blight, cyst nematode, leaf minor, bruchids, cold tolerance, grain nutrients
Pigeonpea	Large seeds, grain nutrients; sterility mosaic virus, <i>Fusarium</i> wilt; pod borer; salinity, water logging	Botrytis gray mold, Ascochyta bight, <i>Helicoverpa</i> , pod fly, pod wasp, sterility mosaic virus, grain nutrients, salinity, root-knot nematode, photoperiod sensitivity, earliness, Phytophthora blight,
Sorghum	Downy mildew, grain mold, anthracnose, leaf blight, rust, charcoal rot, rust, potyvirus spp. shoot fly, stem borer, aphids, drought and grain quality traits (high Fe, Zn, protein, Lysine)	Downy mildew, Shoot fly, stem borer, sorghum midge
Pearl millet	Large seeds, forage, grain nutrients, blast, downy mildew	Leaf spot, downy mildew, drought, insect resistance, forage,

In chickpea, Pande *et al.* (2006) screening mini core accessions for resistance against Ascochyta blight (AB), Botrytis gray mold (BGM), Fusarium wilt (FW), and dry root rot (DRR) also found high levels of resistance to Fusarium wilt (FW), where 21 accessions were asymptomatic and 25 resistant. Totally 3, 55, and 6 accessions were moderately resistant to AB, BGM, and DRR, respectively. ICC 11284 was moderately resistant to both AB and BGM. Combined resistance also was identified for DRR and FW in 4 accessions, and for BGM and FW in 11 accessions. Upadhyaya *et al.* (2013) reported genetically diverse multiple trait specific mini core accessions: ICC 12155 (drought, heat, salinity, FW, BGM) and ICC 5337 (root length density [RLD], TE, and d13); ICC 14402 (drought, heat, salinity, FW, LPB) with ICC 8261 (drought avoidance root traits, salinity, BGM), ICC 9848 (salinity, FW, BGM), and ICC 1915 (drought, FW, AB); ICC 3325 (drought, heat, salinity, herbicide, FW, LPB) with ICC 13816 (drought, salinity, FW, BGM), ICC 7554 (salinity, FW, BGM), ICC 9848 (drought, salinity, FW, BGM); and ICC 13599 (heat, salinity, FW, BGM, LPB)

In groundnut, the published information on 184 mini core accessions revealed 28 accessions resistant to abiotic stress and 30 resistant to biotic stress and 16 were seed nutrient dense (Upadhyaya *et al.* 2014). Genetically diverse germplasm with agronomically beneficial traits



identified in groundnut: ICG 12625 (resistance to drought, low temperature, late leaf spot (LLS), *Aspergillus flavus*, bacterial wilt; high oil and good oil quality) and ICG 442 (resistance to drought, salinity, p deficiency); ICG 12625 and ICG 2381 (resistance to rust, *A. flavus*; good oil quality); ICG 12697 (resistance to LLS, rust, *A. flavus*) and ICG 6022 (resistance to early leaf spot [ELS], LLS); ICG 14710 (high oil, Fe, Zn) and ICG 7963 (high protein, Fe, Zn); ICG 11426 (resistance to ELS, LLS, rust) and ICG 5221 (high Fe and Zn and good oil quality). Waliyar *et al.* (2016) reported 7 accessions, ICGs 13603, 1415, 14630, 3584, 5195, 6703 and 6888 that were found best with very low levels of aflatoxin ( $<4 \mu\text{gkg}^{-1}$ ).

In pigeonpea, *Fusarium* wilt (FW) and Sterility mosaic disease (SMD) are important biotic constraints to pigeonpea production worldwide. In the mini core collection, six accessions (originated from India and Italy) were found resistant to FW ( $<10\%$  mean disease incidence). High level of resistance to SMD was found in 24 accessions (mean incidence  $<10\%$ ). These SMD resistant accessions originated from India, Italy, Kenya, Nepal, Nigeria, Philippines and United Kingdom. Combined resistance to FW and SMD was found in five accessions (ICPs 6739, 8860, 11015, 13304 and 14819). These diverse accessions that are resistant to FW or SMD will be useful to the pigeonpea resistance breeding program (Sharma *et al.* 2012)

In pearl millet, downy mildew (DM), is a highly destructive and widespread disease in Asia and Africa. Sharma *et al.* (2015) reported IP 14537 as resistant to seven pathotypes and also exhibited moderate resistance to pathotype Sg 409. Multiple-pathotype resistance was observed in 62 accessions. Five accessions (IP 9645, IP 11943, IP 14542, IP 14599, and IP 21438) were resistant to six pathotypes, and seven accessions (IP 11930, IP 12374, IP 14522, IP 20715, IP 21187, IP 21201, and IP 21244) were resistant to any five pathotypes.

In sorghum, evaluation of mini core accessions revealed 70 accessions resistant to biotic stress, 12 to abiotic stress, and 13 for bioenergy traits and 27 for nutritional traits. Multiple trait specific sources include: IS 23684 (nutrition traits, diseases, insect pests), IS 1212 (earliness, nutrition traits, drought, seedling vigor, diseases), IS 5094 (yield, drought, diseases, insect pests), IS 473 (earliness, diseases), IS 4698 (yield, Brix, insect pests) and IS 23891 (large seeds, yield, Brix, drought, diseases).

These trait specific sources identified in cultivated germplasm are based evaluating a small set of germplasm such as core/mini core collections. Therefore, expanding screening to a larger set could potentially identify additional sources. Similarly, characterization and evaluation of wild and weedy relatives' germplasm accessions revealed a large variability for morpho-agronomic traits, and also for biotic, abiotic and nutritional traits (Table 2).

## Conclusion

Evaluation of cultivated germplasm of ICRISAT mandate crops has resulted in identification several trait specific sources in agronomically superior background. Use of wild germplasm in the primary gene pool has resulted in transfer of new cytoplasmic male sterility systems in pearl millet and pigeonpea, foliar disease resistance in groundnut cultivars, etc. Valuable traits are also present in the secondary and tertiary gene pool, but, due to strong reproductive barriers that prevent hybridization with cultivated gene pool, the use of those species is so complicated that justify only in the case of traits not found in primary gene pool. The trait specific sources identified in cultivated accessions were based on small sets of germplasm such as core/mini core collections, therefore





expanding screening to a larger set could potentially identify additional sources and use of wild and weedy relatives for those traits for which no variability exist in the cultivated gene pool, in the crop improvement program.

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## **AB06: Wheat Improvement for the heat prone agro-ecologies of Sudan and Sub-Saharan Africa: Breeding and pre-breeding approaches for climate change resilient varieties**

Izzat SA Tahir<sup>1</sup>, Sara EM Meheesi<sup>1</sup>, Ikram ES Mohamed<sup>1</sup>, Yasir SA Gorafi<sup>1,2</sup>, Hisashi Tsujimoto<sup>2</sup>, W Tadesse<sup>3</sup>, Filippo M Bassi<sup>3</sup> and Ahmed Amri<sup>3</sup>

<sup>1</sup>Agricultural Research Corporation, P O Box 126, Wad Medani, Sudan; <sup>2</sup>Arid Land Research Center, Tottori University, 1390 Hamasaka, Tottori 680-0001, Japan; <sup>3</sup>International Center for Agricultural Research in the Dry Areas, Rabat, Morocco

Corresponding Author: [izzatahir@hotmail.com](mailto:izzatahir@hotmail.com)

The importance of wheat (*Triticum aestivum* L.) in Sudan has been steadily escalating due to increased consumption resulted from growing population, urbanization and changing food habits. In 1961, wheat was grown in only about 16000 ha (mostly in areas north of Khartoum) with total production of less than 27000 tons using local varieties and traditional methods. All attempts up to WWII to introduce wheat to the central Sudan failed because of the lack of improved varieties and appropriate technologies. Agricultural Research Corporation in close collaboration with international centers (ICARDA, CIMMYT) and regional research institutes developed more than 70 improved technologies (including more than 33 heat tolerant wheat varieties) for proper integrated crop management. As a result, wheat cultivation has been expanded into areas previously classified as unfavorable environments due to high temperatures during the growing season. Currently, these areas represent more than 70% of the area grown by wheat in the country. The genetic gain in grain yield of wheat varieties released in Sudan from 1960 to 2006 was estimated to be 11.44 kg ha<sup>-1</sup> year<sup>-1</sup>. However, the genetic gain in grain yield was 24.5 kg ha<sup>-1</sup> year<sup>-1</sup> between 1960 and 1990. This indicated that although a reasonable level of improvement was achieved in wheat grain yield mainly through conventional breeding the genetic gain was slowed down in the recent years. During the period from 1960 to 2016, wheat area harvested increased by 3038 ha/year accompanied with increase in the total production rate of 9434 t/year. However, during the same period (1960 to 2016), the increase in wheat consumption rate was 41667 t/year (more than four times the rate of increase in total production). This possesses a big challenge especially in the light of the ensuing climate change which amplifies the challenges for realizing food security. Moreover, an apparent threat to securing food for an ever-growing world population is the existing limited biodiversity and genetic base resulted from cultivation of genetically uniform modern varieties. This is also true in the case of wheat improvement in Sudan and similar agro-ecologies. Therefore, efforts should be directed towards better exploitation of genetic diversity, understanding and mining physiological traits associated with climate change and then utilization these traits via their introduction into new varieties by conventional breeding and/or genetic manipulation. In this regards, different attempts are being made to develop wheat varieties that could cope with the climate change. One of the attempts is utilization of multiple synthetic derivatives (MSD) developed at Tottori University utilizing diverse sources of *Aegilops tauschii* which are being evaluated for heat and drought stress tolerance, nutrient use efficiency, salinity tolerance, etc. Several lines were identified to have better heat-stress adaptive traits, better water saver/drought tolerance, efficient in phosphorus and nitrogen uses. Utilization of these lines through crossing and backcrossing to adapted varieties and then selection using conventional and molecular breeding is underway. Another example is the multi-location evaluation and selection conducted to identify high-yielding better adapted wheat varieties. In this respect, wheat germplasm targeted to heat



stress areas is evaluated and selected under temperature gradients ranging from favorable to very high temperatures. Some stress adaptive traits have been identified and could be used for further improvement and mining the genetic resources for heat stress tolerance. Promising lines identified have been shared among west and east African lowlands experiencing high temperature during the growing season. These efforts have been further supported by the setup of ICARDA-ARC Heat Research Platform (ICARC-HRP) at the Gezira Research Station, Wad Medani, Sudan with the objectives of improving wheat adaptation to hot environments through conventional and molecular techniques and developing efficient methods to genetically improve wheat germplasm by incorporating heat tolerance mechanisms conferring robust performance in irrigated hot environments. Thousands of bread and durum wheat germplasm including accessions from genetic resource units, segregating materials, advanced and elite lines from ICARDA, CIMMYT and different organizations are being evaluated and selected annually and the results are being used to share heat tolerant germplasm with different countries. It could be concluded that innovative and integrated approaches and methodologies will be needed for further improvement especially in the light of the climate change. Efficient breeding methodologies and high precision phenotyping and genotyping will be needed to address the effect of climate change. The main elements could include: Targeted crossing through extensive use of widely known and demonstrated heat-tolerant cultivars in the crossing program; increased use of synthetics for the introgression of variability from both *Aegilops tauschii* and durum wheat to broaden the genetic base for biotic and abiotic (particularly heat) stresses in wheat; genome wide association studies (GWAS) and accurate characterization of relevant physiological traits to permit the identification of molecular markers associated with grain yield and its components under different stress conditions especially heat stress.



## **AB07: Use of tree genetic resources for land restoration: Case study of *Dacryodes edulis* G. Don Lam (Burseraceae) in Cameroon**

*Alain Tsobeng*<sup>1</sup>, *Alice Muchugi*<sup>2</sup>, *Prasad Hendre*<sup>2</sup>, *Ann Degrande*<sup>1</sup>, *Simon Kang'ethe*<sup>2</sup>, *Avana Marie-Louise*<sup>3</sup> and *Ramni Jamnadass*<sup>2</sup>

<sup>1</sup> World Agroforestry (ICRAF), WCA, Cameroon, Yaounde; <sup>2</sup> World Agroforestry (ICRAF), Kenya, Nairobi;

<sup>3</sup> University of Dschang, Cameroon

Corresponding author: Alain Tsobeng ([a.tsobeng@cgiar.org](mailto:a.tsobeng@cgiar.org))

### **Introduction**

The Central African forests, the planet's second largest rainforest block, are key to global environmental health. They influence climate change through their crucial role in carbon sinking and storage, affect weather patterns across Africa, and safeguard unique species and biodiverse communities (Abernethy et al. 2016). The Congo basin represents 70% of world's forest reserves (Noubissié et al. 2008). It is the world's second largest humid forest reserve after the Amazon forest in Latin America. It harbours about 10,000 plant species with about 3,000 endemics (CARPE 2001). Thirty million people directly depend on it for several uses and services such as; food, medicine, wood for building and crafts amongst others. ([http://evb.lacsq.org/fileadmin/user\\_upload/microsites/eav-evb\\_internet/documents/trousses-et-activites/forets-dumonde/fiche\\_5\\_2.pdf](http://evb.lacsq.org/fileadmin/user_upload/microsites/eav-evb_internet/documents/trousses-et-activites/forets-dumonde/fiche_5_2.pdf), consulted on March 21, 2019).

Due to several drivers of the loss of biodiversity such as; land clearing for agriculture, urbanization, forest exploitation for timber, unsustainable harvesting of forest products, mining, overgrazing, bushfires, cash crops (cocoa, coffee, oil palm, rubber) cultivation, the rate of land degradation is disturbing. An average of 40% of total emissions from deforestation and forest degradation are from harvesting of timber in the six Congo Basin countries (Umunay et al 2019). The Global Forest Resources Assessment (FRA), coordinated by FAO, found that the Sub Saharan Africa (SSA) forest area decreased from 31.6% of the global land area to 30.6% between 1990 and 2015. According to the United Nations' Food and Agriculture Organization, an estimated 18 million acres (7.3 million hectares) of forest, are lost each year (FAO, 2018). One of the many adverse consequences is climate change, responsible for the several challenges including food insecurity (FAO 2017, <https://www.livescien>)

Faced with this uncomfortable situation, international conventions such as the United Nations Framework Convention on Climate Change (UNFCCC), seek to provide efficient support like land restoration, to reduce the rate. The Bonn Challenge is a global effort to bring 150 million hectares of the world's deforested and degraded land into restoration by 2020, and 350 million hectares by 2030. This could generate USD 170 billion per year of the net profits from regeneration, and store about 1.7 gigatons of CO<sub>2</sub> per year, and produce other important environmental services. The African Forest Landscape Restoration Initiative (AFR100) is a country-led effort to bring 100 million hectares of land in Africa into restoration by 2030, and this fundamentally contributes to the Bonn Challenge. Many of the African countries have signed onto AFR100 and committed a combined 111 million hectares of land to be restored by 2030 and Cameroon is no exception.

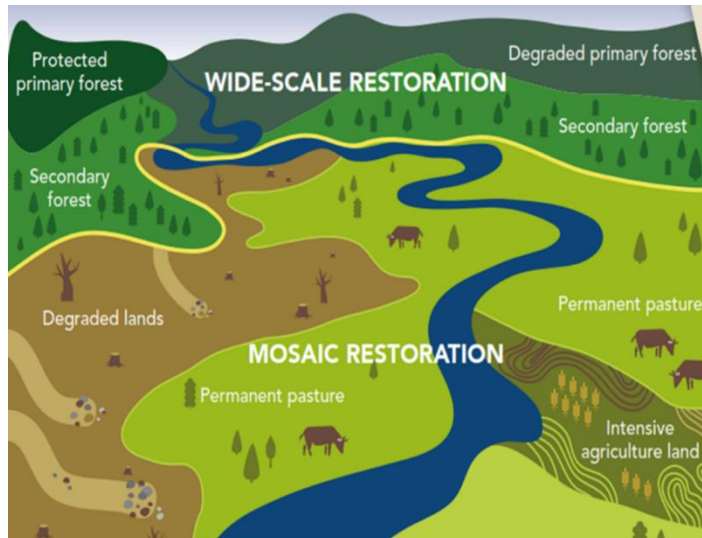


Figure 1 : Wide scale and mosaic approaches (Source: IUCN & WRI 2014)

Considering that Cameroon is one of the countries of the Congo basin, it ranks 5<sup>th</sup> in biodiversity richness and harbours 90% of the ecosystems found in Africa. The country lost approximately 2% of its total forest (defined as 30% canopy cover) over a period of 14 years (2000–2014) (Carodenuto et.al. 2015). It has therefore committed to restoring over 12 million hectares of deforested and degraded land by 2030 as part of the Bonn Challenge initiative including AFR100. As recommended by the national strategic restoration plan of the country, beside the Ministries (Forestry and Wildlife; Environment Nature Protection and Sustainable Development), partners have

to join their efforts to contribute to this objective. One of the main approaches recommended is the mosaic landscape restoration also call integrated approach, or multipurpose approach (contrary to wide scale approach that concerns only the forest). Among the partners, the World Agroforestry (ICRAF) has adopted a participatory tree domestication (PTD) model, recommended during COP 21, because of its efficiency.

Simons (2003) defined PTD as “a process involving accelerated and human-induced evolution to bring originally non-cultivated (wild) species into wider cultivation through a farmer-driven or market-led process”. The domestication of under-utilized tree species through deliberate selection and management of trees with superior traits is a multi-faceted process in which a progressively closer interaction between people and the tree resources takes place (Simons et al. 2004).

Key steps that guarantee the successful implementation of PTD are: priority setting, germplasm collection and conservation in genebanks, diversity assessment and selection of superior trees, development of best propagation options and scaling-up for wider cultivation in farmer fields, including degraded ones.

### Priority setting

Priority setting is the first step of PTD, as it defines the priority species for beneficiaries. This exercise is highly important for wide cultivation of trees because if farmers must play the first role in tree planting, the species to be promoted must be the one that are important to them. The importance can be due to incomes generated, nutritional value and medicinal importance amongst others. According to Franzel et al. (2008), *Dacryodes edulis* occupies the second position of the list of priority species in West and Central Africa. Consequently, it is a good candidate to highlight the contribution of PTD on land restoration.



## Participatory tree domestication of *D. edulis* and land restoration



Figure 2: *D. edulis* fruits on a tree and coal-roasted at roadsides in Cameroon's market

**Taxonomy and importance of *D. edulis* for human wellbeing:** *D. edulis* (Figure 2) is a Burseraceae which is highly represented in tropical Africa. The different common names used in its natural distribution range includes: safu, african pear, african plum, bush butter and native pear. The African pear tree is an oleaginous fruit tree with a generally cylindrical and a rectilinear trunk with early or shallow branching and usually without buttresses (Kengue 2002). It is originated from Central Africa, specifically from the Gulf of Guinea (Silou 1994). Older documentation limits the

geographical distribution to the forest region of Central Africa. While its actual distribution due to anthropic activities extends beyond its zone of origin to Uganda in East Africa, Angola in the South of Africa and the Malaysian peninsular in Asia (Todou et al. 2013). The fruit of *D. edulis* is the organ that gives the true economic value to the species and explains why it is under domestication (Kengue 2002). The contribution of *D. edulis* fruits' pulp to a healthy and nutritious diet is well established (Table 1) (Ajayi and Oderinde 2002).

Table 1: Approximate composition of *D. edulis*

Mean (%)	Pulp
Moisture	18.6
Ash	3
Protein	1.93
Fat	11.94
Fibre	47.5
Carbohydrate	17.03

**Assessment of diversity and conservation of *D. edulis*:** Evidence from phenotypic characterisation studies on *D. edulis* (Leakey et al. 2002, Waruhiu et al. 2004) in Cameroon and Nigeria, reveal a high frequency of intraspecific variation in measured fruit traits (fruit length, width, pulp thickness)



Figure 3: Partial view of *D. edulis* genebank in Mbalmayo

in wild and planted village populations (about 80%). Interestingly, this variation is greatest at village level, but between villages it is modest. Additional information's were raised by Makueti et al. (2015) who described a significant low level of genetic differentiation among the populations and high variability among individual trees. Thus, suggesting that genetic diversity at species level can be maintained by the village-level domestication, with the target on tree level, but not population.

To capture, document and preserve the genetic diversity spread within the natural distribution range of the species, ICRAF has established field genebanks composed of 180 accessions collected in farmers' fields using a random approach. They are established in Mbalmayo demonstration plot.

The genebank not only plays the role of species diversity store, but also progeny and provenance trials as well as seed orchards to ensure long-term availability of improved planting materials in support of the domestication programme. Therefore, monitoring, evaluation and documentation of each accession is being done annually for the past 18 years, focusing on growth and phenological parameters as well as fruit traits inheritability in view of identifying “plus trees”. The main descriptors used for the assessment are selected among those identified by Leakey et al (2002) based on farmer and consumer preferred traits. These included, fruit weight, fruit length and width, pulp thickness and mass as well as flowering and fruiting patterns with the aim of selecting regular fruiting and off-season stands. According to market assessment, off-season fruit prices three times higher than normal season varieties, with a high potential to generate more income to farmers (Schreckenberget al. 2002).

Development of propagation options: Typically, and/or ideally, only the best individuals’ being able to bring into domestication programmes/farmer fields, rooting of leafy stem cuttings and marcotting have been successfully tested with percentage of rooting and survival rate of about 80% on-station (Mialoundama et al. 2002), and about 60% on-farm.

Their key advantages being the ability to obtain a high degree of crop uniformity; production of trees able to fruit earlier (according to Kengue (2002), *D. edulis* trees from marcots bear fruits after three years compared to trees from seed that only fruit after six years); and the ability to perpetuate both pest and disease-resistant forms and climate change resistant of certain plants.

Setting-up of appropriate seeds and seedlings distribution system: One of the major constraints



identified for the PTD in the African Humid Tropics is the limited access to quality and quantity planting material to satisfy planting needs. ICRAF being a research and development institute, setting-up an efficient germplasm distribution pathway is a key concern. It is against this backdrop that community nurseries were established in almost 150 villages across the species’ agroecological zones within the country (nurseries serving more than 10,000 famers). Height of these communities were developed as Rural Resources Centers (RRC) for efficient intervention across communities. Each RRC serves between 5-25 communities (Degrande et al 2012). Moreover, clonal orchards using planting materials from identified “plus trees” have been established around each RRC. This approach endows all tree planters within communities and neighboring villages to have access to improved planting material in the form of seeds, cuttings or marcots according to their specific needs. Among these planting materials, marcots have been found to be preferred by farmers due to their early fruiting behaviour, as well as true-to-type multiplication. Thus, trees from marcots could start producing fruits just two years after planting compared to five and seven years for those from cuttings and seeds respectively (Asaah 2012).

Restoration potential using *D. edulis*: ICRAF efforts on intensification of tree planting using domestication has already been internationally recognized. Three prizes have already been obtained by the institution (Table 2).

Table 2: Prices recorded by ICRAF thanks to high contribution of PTD in improvement of environmental services and wellbeing in livelihoods

Prices	Provider	Date obtained
UNDP Equator Price	United Nation (UNDP)	2010
Award for Leadership in Conservation	National Geographic	2012
Outstanding practice on agroecology	World Food Council	2019

Since 2015, at least 3500 germplasm of *D. edulis* were distributed to diverse users every year. Distribution is free between group members and sold to others (USD 8/plant from marcot, USD4/plant from cuttings and USD 2/plant from seed). These germplasms are mostly used for the diversification of perennial crop farms, or for fencing annual cropping areas in view of increasing the income of farmers. Farmer's strategies to plant African plum trees is either by substitution of less valued species and varieties or by densification of their existing production systems.

From 2015 to date, the total of *D. edulis* trees in demand is estimated at 25,000. That means at least 250 ha of land potentially restored. Main categories of requests are civil-servants preparing for retirement, International NGOs (IUCN, SNV, GIZ) and research institute (CIRAD), and the Institute of Agricultural Research for Development (IRAD) for implementation of their respective projects. ICRAF's projects are not excluded to the list. The Figure 5 highlights key outputs of the FTNS project where several tree species, including more than 50% *D. edulis*, were planted on degraded land in agricultural zones of two protected areas (Lobeke and Dzanga Sangha). One of the key outputs of the project were the restoration of seven hectares (after 1.5 years of project implementation) of land through establishment of tree based cocoa system.

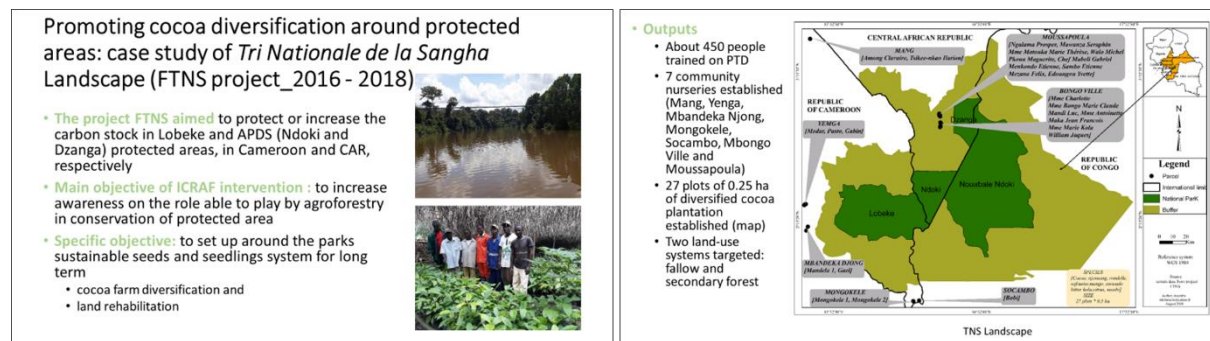


Figure 5: Focus on PTD and restoration in FTNS landscape

The key challenges faced when using PTD to ease land restoration is that annually, less than 60% of the demands is satisfied however, 40% is not met because of insufficiency of good quality planting material. It has been noted that, most of *D. edulis* request are marcots due to shortened maturity period. Unfortunately, they are not always available since farmers are reluctant to set more marcots on a tree because “the cutting of branches when harvesting them reduces the tree yield”. Options of combining marcotting and rooting of cuttings have been tested. Farmers use to establish stock plants composed of marcots, from which cuttings will be collected for mass propagation.





**Way forward: cultivar development:** Lack of environmental adaptation scheme for many tropical plant genetic resources (PGR) is one major reason for the limited use of genetic resources in classical plant breeding. Moreover, a classic approach for PGR breeding may also be considered because those materials without selection for adaptation to the target environment, will not have any immediate use for the breeders (Hallauer and Miranda 1981). To address these plant resilience issues, a multilocation trial has been set-up in view of assessing adaptation capabilities of *D. edulis* clones (Bum/DE/34 is one of these clones). The trial was set-up in 2015 in three agroecological zone and data is being collected yearly on growth (height, stem diameter and crown diameter) and reproductive development (flowering, fruiting, fruit traits parameters) parameters. The information generated will provide orientation for appropriate adding value technologies for improved uses and management of *D. edulis* genetic resources. With the EiB, a CGIAR platform, we have been familiarized with the product profile tools that guides on the process of replacement of old varieties with new one. It is adapting on *Dacryodes edulis* species.



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### Take home message

Within the mosaic restoration approach, PTD can be a highly useful tool for rapid, sustainable and efficient restoration. The key thing is to promote the good quality of the priority species for target farmers. Bringing government on board will provide long-term support to farmers and consequently, considerably boost their effort.

### Acknowledgments

CGIAR Research Program specifically Forests, CGIAR (FTA, EiB), Genebank (supported by Crop Trust), International Fund for Agricultural Development (IFAD); the Belgian Development Cooperation; German Cooperation, European Union, and Institute of Agricultural Research for Development (IRAD, Cameroon).

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## AB09: A glimpse in the domestication history of our cereal crops through genebank genomics

Mona Schreiber<sup>1,2</sup>, Sara G Milner<sup>1</sup>, Nils Stein<sup>1,3</sup>, and Martin Mascher<sup>1,4</sup>

<sup>1</sup> Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Germany; <sup>2</sup> Johannes Gutenberg-University (JGU) Mainz, Germany; <sup>3</sup> Georg-August-University GoOngen, Germany; <sup>4</sup> German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Germany

Correspondence: Mona Schreiber ([schreiberm@ipk-gatersleben.de](mailto:schreiberm@ipk-gatersleben.de))

### Introduction

More than ten thousand years before our time, one of the most significant changes in human history the so-called ‘Neolithic transition’ started. This transition describes the gradual shift from a lifestyle as nomadic hunter-gatherers to sedentary farming and agriculture. The first evidence for this change, mainly characterised by the process of domestication, was found in southwest Asia in the present-day Levant and southern Anatolia, also known as the ‘Fertile Crescent’ (Zohary et al. 2012). Archaeological evidence provides insights into the whereabouts of the initial domestication process, and archaeobotanical remains help trace back the early beginning of plant cultivation. However, archaeology relies on phenotypic characteristics and quantitative differences to distinguish between wild, cultivated and domesticated specimens. Phenotypes can show little beyond those individuals that have already passed the entire process of domestication. But the process of domestication left its traces as well in the genomes of the domesticated species and can offer an additional path to reconstruct the past and to get a better understanding of this momentous change in our history (Shennan 2018). Comparative analysis of population genomic data of large samples of current and past varieties of crops together with their wild progenitors provides insight into the domestication history of our cereals.

To analyse the genomes of cereals, high quality genome sequence assemblies are a prerequisite for the alignment of resequencing data from large diversity panels comprising contemporary crop specimen and their wild relatives. Recent developments in sequencing technology and computational methods have contributed to the construction of high-quality reference sequences for large and complex cereal genomes (e.g. Mascher et al. 2017, IWGSC et al. 2018). Genotyping-by-sequencing (GBS) is a high-throughput sequencing approach that employs complexity reduction by digestion with restriction enzymes to obtain dense genome-wide marker data for a large number of samples at comparatively low costs (Elshire et al. 2012).

Genebanks comprise comprehensive collections of plant genetic resources for crop plants and their wild relatives around the world. The analysis of GBS data for more than 22,000 barley accessions of the German *ex situ* genebank (Milner et al. 2019) together with ~1800 accessions from the International Center for Agricultural Research in the Dry Areas provides insights into the global population structure of domesticated barley. Barley (*Hordeum vulgare* L.) remains are already present in late Mesolithic sites in the Levante and is therefore considered to be a founder crop of agriculture. *Hordeum vulgare* ssp. *spontaneum*, the putative wild ancestor of barley, is still abundant in the Fertile Crescent as well as further east in Central Asia.

A principal component analysis (PCA) of wild and domesticated barley germplasm in the extensive GBS data set shows a clear genetic differentiation according to domestication status as it was



already shown by previous studies. Within the domesticated accessions, PCA and ADMIXTURE analyses highlight geography at the continental scale as the major diversification factor, differentiating at first Eastern and Western barley followed by further geographic subdivisions as groups of Ethiopian and North African together with Southern European barleys. In addition, annual growth habit and morphological characters related to the inflorescence architecture and lemma adherence were major determinants of population divisions. DNA sequences from extraordinarily well preserved archaeobotanical barley remains from Israel dating back to the Chalcolithic (~6000 before present (BP), Mascher et al. 2016) and the Iron Age (~3000 BP) were analysed together with the modern panel genetic. The close genetic proximity of the ancient material with modern barley from the same geographic region indicated genetic continuity within the Near East.

In contrast to barley, rye (*Secale cereale* subsp. *cereale*) is not considered as a founder crop of Neolithic agriculture, even if the wild progenitors of all three cereal crops share the same distribution range in Southwest Asia (Vavilov & Dorofeev 1992). Instead, rye is assumed to be a secondary domesticate that became widely used during the Bronze Age in Central and Eastern Europe only after its introduction as a weed in the course of the expansion of domesticated wheat and barley. Its ability to thrive on poor soils and its high frost tolerance have enabled rye to become a suitable crop under the harsh conditions of Northern Europe (Behre 1992). The combination of genotyping-by-sequencing (extended data set of Schreiber et al. 2019) and whole genome resequencing data (*inter alia* from Bauer et al. 2017) for 1002 *Secale* samples mapped to the recently assembled high-quality reference genome offers the possibility to study the population history of the small genus *Secale*. Overall, the weak genetic differentiation between wild and domesticated rye points to ongoing gene flow and a recent speciation leading to incomplete lineage sorting and low fertility barriers. The analysis of the population genomic history of domesticated rye within the complex population structure of the different wild *Secale* species points to the idiosyncratic characteristics of the domestication process of rye. We identified distinct ancestry groups between Central Europe and the Near East, domesticated rye from the Iberian Peninsula however, is genetically closer to Near Eastern samples than to those from of Europe. A similar pattern can be seen in other cereals as well, likely due to the complex population history of this western most part of the European continent.

The differences in the relationship of barley and rye with their wild relatives emphasize the influence of different reproduction mechanisms on the divergence between wild and cultivated germplasm. Likewise, important are differences in the economic importance of these two cereal crops, diverse end-uses and of course the underlying domestication history. Combining the newly available genomic resources with archaeological, anthropological and linguistic evidence enables us to explore the differences in use and perception of cereals in different geographic areas because of the underlying past processes.

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## **AB10: Improving efficiency in the exploitation of genetic resources using molecular techniques**

*Antonio Costa de Oliveira*

*Full Professor, Universidade Federal de Pelotas – Brazil*

*Email: Antonio DeOliveira ([acostol@gmail.com](mailto:acostol@gmail.com))*

The number of studies showing the gradual plateauing of the genetic advance for most of our crops has been at an increasing rate. This is due to the recurrent use of a very restricted germplasm for crosses and the intense selection pressure for a limited range of phenotypes. This funnel effect is more drastic for bread wheat, a species with naturally narrow genetic diversity. Furthermore, this ongoing narrowing of the genetic variability also means genetic vulnerability at fields. Therefore, there is an urgent need for new sources of genes and alleles to broaden the genetic base of this important cereal, through pre-breeding program. There is a wealth of sources of novelty for wheat breeding, including exotic accessions, landraces and wild relatives, from the secondary and tertiary gene pool. There are, however, difficulties to proper use this richness for crop improvement. One obstacle resides on mining promising donors of improved traits in a universe of thousands of accessions. The other issue is regarding transferring desirable traits into wheat background avoiding all undesirable characteristics, specially from landraces and wild species, the well known *linkage drag* problem. Considering these obstacles, approaches based only on phenotypic assessments present many disadvantages, e.g., they are time-consuming, costly (depending on the trait assayed and number of genotypes) and laborious. These difficulties make its use almost impractical. Improved approaches and tools are needed to counteract this difficulty, improving the efficiency in the exploitation of genetic resources and Molecular Techniques, i.e., methods and tools based on Genomics and Biotechnology, can expressively contribute in this process.

Among several tools, the most important molecular technique is molecular marker, a technology in constant evolution for the last 40 years, which furthermore is experiencing a tremendous advance these days, in which hundreds of thousands of loci can be accessed in hundreds of accessions in short time at a dropping cost. Molecular markers can be used to diagnose the level of narrowing of the genetic base in a given elite germplasm with higher precision. These markers can also be used to characterize the genetic variability available in other germplasm for reducing redundancy and selecting potential parents for use in crosses. Also, these loci can be used in Marker Assisted Selection, tracking segments of interest in segregating or non-segregating genotypes, aiming a more precise selection, saving time and resources. Nowadays, they can also be used in the “improved version”, the cutting-edge Genomic Selection approach, in which all loci available are considered simultaneously for accurate selection. Indeed, there is a considerable variability regarding types of markers, for different necessities and available resources. SSRs (Simple Sequence Repeats) loci have been used as molecular markers for more than two decades and are still being used, and their advantages reside on the cost (regarding both equipment and reagents) but also their expressive quality, as they are highly polymorphic, multi-allelic, numerous across genomes and not directly affected by either natural or artificial selection.

SNP (Single Nucleotide Polymorphism) markers are, however, the markers of choice these days. The abundance and distribution of these “single letter variations” across genomes make these loci a powerful tool for accessing genetic variability for any purpose and tracking genes and alleles of



interest with high precision. There are numerous variations in which these loci can be assayed. Genotyping by Sequencing and array-based platforms deliver an immensurable amount of data for hundreds of genotypes in short time and are the best choice, although still costly (but the cost is dropping out), when accessing a germplasm for the first time (a throughout “read” of these genotypes) and when the objective is applying Genomic Selection. To track segments already introgressed or transferred to breeding lines, individual or few loci linked to segments of interest can be used, through conversion of these SNPs from high-throughput equipment to other technologies, such as KASP™ or CAPs markers. There is a vast number of other molecular markers which can also be used in pre-breeding and breeding programs, giving good results, especially when the technology is already in use in the lab. Molecular markers have been proven to be a valuable tool for crop breeding, but also pre-breeding, and are a key component to fight against the narrowing of the genetic diversity of our crops that feed the world.



## **AB12: Can we find abiotic stress tolerance in wheat related-wild species? - Experiences and lessons learned in pre-breeding of wheat with drought and heat stress tolerance**

*Hisashi Tsujimoto*

*Arid Land Research Center, Tottori University, 1390 Hamasaka, Tottori 680-0001, Japan*

*Email: tsujim@tottori-u.ac.jp*

World genebanks maintain many accessions of crop-related species as genetic resources for crop improvement. In the breeding of bread wheat, genes originating from wild species have been used for biological stresses resistance, such as disease and insects. These wild species are expected to have promising genes also for abiotic stress tolerance such as drought and heat. We have been trying to find abiotic tolerance in the diversity of *Aegilops tauschii*, a wheat-related species. However, it was difficult to find the genes because of their grassy morphology and tough and brittle spikes. We used many 'synthetic hexaploid wheat (SHW)' lines that were produced by crosses between a durum wheat variety (cultivar Langdon) and many *Ae. tauschii* accessions. However, because the SHW lines still kept grassy morphology and hard spikes, we could not evaluate the stress tolerance based on the yield potential. Also, we found that there is no correlation between the tolerance-related traits observed in *Ae. tauschii* and the corresponding SHWs. This result meant that from the morphology of *Ae. tauschii* accessions we cannot predict the traits of the corresponding SHWs. We crossed and backcrossed many SHWs with a bread wheat variety, Norin 61 (N61), and mixed the obtained BC1 seeds to make a bulked population. By self-pollinating several generations, we made a mixed pure-line population and named it 'multiple synthetic derivative (MSD) population' in the genetic background of N61. We cultivated this population in a heat-stressed field in Sudan and successfully selected heat-tolerant genotypes. We crossed the tolerant lines with N61 for QTL analysis in the genetic background of N61. Also, we crossed the lines with Sudanese varieties. The molecular markers that will be produced by the QTL analysis will be used to follow the presence of QTLs even in the miscellaneous segregating genetic backgrounds that appear in the crosses between the tolerant lines and Sudanese varieties.





## **AB13: Chromosome engineering to leverage alien genetic variation and make a better wheat in the face of current and future challenges**

*Carla Ceoloni and Ljiljana Kuzmanović*

*Department of Agricultural and Forest Sciences (DAFNE), University of Tuscia, Viterbo, Italy*

*Corresponding author: Carla Ceoloni ([ceoloni@unitus.it](mailto:ceoloni@unitus.it))*

Nowadays, there is a common concern on how fast and alarmingly is the world changing. The population continues to grow, and societal behaviour is experiencing rapid evolution, particularly in developing countries. Demand for and pressure on resources (mainly land and water) continues to increase, in the face of their progressive shortage because of population growth, overconsumption in parts of the world, land degradation and increasing urbanization. Climate changes pose further and less-predictable challenges. As a result, intensified agricultural production will be required to ensure food and nutrition security and to compensate for production lost in environmentally stressed areas. However, adequate and diversified measures to be adopted must be environmentally sustainable.

Crop breeding, besides management, is the main pillar of efforts to tackle the present and future challenges of food production. Although it is obvious that crop improvement is dependent upon genetic diversity, strategies of agricultural intensification followed in the recent past (such as during the Green Revolution), have had, alongside highly positive outcomes on world's food crop production, negative implications on genetic variation. Contemporary globalization processes, then, are eroding genetic diversity faster than ever, with industrialization of agriculture, as well as progressive tendency towards uniformity in eating habits, emerging as the main factors in accelerating the process. Today, of the 150 crops that are commercially important, rice, wheat and maize account for 60% of the world's food supply. On the other hand, the same process of globalization is also making that very diversity essential for the continuation of human well-being into the future. Hence, the currently globalizing humanity, to some extent also contributing to climate change, must put all its efforts, for itself and for future generations, to safeguard the existing diversity, as well as to generate novel and ample crop genetic diversity to satisfy the new, diversified and foreseen (or even just potential) requirements.

The rate of genetic gain in breeding programs can greatly increase by extending the amount and nature of variation using landraces and wild relatives. Despite the range of undesirable traits that exotic germplasm is known to carry alongside beneficial traits, we are fortunate to live in the era of "omics revolution", which is increasingly enabling genome-based breeding even for the complex cereal genomes. Advancements include all the necessary tools for accurate characterization and selection of desired products, from identification and annotation of genes/QTL underlying the genetic architecture of relevant traits, to high-throughput phenotyping, as well as molecular marker technologies.

With all these tools available, access to the still poorly exploited gene pools of wild crop relatives has become feasible as ever before. As a relevant example, wild wheat relatives (WWRs), equipped with inherent resilience to a variety of harsh environments, but also with a wealth of unpredictable or only recently uncovered and valued positive effects on wheat yield and quality-related traits, are being increasingly targeted in a variety of "smart breeding" designs. The foundations of the main



cytogenetic strategies to harness useful genetic variation from WWRs were laid in the '70-80's, when ER Sears coined the term “chromosome engineering” to describe the “transferring of segments of alien chromosomes carrying particular desired genes to wheat chromosomes”, and mutants at the wheat *Ph1* locus, whose absence allows recombination between homoeologous Triticeae chromosomes, hence gene transfer from even distantly related gene pools, were made available to the “wheat chromosome engineers”. As considerable synteny and colinearity, at least at a regional level, is shared by cultivated and wild Triticeae genomes, genetically-promoted homoeologous recombination enables attainment of largely balanced wheat-alien transfers. Aided by use of highly diagnostic tools, their size and consequent linkage drag can in most cases be conveniently minimized. The latter represents a generally valid requirement for the alien segment introgression to be well accepted by the recipient genome, and hence “breeding-friendly”.

Nonetheless, given the polyploid nature of cultivated wheats, multiple segment introgressions can also be pursued, more amenable into the hexaploid bread wheat (*Triticum aestivum*) and, to a lesser extent, into the tetraploid durum wheat (*T. durum*). Simultaneous incorporation of multiple segments may be the goal of “untargeted” approaches, applicable when prior knowledge of the chromosomal location and map position of genes/QTLs underlying a complex trait or a variety of traits, known to be present in a given alien donor, is lacking, or even when the very purpose is the fractionated introgression of ideally the entire wild genome, later followed by phenotypic analysis of all introgression products.

Alternatively, creating a pyramid of segments can be part of the deployment process of “targeted” genetic variation originating from multiple alien donors and for multiple traits that can thus be simultaneously enhanced in the recipient genotype. A successful example of this design is that of a triple recombinant line that we have created in a durum wheat background, in which the *Lr19+Sr25+Yp* genes (from the 7e1L arm of *Thinopyrum ponticum*), *Glu-D3* from 1DS of *T. aestivum* and *Pm13* from 3S<sup>1</sup>S of *Aegilops longissima* have been introduced on recipient chromosome arms 7AL, 1AS and 3BS, respectively. The three segments, previously incorporated into separate recombinant lines via *ph1*-mediated homoeologous recombination, were each associated with robust selection markers, invariably linked to the respective segment in a restored *Ph1* condition. With its package of highly effective resistance genes, and a more suitable gluten for “dual purpose” transformation (pasta and bread making, thanks to the *Glu-D3* effect), as well as a high yellow pigment content (*Yp*), this triple recombinant line, crossed and backcrossed with several durum wheat varieties adapted to Northern and Southern regions of durum wheat cultivation areas (including those under ICARDA mandate), is currently at an advanced breeding stage, with concrete promises to provide field-ready materials in the short term run (Ceoloni et al. 2017, doi:10.3390/d9040055). Interestingly, in recent field trials carried out in a range of contrasting environments (Kuzmanović et al. 2018 doi: 10.1016/j.fcr.2018.08.014), the line (named R5) contributing the *Th. ponticum* segment to the triple recombinant, showed a grain yield advantage compared to its null control in Central Italy, Morocco and South Australia sites, in coincidence with seasons characterized by very low rainfall and high temperatures from heading onward. In a global warming scenario, this evidence enhances the value of such chromosome engineering interventions addressing durum wheat, a strategic commodity for many countries of the Mediterranean Basin, but also expanding its cultivation on a vast array of agroenvironments in many world areas.

Another strategy through which multiple traits from different alien sources can be combined in a



single wheat genotype is practicable when the target genes/QTL reside in related alien chromosomes, hence capable of recombination. Thus, depending on their relative position, a single segment can be made up with stacked genes/QTL. We resorted to this strategy to incorporate into the very distal tip of *Th. ponticum* 7e<sub>1</sub>L segments (previously transferred into durum wheat 7AL, see above) a small 7e<sub>2</sub>L portion, originating from a quasi-homologous arm of a different *Th. ponticum* accession. This contains a very effective QTL for resistance to *Fusarium* diseases, both Fusarium head blight (FHB, hence the designation of *Fhb-7e<sub>2</sub>* and later *Fhb7*; Forte et al. 2014, doi:10.1007/s11032-014-0175-3) and also crown rot (FCR, unpublished). The efficacy of *Fhb7*, as measured in controlled inoculation tests, is remarkable, amounting to over 80% and 40% reduction of FHB and FCR disease severity, respectively. However, *Fusarium* diseases, severely affecting grain yield and quality of the wheat crop, are considerably expanding worldwide. In this, they are favoured by climate changes, particularly rising of minimum winter temperatures and warmer-wetter conditions during flowering, the most sensitive stage to pathogen attack. Furthermore, the current scarcity of resistance sources in wheat germplasm is particularly alarming for durum wheat, mainly used for human consumption, hence at high risk of kernel contamination by health-dangerous mycotoxins (e.g. deoxynivalenol = DON). Indeed, susceptibility against *Fusarium* spp. infection seems to be higher in durum wheat than in bread wheat cultivars, one contributing factor probably residing in the limited exposure to relevant disease pressure of current durum wheats, mostly descending from germplasm cultivated in relatively warm and dry environments. Thus, to widen the array of *Fusarium* resistance sources for wheat improvement, we have recently undertaken a further pyramiding exercise, eventually accomplishing the introgression of an exceptionally valuable FHB resistance QTL (*Fhb-7EL*) from the 7EL chromosome arm of *Th. elongatum* onto *Th. ponticum* 7e<sub>1</sub>L segments, stably inserted into the 7DL and 7AL arms of bread and durum wheat primary transfer lines. At first, an introgression mapping work was carried out at the hexaploid level, which showed the *Fhb-7EL* to be distally located on 7EL with respect to the position of homoeologous 7e<sub>1</sub>L target genes (Ceoloni et al. 2017, doi:10.1007/s00122-017-2939-8). Without resorting to any genetic pairing promotion but relying on the close 7EL-7e<sub>1</sub>L homeology (20% pairing frequency), several 7EL+7e<sub>1</sub>L bread wheat recombinant lines could thus be obtained. Those possessing telomeric 7EL chromatin, evidently including the *Fhb-7EL* locus, had over 95% reduction of FHB severity, and over 70% reduction of FCR symptoms. Two such lines were crossed with 7AL-7e<sub>1</sub>L durum wheat recombinants and, as proved by GISH analysis of meocytes of pentaploid hybrids, homologous pairing occurred at high frequency in the 7e<sub>1</sub>L region common to the bread and durum wheat recombinant chromosomes. Aided by 7EL/7e<sub>1</sub>L-linked markers, 7EL+7e<sub>1</sub>L tetraploid recombinant types could thus be isolated in BC<sub>1</sub> progeny to durum wheat. Results of controlled inoculations confirmed what previously observed in bread wheat, with over 90% reduction of disease severity associated with *Fhb-7EL* presence compared to null controls. The latter exhibited nearly 80% reduction of seed set and weight compared to *Fhb-7EL* carriers. In parallel, DON content resulted from 200 to 1000-fold reduced in flour extracts of resistant vs. susceptible genotypes. Moreover, the same *Fhb-7EL* also conferred the novel durum wheat recombinants with the ability to reduce by 50% FCR disease symptoms. Thus, a very effective barrier has been built in durum wheat towards different *Fusarium* diseases, which, combined with the positive attributes of 7e<sub>1</sub>L derivation, makes a super-combination of tactical traits for crop security and safety.

Not underestimating possible future impacts on breeding gains of even finer manipulations (e.g. genome editing), the above examples should provide convincing evidence of the contribution that, in the frame of a cutting-edge technological and holistic approach, ‘smart’ chromosome



engineering strategies involving WWRs are fully ready to provide in order to meet the current and future requisites for agricultural production. This is particularly relevant for wheat, which covers more of the earth than any other crop and is in increasing demand in countries undergoing urbanization and industrialization. Therefore, it is hoped that investments in pre-breeding programs and human capacity development for the ongoing “second Green Revolution” will include in their priorities the expertise in certain fields, primarily cytogenetics, so to reinvigorate the vanishing community of “chromosome engineers”.



## **AB14: Contribution of synthetic wheats for the development of elite spring bread wheat genotypes at ICARDA**

*W. Tadesse, M. El-Bouhssini, A. Hamwieh, A. Niane, Z. Bishaw, S. Rehman, K. Nazari, M. Keser, R. Sharma, A. Al-Abdallat, A. Amri, F. Ogbonnaya and M. Baum*

*International Center for Agricultural Research in the Dry Areas (ICARDA), Rabat-Morocco;*

*Corresponding author: Wuletaw Tadesse ([w.tadesse@cgiar.org](mailto:w.tadesse@cgiar.org))*

Wheat production is constrained by biotic and abiotic stresses at global level in general and Central and West Asia and North Africa (CWANA) and Sub Saharan Africa (SSA) regions. With changing climate, biotic (rusts, septoria, insects) and abiotic (heat and drought) stresses are increasingly important causing reduction in photosynthesis, pollen viability, grain number and weight, and hence lowering yield and quality. Landraces, wild relatives, and synthetic wheats provide useful sources of resistance to most of these biotic and abiotic stresses. Most of the landraces and wild relatives are conserved *ex-situ* in genebanks. Global reports indicate ~10% of the genetic resources utilized in wheat breeding programs. The low percentage of germplasm utilization is due to: (a) gene bank materials are too wild, obsolete and difficult to breed, (b) germplasm characterization and evaluation data are not of good quality, (c) elite lines and cultivars possess different traits breeders are looking for, (d) gene bank accessions data might not be user friendly and easily accessible, and (e) it is difficult to transfer genes from wild relatives into elite genotypes/cultivars. Sometimes, the transferred genes might also result in linkage drags. Nevertheless, genetic resources have been utilized in breeding programs contributing to improved yield, resistance to diseases and insect pests and nutritional quality.

Wheat breeding program at ICARDA uses conventional and molecular approaches such as Focused Identification of Germplasm Strategy (FIGS), mega environments, shuttle breeding, wide crosses using synthetic wheats, doubled haploids, marker-assisted selection, genomic selection, speed breeding and key location phenotyping to identify sources of resistance and develop elite genotypes with high yield and resistance to biotic and abiotic stresses. Spring bread wheat breeding program has used synthetic wheats extensively for improving grain quality, drought and heat tolerance, and resistance to rusts, Hessian fly, and sunn pest. Currently, more than 25% elite genotypes in the program have synthetic wheat backgrounds in their pedigrees.

Yield of the top yielding elite spring bread wheat genotypes ranges up to 6 t/ha at Wad Medani in Sudan under extreme heat stress, 7 t/ha at Marchouch in Morocco under terminal moisture stress (260 -300 mm) and 11 t/ha at Sids in Egypt under optimum conditions. Genetic gain from 1980 to 2017 showed 2.5, 1.3 and 2.3% per year increment at Marchouch, Wad Medani and Sids station, respectively. Significant MTAs for yield under heat stress have been identified through genome wide association mapping studies for heat and drought tolerance, resistance to yellow rust, stem rust, Hessian fly, and quality traits. Pedigree analysis showed that tolerance to heat and drought, and resistance to rusts, Septoria, Hessian fly and sunn pest in such elite wheat germplasm were introgressed from synthetic wheats and wild relatives mainly *T. dicoccoides*.

ICARDA distributes more than 400 of such genotypes to its partners through international nurseries. In the last 5 years, more than 60 bread wheat varieties of ICARDA origin have been released by National Agricultural Research System (NARS) in the CWANA and SSA regions. Some of the released varieties have synthetic wheat backgrounds in their pedigrees.



## AB17: The use of wheat wild ancestors for wheat pre-breeding in CIMMYT

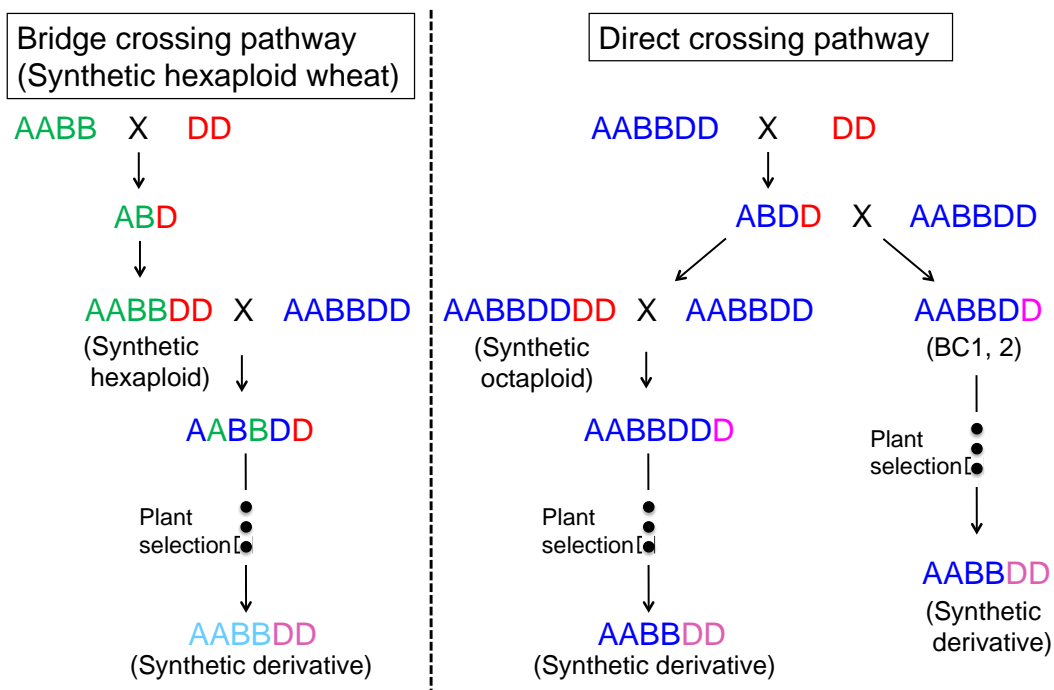
Masahiro Kishii

International Maize and Wheat Improvement Center (CIMMYT), El Batán, Mexico

Email: [m.kishii@cgiar.org](mailto:m.kishii@cgiar.org)

The use of crop wild relatives in wheat breeding often associates with additional difficulties such as crossability problem, incompatibility and less meiotic pairing frequency between wheat and wild species chromosomes. We also see expression differences between diploid ancestral species and hexaploid bread wheat, in which a trait in a diploid ancestor will not always express in bread wheat. Despite these difficulties, crop wild relatives have brought so many impacts in wheat breeding.

Among various wheat wild relatives, CIMMYT has been focusing on the use of D genome ancestor (*Aegilops tauschii*), because we can have faster outputs. It has two ways in the use of D genome ancestor in wheat breeding, either through bridge or direct crossing pathway. While Kansas State University adapted the direct pathway, CIMMYT adapted the bridge pathway.



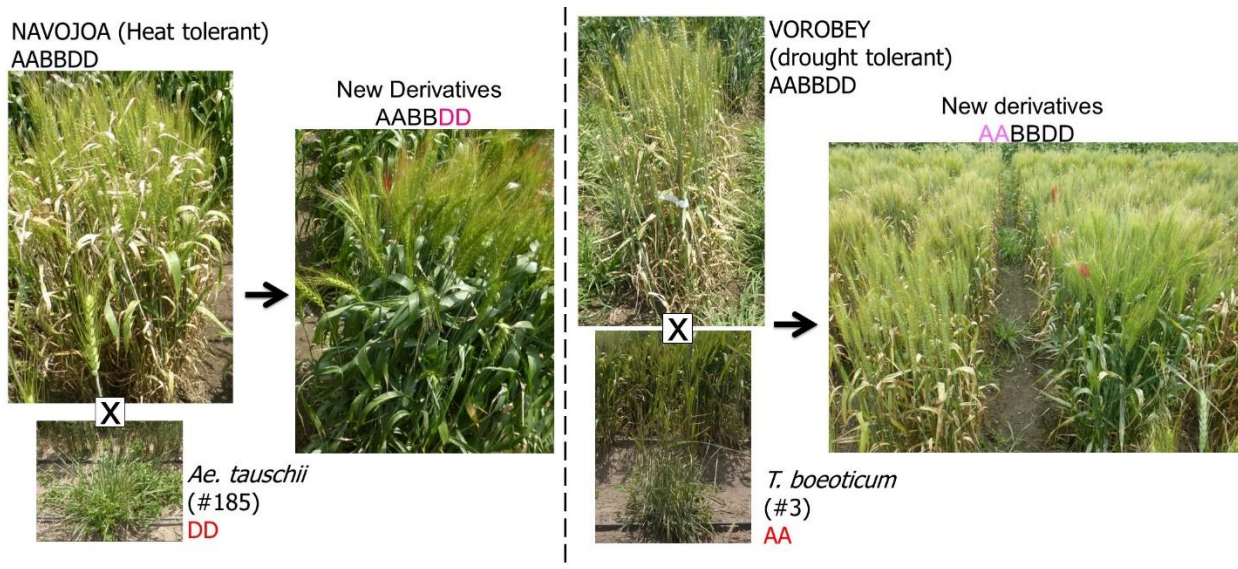
In the bridge cross, durum wheat and D genome ancestor are crossed to produce so-called synthetic hexaploid wheat (SHW), and then SHW are crossed with bread wheat lines to produce synthetic derivative lines. Since 1980's, CIMMYT has produced more than 1,500 SHW lines using various *Ae. tauschii* (DD) accessions. The production has not been trait-specific but rather random crossing. This is partly because we have seen some problems in crossability and incompatibility during the crosses between tetraploid wheat and *Ae. tauschii*. The large number of SHW lines provides an advantage to screen many things including newly emerging threats.



Impact of synthetic wheat for wheat breeding is remarkable. At least, 60 wheat varieties from synthetic wheat have been released around the world from China, India, the Middle East and Africa. Synthetic derivatives lines have been also occupying a large portion of disease resistance and rain-fed nurseries of CIMMYT for years. One limitation is that it has not contributed much for yield potential, although some studies show that it can have positive effects on yield improvement. Another concern is that the number of synthetic wheat lines frequently appeared in nurseries has been limited. To find out a reason behind this, we have started doing molecular research. Preliminary data shows that some *Ae. tauschii* fragments retain in synthetic wheat derivatives more than other parts, implying that these segments have positive values for the breeding, but we have not obtained evidences that those *Ae. tauschii* segments are contributing for improvement of specific traits.

Since we have produced many synthetic wheat lines using D genome ancestor, we have shifted our focus more on the use of diploid ancestral species of A and B through octaploid synthetic wheat. We have also started producing octaploid synthetic wheat using D genome ancestor, aiming direct improvement of CIMMYT best lines and to see only effect of D genome. This is because some wheat breeding programs have difficulties to use SHW due to *Ne1-Ne2* necrosis and partly because we can see only effects of D genome of *Ae. tauschii* octaploid pathway.

We have produced more than 200 lines of octaploid synthetic wheat using A, B and D genome ancestors in the last five years. Preliminary results from octaploid synthetic derivative are encouraging. We have seen improvements of disease resistance in almost all cross combinations we tested. We have also confirmed that A genome octaploid synthetic wheat is useful for wheat breeding. Yet one of additional difficulty in the use of A genome ancestor will be a significant reduction in recombination rate between wheat A genome chromosomes.



Our main work at CIMMYT has been the use of ancestral species. But we have observed new alien translocations from rye and *Thinopyrum* have positive effects on grain yield in irrigated and drought conditions. This, along with the 2NS translocation, reminds us that there are still many untouched useful genes in wild relatives for wheat improvement. The use of wild alien species will take much more time and risky, but it can bring tremendous impacts that we can expect in ordinal breeding and which will become more important in the world of climate change.



## AB18: The magic of crop wild relatives in durum wheat breeding

Filippo M. Bassi<sup>1</sup>, Khaoula El Hassouni<sup>1,2</sup>, Samir Alahamad<sup>3</sup>, Hafssa Kabbaj<sup>1,2</sup>, Meryam Zaim<sup>1,2</sup>, Mounira Azouz<sup>1,4</sup>, Amadou Tidiane Sall<sup>5</sup>, Zakaria Kehel<sup>1</sup>, Ayed Al Abdallat<sup>6</sup>, Veronique Alary<sup>1</sup>, Abdul Aziz Niane<sup>1</sup>, Bouchra Belkadi<sup>2</sup>, Abdelkarim Filali-Maltouf<sup>2</sup>, Lee Hickey<sup>3</sup>, Rodomiro Ortiz<sup>7</sup>, Michael Baum<sup>1</sup>, and Ahmed Amri<sup>1</sup>

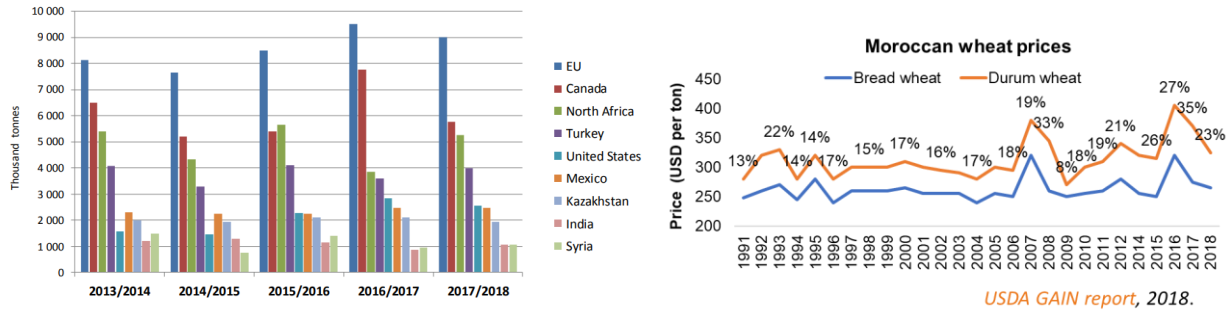
<sup>1</sup>International Center for Agricultural Research in the Dry Areas (ICARDA), Rabat, Morocco; <sup>2</sup>The University of Mohammed V, Faculty of Sciences, Rabat, Morocco; <sup>3</sup>The University of Queensland, QAAFI, Brisbane, Australia; <sup>4</sup>Institut National de la Recherche Agronomique (INRA), Algiers, Algeria; <sup>5</sup>Institut Sénégalais de Recherches Agricoles (ISRA), Saint-Louis, Senegal; <sup>6</sup>University of Jordan, Amman, Jordan; <sup>7</sup>Sveriges lantbruksuniversitet (SLU), Alnarp, Sweden

Corresponding author: Filippo Bassi ([f.bassi@cgiar.org](mailto:f.bassi@cgiar.org))

### Introduction

Durum wheat (*Triticum durum* Desf.,  $2n = 4x = 28$ , AABB) is the tenth most important crop in the world in terms of production with an achieved annual harvest of 40 million tons in 2017 (**Fig 1**). Its global cultivation places it as the 15<sup>th</sup> crop in terms of surface with over 18 million hectares cultivated in 2018 (Leff et al. 2004). Canada is the largest cultivator with 2.1 million ha, followed by Italy, Algeria and India with approximately 1.4 million ha each, then Turkey, Morocco, and Syria at 1.0-1.2 million ha, and in order of surface: Ethiopia, Tunisia, Iran, France, Spain, Pakistan, and Greece cultivating 0.3-0.5 million ha each. Durum wheat is considered a *staple food* of the Mediterranean diet in the form of unleavened breads, bulgur (concassed grains), pasta, couscous (or Dalhia in India), and its straw is very important as feed for the livestock. However, bulgur, pasta, and couscous are now also produced on industrial scale and the market value of the pasta industry alone was estimated at 11 billion USD in 2016, with an expansion trend projected at +20% in the next 5 years. This industry relies on durum wheat to produce high quality semolina. The average price of durum wheat is typically 15-20% higher than bread wheat (**Fig 1**), and premium price of 10-20% over the basic price are paid in developed countries when selling varieties with good grain color (yellow pigment), high grain size (hectoliter weight), and especially protein content exceeding 13%. In that sense, durum wheat has also become a major *cash crop* that can provide good returns if the right varieties and agronomic practices are used.

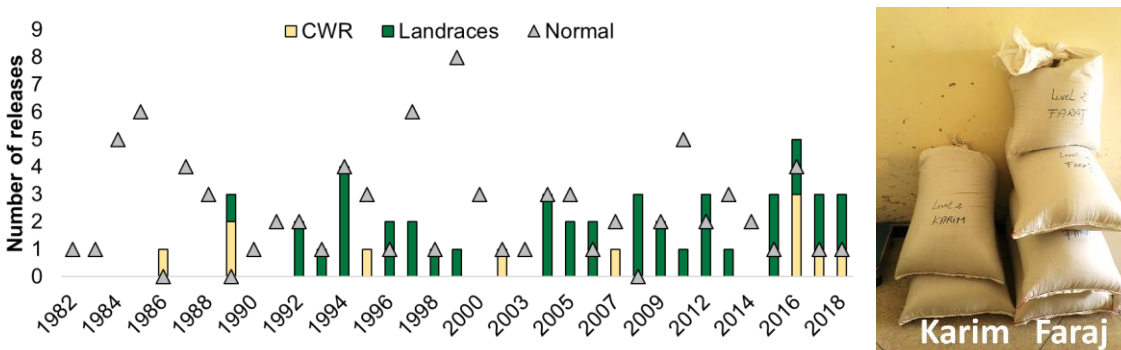
Farming of this tetraploid cereal crop occurs in several different agro-climatic regions, varying from warm and dry to cool and wet environments. These are mostly located in areas subject to alternating favorable and stressed conditions depending on annual rainfall and temperatures (Mediterranean-type). Therefore, genetic improvement for tolerance to biotic and abiotic stresses remains a strategic breeding target to improve durum wheat productivity and stability. In the last decades, many durum wheat varieties have been developed based on field assessment for higher yield, disease resistance and technological seed qualities. However, the strong selection pressure imposed through genetic improvement has eroded a large part of the genetic diversity, hence resulting in a germplasm less prone to adapt to new environmental stresses, diseases and pests. Compared with domesticated varieties, crop wild relatives (CWR) and primitive wheats have been challenged in natural environments for thousands of years and maintained a much higher level of diversity. Hence, interspecific hybridization between durum elite lines and wild relatives of the *Triticea* family is a promising method to restore variability to the modern breeding germplasms.



**Fig 1.** Global producers of durum wheat (adapted from International Grain Council report 2018) and Moroccan annual average prices for durum and bread wheat (adapted from USDA GAIN report 2018).

### Breeding varieties via Crop Wild Relatives (CWR)

Several useful traits have been identified in species related to durum wheat. Regardless of the proven usefulness of CWR for trait discovery and deployment in pre-breeding, wheat breeders have often regarded this type of crosses as a “last resource” that will inevitably require several years and multiple recurrent cycles before delivering a promising candidate for variety release. This vision is mostly associated with the risk indicated by several authors that undesirable linkage drags exist between useful wild traits and low yield or quality alleles. One of the exceptions to this breeding trend has been the ICARDA durum wheat program that broadly utilized CWR and landraces into their hybridization strategy (Nachit and Elouafi, 2004). ICARDA’s gene bank collection is among the largest in the world with over 43,000 accessions of wheat, mostly composed of landraces and CWR. This treasure trove has been extensively utilized by ICARDA’s breeders to generate huge impact in farmers’ fields, with a special focus to those dry areas where the harsh conditions made it impossible for other breeding strategies to work effectively. The durum wheat germplasm supplied by ICARDA has allowed national programs in 22 countries to release 125 varieties, of which 8% were derived from crosses with CWR, and 30% from crosses with landraces (Fig 2). Among the ten most recent varieties released in 2016 and 2017, four were derived from top-crosses with CWR and two from crosses with landraces. Morocco released in 2017 the variety ‘Nachit’ (Amedakul 1/*Triticum dicoccoides* Syrian collection//Loukos), well adapted to the dry conditions of the high-plateaus thanks to its deep root system and large grains. On-farm and on-station testing on 0.1 ha plots of this new variety revealed a significant 25% yield advantage over the most grown variety ‘Karim’. But Morocco is not new of releasing ICARDA’s wide crosses. Already in 2007 a variety under the name ‘Faraj’ was derived from crosses with *Triticum araraticum*. This variety was specifically released to target the driest areas of Morocco, where Hessian fly is a major issue. Testing of ‘Faraj’ on-station and on-farm revealed a clear yield advantage under dry conditions (Fig 2).



**Fig 2.** Origin of 125 durum wheat varieties released from ICARDA's germplasm, and comparative performances of the most grown Moroccan cultivar 'Karim' against the 2007 release from CWR 'Faraj'.

In Mauritania and Senegal, two varieties were released in 2017: 'Amina' (Korifla/*Ae. speltoides*//Loukos) and 'Haby' (Mrb5/*T. dicoccoides* Aleppo Col//Cham1) also derived from simple top-crosses with *A. speltoides* and wild emmer, respectively. These new varieties were selected under relentless high temperatures for a period of four years, to reveal the ability of maintaining high spike fertility under heat stress, a trait that was likely derived from their CWR introgressions. The release of durum wheat varieties for the Senegal River region was recognized as a major achievement and awarded the 2017 OLAM Prize for Innovation in Food Security (<http://olamgroup.com/sustainability/food-prize-re-imagine-agriculture/>).

### CWR for pre-breeding efforts

The direct use of CWR as parental lines for the ICARDA's breeding program has resulted in a genetic diverse germplasm, capable of adapting to extreme agro-ecological stresses. However, each new cultivar includes only some combination of positive wild and elite alleles, but not all. Hence, there is scope to attempt to pyramid the most useful alleles from different sources into a superior cultivar. The first step in that sense is to define the specific traits and genomic regions involved. To do so, a panel including ICARDA's CWR-derived elites as well as germplasm from around the world was assembled and genotyped with 35K Axiom array. This set of germplasm was tested under different conditions to reveal QTLs and traits of interest. The studies conducted and their outcomes are summarized below:

1. *Genetic diversity*: a study by Kabbaj et al. (2017) revealed the existence of 6 sub-populations among the modern germplasm, with ICARDA's being part of 4 of them. This study also revealed that the CG programs have been able to capture up to 21% of the variation for rare alleles (CIMMYT), and 51% of the diversity for common alleles (ICARDA), which is significantly lower than what observed for Central and South Asia landraces, which reached 62% and 84%, respectively, but superior to other breeding programs.
2. *Hessian fly resistance*: a study by Bassi et al. (2019) revealed a new source of Hessian fly resistance derived from *Triticum araraticum* and located in the telomeric portion of chromosome 6BS in the progenies derived from the 'Faraj' cultivar.
3. *Heat tolerance along the Senegal River*: a study by Sall et al. (2018a, b) identified that the capacity of maintaining high fertility (grain number per spike) under severe heat was the key to ensure tolerance to constant daily temperatures above 34°C. Further, it was possible to identify seven genomic regions linked to this trait, with one QTL on chromosome 4AS and





originated from *A. speltoides* accounting for an average yield advantage 669 Kg ha<sup>-1</sup> under severe heat.

4. *Heat tolerance under plastic tunnels*: a study by El Hassouni et al. (unpublished) revealed that applying a plastic tunnel at the time of flowering, raises the daily temperatures to up to 46° C and causes a yield reduction of up to 60%. As per the study by Sall et al. (2018a, b), the key trait for tolerance was the maintenance of spike fertility. Further, this study was able to identify 6 genomic regions controlling heat tolerance, 3 of which overlap with the study along the Senegal River.
5. *Drought tolerance via root architecture*: a study by El Hassouni et al. (2018, unpublished) revealed that mature and seminal root angles are controlled genetically and a narrower root angle (deeper roots) can ensure up to 39% yield advantage under terminal drought conditions. Genomic scan revealed three regions controlling this trait with additive effects, but just one major locus on chromosome 7B, originated from wild emmer wheat, ensures up to 500 Kg ha<sup>-1</sup> yield advantage when terminal drought occurs.
6. *Drought tolerance via yield testing*: a study by Zaim et al. (2017, unpublished) conducted yield trials across 21 drought-prone and favourable sites to reveal that grain size is the most critical yield component for tolerance against terminal drought, together with high biomass. The combination of G and GxE components (Bassi and Sanchez-Garcia, 2017) has allowed to identify CWR-derived elites as the best performing ones, thanks to four major QTLs on chromosomes 2B, 5A, 5B, and 7B.
7. *Industrial quality across 5 sites*: a study by Azouz et al. (unpublished) used the harvest across 5 sites (2 drought-prone, 1 flood irrigated, and 2 with fertigation) to measure the milling and transformation property. It was possible to confirm that CWR-derived lines had significantly larger grain size, better semolina yield, higher protein content, superior virtuousness and yellow pigment. Gluten strength was instead superior in germplasm coming from Canada and Australia. The line 'Syriopsis', derived from *A. speltoides*, was identified as accumulating the highest rate of selenium in the grains, an important micronutrient that can be used to raise market value.
8. *Conversion and validation for MAS*: over 120 marker-trait associations for the different stresses described above were converted to KASP markers and used for validation on an independent germplasm set. A total of 20 markers explained more than 10% of the phenotypic variation targeted and can now be used for MAS (**Table 1**).

## Conclusions

Several field and artificial assessments for different traits and different stresses have been completed. The results are extremely clear: CWR-derived elites are superior to elite-by-elite lines. Their superiority is mainly linked to higher spike fertility under extreme heat, deeper root system under drought, larger grain size, and several added-value characteristics. In addition, 20 KASP markers have been validated and linked to these traits, making pyramiding easier. Hence, the results presented here suggest that the only true limitation that shall prevent durum wheat breeders from taking full advantage of CWR would be their unwillingness to do so. For that reason, ICARDA, its national partners in Morocco, Lebanon, Senegal, and Ethiopia, and the Crop Trust have partnered with funding from NORAD (Norway) to focus on the delivery to farmers' fields of these superior CWR-derived cultivars, with a special focus to those regions that suffer from the harshest drought and heat damages.

**Table 1** Validated KASP from association mapping studies

KASP code	QTL	Scope	Chr
BS00009709	QTL.ICD.RL3.001	Drought tol	1A
AX-94733268	QTL.ICD.RL2.001	Drought tol	1B
AX-95176186	QTL.ICD.RA.006	Drought tol	3B
BS00022364	QTL.ICD.GYKFD17A.001	Drought tol	4B
AX-94383178	QTL.ICD.RA.011	Drought tol	6B
AX-94549122	QTL.ICD.HSI-GY.002	Heat tol	1B
AX-94509297	QTL.ICD.HSI-TKW.002	Heat tol	1B
BS00004224	QTL.ICD.PlstTun.TKW.001	Heat tol	2B
AX-94681091	QTL.ICD.PlstTun.GY.003	Heat tol	3A
AX-94932858	QTL.ICD.HSI-GY.003	Heat tol	3B
AX-94679648	QTL.ICD.GYheat.004	Heat tol	4B
AX-95260810	QTL.ICD.PlstTun.GY.006	Heat tol	5A
AX-94432276	QTL.ICD.PlstTun.GY.006	Heat tol	5A
AX-95182463	QTL.ICD.PlstTun.GY.007	Heat tol	5B
AX-94622179	QTL.ICD.HSI-GY.007	Heat tol	7B
BS00072387	QTL.ICD.Hara.6B	Hessian fly res.	6B
AX-94385320	QTL.ICD.Ppd.002	Phenology	2A
AX-95213349	QTL.ICD.Vrn.006	Phenology	5A
AX-95140644	QTL.ICD.Vrn.007	Phenology	5B
AX-95115092	QTL.ICD.AWAI.007	Stability	4B

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## AB19: Excellence in pre-breeding efforts in barley

Ramesh Pal Singh Verma, Sajid Rehman and Ahmed Amri

International Center for Agricultural Research in the Dry Areas (ICARDA), Morocco

Corresponding author: Ramesh Pal Singh Verma ([r.verma@cgiar.org](mailto:r.verma@cgiar.org))

Barley is a critical crop of the dry areas, especially in the dry region, where the grains are used as food and feed, grazing and straw as feed for the livestock. ICARDA holds the global mandate for the genetic improvement of barley germplasm, with a special focus to its adaptation to the non-tropical dry areas. Advance breeding materials and segregating populations are constantly supplied to national programs for testing and as source of specific traits. The introduction of ICARDA's elite germplasm has led to the release of 269 varieties in 45 countries directly derived from it, excluding the use of enhanced germplasm in the hybridization program by NARS partners for variety development/release. ICARDA's gene bank collection for barley totaling 32,560 of barley, is mostly composed of landraces and more than 2000 wild relatives. The wild *H. spontaneum* has been extensively utilized by ICARDA's breeders with a special focus to areas with drought and cold, where the harsh conditions made it impossible for other breeding strategies to work effectively.

The near exhaustion of the available genetic diversity for abiotic and biotic stresses has pushed breeders to recur to wild species from primary (*H. spontaneum*) and secondary (*H. bulbosum*) gene pools. ICARDA's list of released cultivars includes several derived from landraces, though many top performing elite germplasm were derived from crosses to *H. spontaneum*. Several *H. spontaneum* derivatives from accessions *H. spont.96-3*, *H. spont.41-1*, *H. spont.21-3*, *H. spont.19-15* and *H. spont.41-5* have been evaluated in recent years in Morocco, Lebanon and other places indicating that apart from constituting sources of specific traits, they have good yield potential and, in some instances, have out-yielded the best commercial checks by 10 to 15 % in the trials under limiting environments.

Pre-breeding activities to mobilizing novel alleles for biotic stresses from wild relatives have been initiated recently at ICARDA. Identification of sources of novel diversity for diseases like leaf rust, spot and net blotch has been taken up through efficient evaluation and precision phenotyping at hot spot locations in Morocco and Ethiopia. Out of 114 accessions of *H. spontaneum* evaluated for resistance to major diseases in the field and greenhouse, nine accessions showed adequate levels of resistance to the most virulent population of leaf rust and 44 accessions were resistant to net blotch. Ten accessions showed combined resistance to spot blotch and net blotch at seedling stage. A total of 159 new interspecific crosses to *H. spontaneum* were generated, and currently the Top Cross<sub>1</sub> F<sub>3</sub> (TC<sub>1</sub>F<sub>3</sub>) generation is under field testing. In addition, more than 150 *H. bulbosum*-derived lines (NordGen lines) have been grown during current season for screening at different stations in Morocco and preliminary observations on net blotch indicates some promising genotypes from these accessions. The quality evaluation has also indicated *H. spontaneum* accession with higher  $\beta$ -glucan content, providing an opportunity for increasing the food quality of the cultivated barley through pre-breeding.



## **AB20: Spring wheat pre-breeding in CIMMYT from exotics: Synthetics and landraces**

*Sivakumar Sukumaran<sup>1</sup>, Gemma Molero<sup>1</sup>, Carolina Sansaloni<sup>2</sup>, Francisco Pinto<sup>1</sup>, Deepmala Sehgal<sup>1</sup>, Francisco Pinera<sup>1</sup>, Susanne Dreisigacker<sup>1</sup>, Jose Crossa<sup>2</sup>, Tomas Payne<sup>2</sup>, Matthew Reynolds<sup>1</sup>, Kevin Pixley<sup>2</sup>*

*<sup>1</sup>Global Wheat Program and <sup>2</sup>Genetic Resources Program, International Maize and Wheat Improvement Center (CIMMYT), El Batán, Mexico*

*Corresponding author: Sivakumar Sukumaran ([s.sukumaran@cgiar.org](mailto:s.sukumaran@cgiar.org))*

### **Introduction**

To meet future demand in wheat, higher rates of genetic gains must be achieved through collaborative cross cutting approaches in genetics, physiology, and breeding. This involves exploring new genetic diversity as well as understanding genetic and physiological bases of traits contributing to higher yields. In addition, higher and stable yields are needed to feed the burgeoning human population and to mitigate the effects of climate change on wheat production.

### **Genetic diversity and use by breeders**

CIMMYT germplasm bank holds 150,000 accessions of wheat, the largest unified collection in the world for a single crop from more than 100 countries. Recent genetic diversity analysis of the CIMMYT and ICARDA wheat germplasm bank has shown that, breeding programs have greatly reduced diversity of tetraploid wheat, although a few elite lines explore a wide range of the diversity found among landrace materials. Within hexaploids, elite breeding lines and synthetic derivatives formed genetically distinct groups, and many landraces remain completely unexplored, representing great potential reservoirs of novel alleles for breeding (Sehgal et al., 2015). Landraces are sources of genetic diversity and might have specific adaptive value to specific environments due to their cultivation for thousands of years (Lopes et al., 2015).

### **Phenotyping genetic resources**

Phenotyping is a challenge in the current post genomics era. To facilitate novel gene discovery and utilization, we screened 70,000 wheat accessions from the CIMMYT's genebank funded by the MasAgro Biodiversidad project from Mexican government (<https://seedsofdiscovery.org/>). The screened resources were assembled into different panels based on trait (High Biomass Association Panel), diversity (Bread Wheat Diversity Panel), and novel genome (Synthetic wheat panel) - for genetic and physiological studies (<https://iwyp.org/>), with the aim of increasing yield potential of wheat by 50% in the next two decades. Genome-wide association mapping (GWAS) are being performed on these and related materials to identify stable genomic regions for a wide range of traits lined to grain yield, eg. biomass, harvest index, partitioning, phenology.

### **Crossing and selection schemes**

To mobilize novel genetic diversity for higher yield, physiological trait (PT) based crossing was performed to combine source and sink traits in a complementary way in pre-breeding; for example, source traits included high radiation use efficiency (RUE), biomass, and cool canopies, while sink included harvest index and other spike-fertility related traits (Reynolds and Langridge, 2016). Several strategies including trait × trait, trait × elite, linked top cross (LTP), marker assisted



backcrossing, marker assisted selection, and genomic selection are being used in pre-breeding. Other promising approaches include haplotype-based pre-breeding and speed breeding, which will be explored in coming years when enough genetic information is available for use in pre-breeding.

### **Delivery to breeders and national programs**

Pre-breeding lines that have exotic genome with higher trait value and acceptable level of disease resistance and agronomy type are distributed to breeders and national programs worldwide as two nurseries: one for yield potential traits -Wheat Yield Collaboration Yield Trial (WYCYT) and another one for drought and heat stress-Stress Adaptive Trait Yield Nursery (SATYN). Nurseries were distributed through the International Wheat Improvement Network (IWIN) and grown by national partners in more than 200 locations over the past 9 years. Many lines, especially from more recent nurseries have shown yield advantage over the CIMMYT and local checks (Reynolds et al., 2018; Singh et al., 2018).

### **Impact of exotics in yield advantage**

How much landraces and synthetics can contribute to mainstream breeding is still an unanswered question. Singh et al. (2018) recently revealed exotic specific imprints of 16.1 to 25.1% in pre-breeding lines and association of 3% of exotic-specific haplotypes with abiotic and biotic stress tolerance. Similarly, analysis of the HIBAP has shown contribution of synthetics and landraces in increasing grain yield under yield potential conditions (Molero et al., 2018). Comparing the lines that outperformed the checks indicated that 90% of these lines has exotic genome in their pedigree. From the analysis of WYCYT and SATYN data from 126 locations, most of the lines that outperformed or performed like local checks in different countries have synthetics or landraces in their pedigree. This indicates the importance of using diverse genetic resources to add value to the current elite wheat germplasm through novel alleles and traits.

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## AB21: Pre-breeding to enhance genetic gains in lentil, chickpea and grass pea

Shiv Kumar<sup>1</sup>, Jitendra Kumar<sup>2</sup>, Harsh Kumar Dikshit<sup>3</sup>, Moez Amri<sup>1</sup>, Aladdin Hamwiah<sup>4</sup>, Ashutosh Sarker<sup>5</sup>, Ahmed Amri<sup>1</sup>, and Michael Baum<sup>1</sup>

<sup>1</sup>International Center for Agricultural Research in the Dry Areas (ICARDA), Rabat, Morocco; <sup>2</sup>Indian Institute of Pulses Research, Kanpur, India; <sup>3</sup>Indian Agricultural Research Institute, New Delhi, India; <sup>4</sup>ICARDA, Cairo-office, Egypt; <sup>5</sup>ICARDA, Delhi-office, India

Corresponding author: Shiv Kumar [sk.agrawal@cgiar.org](mailto:sk.agrawal@cgiar.org)

### Introduction

Lentil (*Lens culinaris* ssp. *culinaris* Medik), chickpea (*Cicer arietinum* L.) and grass pea (*Lathyrus sativus* L.) are important cool-season legume crops grown on 22 million ha in more than 50 countries across the globe with 23 million tonnes production. During the last five decades, chickpea and lentil crops have experienced spectacular growth in global production, thanks to their expansion in new areas and enhanced productivity. This was possible due to adoption of improved varieties emanating from international and national programs. However, the genetic base of these varieties remains narrow due to repeated use of a handful of germplasm. Pedigree analysis of chickpea and lentil varieties released in India confirmed the extensive and repetitive use of a few genotypes as one of the parents in hybridization (Kumar et al. 2004). Mainstreaming wild species and landraces in the process of trait discovery and deployment is necessary to widen the genetic base of cultivated germplasm and make lentil, chickpea and grass pea varieties adapted to challenges posed by biotic and abiotic stresses under climate change and variability. Recently, grass pea has emerged as a crop of choice in those areas where major agricultural crops fail to perform under harsh and fragile agro-ecologies. Pre-breeding helps identify novel traits/alleles in exotic germplasm and increase the extent of useful diversity through introgression of those alleles into breeding material more readily accessible by breeders to broaden the genetic base of cultivars.

### Present Status

The ICARDA's genebank holds 14,500, 15,200 and 4,450 accessions of lentil, chickpea and grass pea, respectively. The current holdings also include crop wild relatives of lentil (619), chickpea (547) and grass pea (1555). In the process of domestication and directed breeding programs, many alleles are left behind in landraces and wild species and these need to be introgressed in improved germplasm using innovative breeding tools. Previous research shows marked genetic variability for desired traits among landraces and wild species in lentil, chickpea and grass pea. 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. 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 the 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 agri-ecological zones. To widen the genetic base, ICARDA breeding program uses 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 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).

### Screening of CWR

Screening of CWR of lentil, chickpea and grass pea has resulted in identification of useful donors at ICARDA. wild *Lens* has shown to carry resistance/tolerance for key stresses including drought, cold, salinity, Ascochyta blight, Stemphylium bight, 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). Genes for yield traits like 100-seed weight and pods/plant have been discovered in *L. lamottei* and *L. culinaris ssp orientalis* (Ferguson et al. 1998, Singh et al. 2014). Sources of extra earliness in 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 prebreeding project in lentil, which has been instrumental in introgressing useful genes in mainstream breeding (Kumar et al. 2011). Transgressive segregants for agronomically important traits have been mined from wide crosses (Singh et al. 2013 and 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* (Bucak et al. 2014) and anthracnose (Fiala et al. 2009; Tullu et al. 2011). Similarly, alien genes were introgressed for resistance to Ascochyta blight, anthracnose and cold into cultivated lentil (Fiala et al. 2009).

Annual *Cicer* species have been evaluated for reaction to ascochyta blight, fusarium wilt, cyst nematode, leaf miner, seed beetle, and cold tolerance at 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.

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* at ICARDA showed a range of 0.073–0.513% for ODAP content which is much lower than that in the cultivated species (Kumar et al., 2010). Accessions of *L. cicera* are also good source of earliness, orobanche tolerance and cold tolerance. Evaluation of 490 accessions of *Lathyrus* species for ODAP content revealed significant variation for ODAP content ranging from 0.024 to 0.456% with an overall mean of 0.129. 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 (Fig. 1) showed good sources of resistance not only in wild accessions (*L. ochrus* and *L. cicera*) but also in cultivated species.



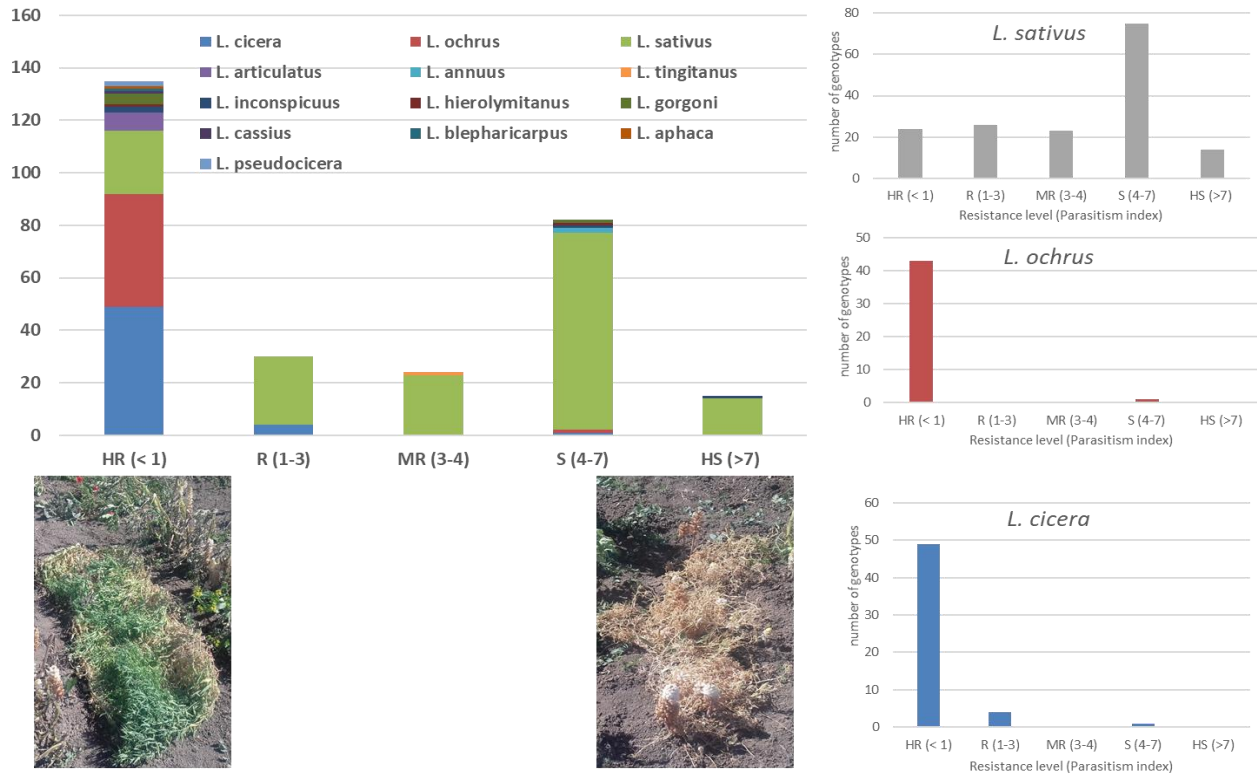


Figure 1. Screening of *Lathyrus* CWR against *Orobanche crenata* during 2017

### Use of CWR in breeding program

Recently, successful use of *Lens orientalis* and *L. ervoides* as a source of genes for resistance to key diseases, phenology, micronutrients 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 90 days (Fig. 2).

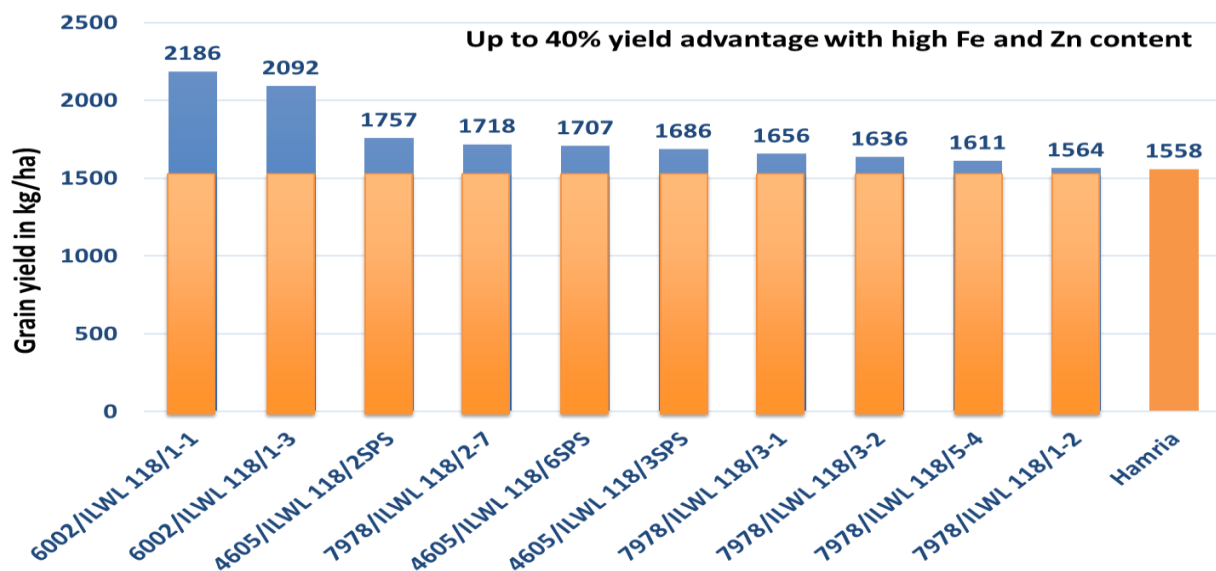


Fig. 2. Yield performance of prebred lines of lentil (*L. culinaris* x *L. orientalis*) at Marchouch in Morocco during 2017.





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 chickpea in grain yield increase (Fig. 3).

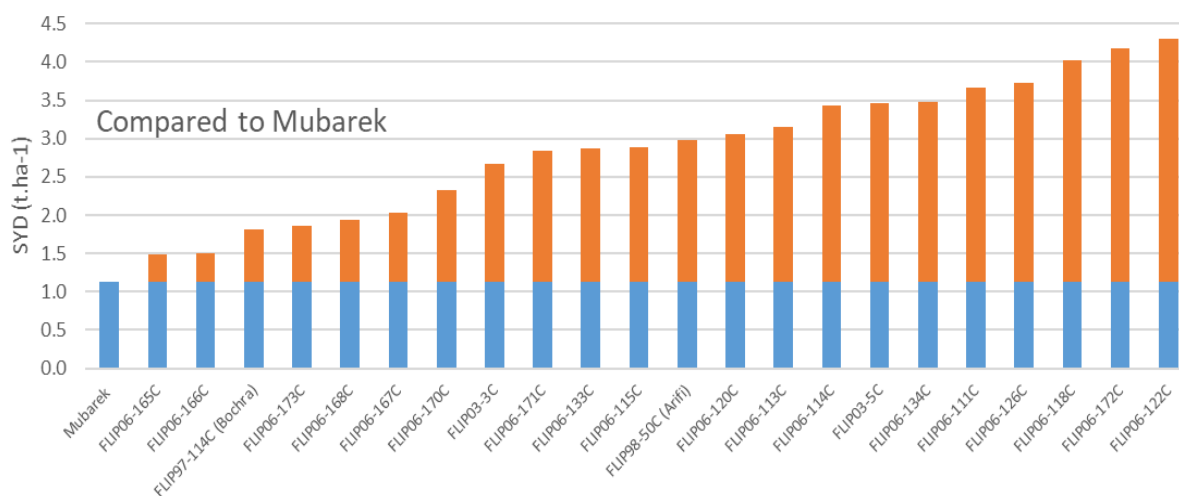


Fig. 3. Performance of prebred lines of chickpea in yield trials at Terbol, Lebanon

## 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 and biotechnology tools, the prospect of transferring useful traits from wild gene pools in these 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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## AB22: Utilizing the underutilized CWR: Pigeonpea pre-breeding from discovery to delivery

Shivali Sharma

Theme Leader- Pre-breeding, International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru-502324, India

Email: [Shivali.sharma@cgiar.org](mailto:Shivali.sharma@cgiar.org)

Pigeonpea (*Cajanus cajan* (L.) Millsp.), the sixth most important grain legume crop, is cultivated on 7.02 million ha area with a production of 6.81 million tones and 969 kg ha<sup>-1</sup> productivity, mainly in Asia (India and Myanmar) and Eastern and Southern Africa (Kenya, Tanzania, Malawi, Uganda, and Mozambique). It is an often cross-pollinated diploid ( $2n = 2x = 22$ ) crop which is a major source for proteins, carbohydrates, B-group vitamins, and minerals. It is grown for its multiple uses such as food, feed, medicine, fuel, fencing, roofing, basket making, etc. and as soil enricher and soil binder.

In spite of enormous breeding efforts in India and elsewhere, genetic enhancement in pigeonpea is not adequate, which is mainly due to its narrow genetic base and various biotic and abiotic stresses adversely affecting crop productivity. Equipped with many useful genes including biotic and abiotic stress resistance, wild *Cajanus* species provide novel genetic variations for the genetic upgradation of pigeonpea cultivars. Evaluation of wild *Cajanus* species has led to the identification of accessions having resistance to important biotic stresses such as sterility mosaic disease (SMD), fusarium wilt (FW), phytophthora blight, pod borer (*Helicoverpa armigera*), pod fly, root-knot nematodes and tolerance to abiotic stresses such as salinity, drought, and photoperiod insensitivity. Utilization of these wild species in pigeonpea breeding programs will not only help in introgression of useful alleles/genes but also result in broadening the genetic base of pigeonpea cultivars. ICRISAT has the global responsibility of collecting, conserving and distributing the pigeonpea germplasm comprising of landraces, modern cultivars, genetic stocks, mutants, and wild *Cajanus* species, and conserves about 13,220 accessions of cultivated pigeonpea and 555 accessions of wild species in the genus *Cajanus* from 60 countries.

The most significant achievement of utilizing wild crop relatives of pigeonpea is the discovery of cytoplasmic-nuclear male sterility systems (CMS). The world's first commercial pigeonpea hybrid, ICPH 2671 was developed by using the A<sub>4</sub> sterility system derived from secondary genepool species, *C. cajanifolius*. Apart from A<sub>4</sub>, other CMS systems have been developed with cytoplasm derived from wild *Cajanus* species belonging to secondary and tertiary genepool species that includes A<sub>1</sub> cytoplasm derived from *C. sericeus*, A<sub>2</sub> cytoplasm from *C. scarabaeoides*, A<sub>3</sub> cytoplasm from *C. volubilis*, A<sub>4</sub> cytoplasm from *C. cajanifolius*, A<sub>5</sub> cytoplasm from *C. cajan*, A<sub>6</sub> cytoplasm from *C. lineatus*, A<sub>7</sub> CMS from *C. platycarpus*, A<sub>8</sub> CMS from *C. reticulatus* and A<sub>9</sub> cytoplasm from *C. lanceolatus*.

Efforts have been made to introgress useful genes/alleles for important traits such as tolerance to salinity, pod borer resistance, phytophthora blight resistance, extra-early flowering and maturity and photoperiod-insensitivity from wild *Cajanus* species into cultivated pigeonpea. Using cross compatible secondary genepool species, *C. cajanifolius*, *C. acutifolius*, *C. scarabaeoides*, and cross incompatible tertiary genepool species, *C. platycarpus*, and *C. volubilis* as donors and popular



pigeonpea cultivars, ICPL 87119 (also known as ‘Asha’) and ICPL 85010 as recipients, advanced backcross populations have been developed at ICRISAT.

Among abiotic stress, salinity is ever-increasing abiotic stress to the cultivated land, which affects plant growth, development, and yield. The pigeonpea production in India and elsewhere is being affected by the salinity. An advanced backcross population consisting of 138 introgression lines (ILs) derived from *C. platycarpus* was evaluated in pots under salt (20-30 mM NaCl) and well-watered conditions. Based on plant growth, 20 ILs exhibiting good growth both under salinity and well-watered conditions were selected as salinity tolerant lines. To identify salinity tolerant ILs with good agronomic performance, these 20 ILs were evaluated across locations for yield and component traits and high-yielding salinity tolerant accessions have been identified for use in pigeonpea improvement programs.

Among biotic stress, insect-pests lead to an approximate economic loss of 15 percent in terms of yield worth \$2285.29 million in India. Pod borer (*Helicoverpa armigera*)—the single largest yield reducing factor in food legumes, causes an estimated loss of US\$ 317 million in pigeonpea. Worldwide, it causes an estimated loss of over \$2 billion annually, despite over \$1 billion value of insecticides used to control *H. armigera*. Efforts are in progress to introgress pod borer tolerance from wild *Cajanus* species into cultivated pigeonpea following simple- and complex-cross approaches. In wild *Cajanus* species, there are different components of tolerance for pod borer such as antixenosis (oviposition non-preference), antibiosis and morphological and biochemical compounds. Using the *C. acutifolius* having high levels of antixenosis for oviposition and antibiosis and the *C. scarabaeoides* accession, ICPW 281 having high density of C-type trichomes as donors - and the two popular pigeonpea varieties, ICPL 87119 and ICP 8863 as recipients, two advanced backcross populations have been developed. Besides this, with a view to combine different components governing pod borer tolerance into a common genetic background, two backcross populations derived from complex four-way F<sub>1</sub> crosses have been developed in two different genetic backgrounds. Preliminary evaluation of these four populations resulted in the identification of 106 ILs having least pod damage compared to tolerant check under un-sprayed field conditions. These ILs will be further evaluated for leaf, flower and pod bioassay to confirm pod borer tolerance.

In the climate change scenario, phytophthora blight is emerging as one of the important diseases of pigeonpea. Three advanced backcross populations consisting of 138, 149 and 183 ILs derived from *C. platycarpus*, *C. acutifolius*, and *C. cajanifolius*, respectively were screened for phytophthora blight resistance under controlled environmental conditions at ICRISAT. Eighteen ILs derived from *C. acutifolius* and five ILs derived from *C. platycarpus* were found moderately resistant to phytophthora blight. These 23 ILs were also evaluated for agronomic performance across locations, and high-yielding ILs with moderate phytophthora blight tolerance have been identified for use in pigeonpea improvement programs.

Pigeonpea has a large temporal variations for maturity (90-300 days) and each maturity group such as super-early (matures in < 100 days), extra-early (mature in 100-120 days), early (120-140 days), mid-early (matures in 140-160 days), medium (160-180 days) and long duration (>180 days) group is suited to a particular agro-ecosystem, which is defined by altitude, temperatures, latitude and day length. Most of the pigeonpea cultivation is under medium maturity duration and Asha is the most dominant cultivar being grown in these areas. Breeders are working to develop new high-yielding varieties as a replacement for Asha. Further, due to short cropping seasons, pigeonpea breeding



programs are also focussing on developing new cultivars having mid-early maturity duration. Preliminary evaluation of two advanced backcross populations derived from *C. cajanifius* and *C. acutifius* resulted in the identification of 91 high-yielding ILs in early, mid-early and medium maturity duration group. Further evaluation of these selected ILs across locations for yield and related traits resulted in the identification of 12 ILs, ICPL 15017, ICPL 15019, ICPL 15023, ICPL 15028, ICPL 15042, ICPL 15043, ICPL 15048, ICPL 15061, ICPL 15062, ICPL 15067, ICPL 15072, and ICPL 15075 having high yield across locations. These ILs will be used for conducting Farmer Participatory Varietal Trials (FPVT) in 2019-20 crop season. In pigeonpea, resistance for sterility mosaic disease (SMD) and fusarium wilt (FW) is the must-have trait. Five salinity tolerant ILs, 14 phytophthora blight tolerant ILs and 11 high-yielding ILs were also found to have high levels of resistance for SMD and wilt.

The superiority of few ILs over local and/or national checks has provided an opportunity to the breeders to include promising ILs in the Initial Varietal Trial (IVT) of AICRP (All India Coordinated Research Project) on Pigeonpea for potential release as a variety/(ies). E.g. ICPL 15072 has been nominated for IVT of AICRP during 2019 rainy season from Gulbarga, Karnataka, India due to its better performance than the checks ICPL 87119 (Asha), ICPL 8863 (Maruti) and local check over three consecutive years 2016, 2017 and 2018. The high-yielding ILs as well as salinity and phytophthora blight tolerant ILs have been shared with breeders in Myanmar to study the adaptability and performance of these ILs for yield-related traits.

In conclusion, pre-breeding using crop wild relatives (CWR) has a great potential in enriching genetic variability in primary genepool for ready use by the breeders for developing new climate resilient cultivars with a broad genetic base. However, the success of pre-breeding programs is hindered by several challenges. Due to the involvement of un-adapted and incompatible CWR, it takes many years to develop breeding populations, thus making pre-breeding a time-consuming, long-term, resources demanding and less attractive research area. Further, specialized expertise is required to deal with several technical challenges, especially for using cross-incompatible wild species. To harness the full potential of CWR conserved in genebanks through pre-breeding, unrestricted, assured, and long-term funding is required. There is an urgent need for active engagement with the stakeholders to strengthen the prebreeding programs, which will ensure the continuous supply of new genetic variability into the main breeding programs to accelerate genetic gains and to improve nutrition and resilience of modern crop varieties.





## AB23: Utilizing the underutilized CWR for cowpea improvement

Ousmane Boukar<sup>1</sup>, S. Chamarthi<sup>1</sup>, B. Nouhoun<sup>1</sup>, A. Togola<sup>1</sup> and C. Fatokun<sup>2</sup>

<sup>1</sup>International Institute of Tropical Agriculture (IITA), Kano, Nigeria; <sup>2</sup>International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

Corresponding author: Ousmane Boukar ([O.Boukar@cgiar.org](mailto:O.Boukar@cgiar.org))

Compared with many other crops, genetic improvement activities in cowpea (*Vigna unguiculata* (L.) Walp.) is relatively recent. Most if not all the available improved cowpea varieties have been derived from direct selections from cultivars or from crosses made between cultivars. It is also worth noting that many of recently improved cowpea varieties have resulted from few parental lines as released varieties served as their parents in many instances. Narrow genetic base is therefore characteristic of several improved cowpea varieties.

Cowpea has a number of wild relatives none of which has been reportedly used in the development of improved varieties. Some of the wild relatives possess genes that could be of benefit to cultivated cowpea such as for example, *Vigna vexillata* with resistance to cowpea insect pests (*Maruca vitrata*, *Callosobruchus maculatus* etc.). Due to strong cross incompatibility between cowpea and *vexillata*, it has not been possible to take advantage of the genes in this wild relative. There are however, some cross compatible wild relatives that can be used to broaden the genetic base of cultivated cowpea. Nevertheless, breeders have hardly used these because the former have very small seeds (which is dominant to large seeds) with unattractive seed coat colour and texture. The climate change being experienced with reduced amount of rainfall and increased temperature especially in the drought prone areas of sub-Saharan Africa (SSA) calls for increased need for developing climate smart cowpea varieties that will be adapted to the dry savannah agro-ecologies. To this end, 173 Aph1- recombinant inbred lines (RILs) obtained from a cross between TVNu-1158 (wild parent) and IT99K-573-1-1 (cultivated parent) along with drought resistant and susceptible checks, Danila and TVu7778 respectively were evaluated for seedling stage drought tolerance in the screenhouse using the wooden box technique (Singh et al. 1999) at IITA-Kano. A completely randomized design with four replications was used. The test lines were planted and watered for approximately 10 days (days from planting to initiation of first trifoliates) after which irrigation was stopped to expose the seedlings to drought stress for 14 to 20 days until the drought-sensitive check reached permanent wilting point. Number of plants per line, number of trifoliates per plant, leaf senescence score, stem greenness, and seedlings recovery rates were recorded at drought imposition (I), 14 to 21 days after stress initiation (S) and finally 7 to 10 days after re-watering (R). There were substantial phenotypic variations among the tested lines for the measured traits. Eleven lines, namely Aph1-240, Aph1-221, Aph1-91, Aph1-103, Aph1-118, Aph1-163, Aph1-210, Aph1-115, Aph1-176, Aph1-24 and Danila showed best seedling drought tolerance based on their percent seedling recovery (65 to 100%) and leaf senescence score.

In addition, field trials were conducted under two water treatments, i.e., well-watered (WW) and post-flowering water-stressed (WS) from March to June and September to December 2017 using a set of 180 recombinant inbred lines (Aph-1 RILs) to determine genotypic variation in agro-physiological and yield performance among them. The field layout was an alpha-lattice design with three replications. Crop phenology, growth, agro-morphological, physiological, major insect pests and diseases, and yield data were recorded on the test lines. Overall, Aph1-209, Aph1-242, Aph1-





227, Aph1-163, Aph1-105, Aph1-17, Aph1-24, Aph-210, Aph1-178, Aph1-79, Aph-189, Aph1-88, Danila, and IT99K-573-1-1 performed best under drought and heat stressed conditions based on their high grain yield and low pod abortion rates. Aph1-163, Aph1-105, and Aph1-17 were identified as best genotypes under drought in the field in 2016 trial.

Two hundred and seventy lines of wild cowpea relatives from IITA gene bank were assessed in the field under water-stressed (WS) conditions using an alpha-lattice design with three replications. The trials were carried out between September and December 2017. Crop plant phenology, growth and development attributes, agro-physiological parameters, and yield-related data were collected. The results of this trial revealed significant differences between test lines for the parameters measured. Based on their relatively high number of pods per peduncle and high pod and grain yields, the following wild cowpea relatives TVNu-1129, TVNu-1422, TVNu-417, TVNu-396, TVNu-420, TVNu-136, TVNu-691, TVNu-699, TVNu-416, TVNu-1153, TVNu-697, TVNu-687, TVNu-695, TVNu-428, and TVNu-696 were identified as the most drought tolerant. In addition, lines TVNu-136, TVNu-403, TVNu-414, TVNu-420, TVNu-428, TVNu-693, TVNu-695, TVNu-696, and TVNu-701 were found to be the best genotypes under drought and heat stress conditions.

Based on two years (2016 & 2017) of field and one year (2017) of screenhouse (wooden box experiment) screening, all partners (IITA, NACGRAB, INERA and INRAN) selected six common wild accessions TVNu 403 and TVNu 428 for drought, heat, and seedling stage drought tolerance, TVNu 693 for drought and seedling stage drought tolerance, and TVNu 414, TVNu 420, and TVNu 701 for drought and heat tolerance. These accessions were used in crosses to 10 elite cultivars from the participating institutions to initiate the introgression of the drought and heat tolerance genes from wild relatives into cultivated cowpea.  $F_{3:4}$  lines have been generated and will be tested to confirm their drought and heat tolerance.

Utilization of cowpea wild relatives will broaden the genetic diversity of cultivated cowpea. This will be critical for the efforts being made to mitigate the effects of climate change including drought and heat through breeding. The ultimate outcome of the present project will be very important to cowpea genetic improvement in the near future thereby justifying the investments made in this project.



## AB25: Successful Multipartite Collaboration on Pre-breeding and Breeding for Hessian fly Resistance: INRA- Kansas State University-ICARDA-CIMMYT

Ahmed Amri<sup>1</sup>, Naserlehaq Nsarellah<sup>2</sup>, Mustapha El-Bouhssini<sup>1</sup>, Jamal El-Haddoury<sup>2</sup>, Mohamed Jlibene<sup>2</sup>, Moha Ferrahi<sup>2</sup>, Saadia Lhaloui<sup>2</sup>, Miloudi Nachit<sup>1</sup>, Filippo Bassi<sup>1</sup>, Degu Wuletaw Tadesse<sup>1</sup>, Osman Abdallah<sup>1</sup>, Bikram Gill<sup>3</sup>, Jimmy Hatchett<sup>3</sup>, Stan Cox<sup>3</sup>, Mohamed Mergoum<sup>4</sup>, Karim Ammar<sup>4</sup>, Memo Ortiz Ferrara<sup>4</sup>, Ravi Singh<sup>4</sup> and Sanjaya Rajaram<sup>4</sup>

<sup>1</sup>International Center for Agricultural Research in the Dry Areas (ICARDA), Rabat-Morocco; <sup>2</sup> Institut National de la Recherche Agronomique (INRA), Morocco; <sup>3</sup> Kansas State University, Manhattan Kansas, United States of America; <sup>4</sup> International Maize and Wheat Improvement Center (CIMMYT, Mexico)

Corresponding author: Ahmed Amri ([a.amri@cgiar.org](mailto:a.amri@cgiar.org))

### Introduction

Wheat is a pillar crop in ensuring food security globally and in Morocco. The use of improved varieties and their certified seeds are key to enhancing wheat productivity and production. In Morocco, wheat is grown over three million hectares with an average grain yield not exceeding two tons/ha making the country a net importer of wheat grain. Low yields are due to the unpredictable environmental conditions with frequent drought and the prevalence of diseases and insects, combined with limited adoption of new varieties and appropriate technological packages. Among biotic stresses, Hessian fly (*Mayetiola destructor* (Say)) is the most damaging insect causing an average loss of 30% on both bread wheat and durum wheat (Amri et al., 1992; Lhaloui et al., 1992). This insect is also prevalent in the Great Plains of United States of America where intensive research on the development of resistant varieties has been undertaken since early 1970's. Although the first Hessian fly resistance genes were identified in landraces of bread wheat and durum wheat, substantial and successful pre-breeding efforts were initiated since 1980 to introgress resistance genes from *Aegilops tauschii* through the development of synthetic hexaploidy wheat. Morocco has benefited largely from the collaboration with Kansas States University in terms of capacity development and exchange of resistant germplasm. The multipartite collaboration involving INRA-Morocco, KSU-USA, ICARDA and CIMMYT has allowed over the years the establishment of reliable pre-breeding and breeding efforts supplying Hessian fly resistant elite germplasm to Morocco and other countries facing this challenge.

### History of breeding for Hessian fly resistance in Morocco

Wheat breeding in Morocco started in 1914 and the first Hessian fly damages were reported in 1930's and the first Hessian fly resistant accession "Reibero" was identified in 1970. Galun sent the Hessian fly special nursery to Morocco in 1972 but was never planted for evaluation. It is in 1983, within the collaborative Dryland Agriculture Applied Research Project (DAARP) in Morocco (1978–1994) between INRA-Morocco and the Mid-America International Agricultural Consortium (MIAC), that intensive research was initiated on the biology and integrated management of breeding for Hessian fly resistance (Rayan, 2007). Major research was undertaken as part of MSc and PhD research of INRA researchers at Kansas State University. The first screening of the Hessian fly special nursery showed that H5, H11 and H13 genes were effective against the Hessian fly population in Morocco (El Bouhssini et al., 1988) and the first breeding germplasm acquired allowed the identification of a resistant line from South Dakota (SD8036),



released in 1985 as first HF resistant variety in Morocco (called Saada), and of two lines L222 and L254 from the Regional Disease Screening Nursery (RDSN) supplied by CIMMYT/ICARDA. L254 was released in 1997 as Arrihane. The evaluation of genetic stocks, landraces and crop wild relative species against GP and L biotypes in USA and against Moroccan Hessian fly population in Morocco allowed to identify large number of resistant durum wheat landraces against USA biotypes and several *Ae. tauschii* derivative lines resistant to Moroccan fly population. These later lines were used in the first crosses and backcrosses initiated in 1985 and allowed to develop large number of lines of bread wheat resistant to Hessian fly. The variety “Aguilal” with H22 Hessian fly resistant gene and resistant to UG99 strain of stem rust was released in Morocco from this germplasm. This germplasm was further used as parental by INRA, ICARDA and CIMMYT breeders to develop elite germplasm combining resistance to yellow rust, leaf rust, Septoria and Hessian fly. The synthetic hexaploidy germplasm supplied by CIMMYT and KSU provided additional sources of resistance to Hessian fly and to other insects and diseases which are extensively used in breeding efforts (El Bouhssini et al., 2012). In case of durum wheat, for which no effective sources of resistance to Moroccan population were identified in landraces, the first crosses were performed to transfer H5 gene, followed later by the transfer of H21 and H25 genes from wheat-rye translocations. Interspecific crosses using Hessian fly resistant accessions of *Aegilops* and wild *Triticum* species started in 1989 in Morocco, in close collaboration with ICARDA (Nsarellah et al., 2003; Bassi et al., 2019).

### Outcome of the multipartite collaboration

Breeding efforts for Hessian resistance in Morocco has offered the best example of successful pre-breeding using crop wild relatives, strengthened collaboration among International Centers (ICARDA and CIMMYT), Kansas State University and INRA-Morocco and enhanced multi-disciplinary and multi-institutional team efforts among national researchers. This collaboration has allowed:

- Development of greenhouse facilities and capacities for the evaluation and screening of wheat germplasm for resistance to Hessian fly using artificial infestation;
- Identification of a large number of sources of resistance in the *Ae. tauschii* derived germplasm obtained from CIMMYT, KSU and other partners;
- Identification of large number of sources of resistance in various *Aegilops* species and in few accessions of durum wheat landraces, *Triticum boeoticum* and *T. araraticum*. Some of these sources were included in the crossing blocks at INRA, ICARDA and CIMMYT;
- Transfer of new genes of resistance to Hessian fly from wheat wild relatives through strengthening pre-breeding activities at INRA and ICARDA;
- Crosses targeting Hessian fly resistance were undertaken by ICARDA, CIMMYT and INRA, which has led to a large number of elite lines available to breeding programs in Morocco and other countries;
- Five bread wheat and six durum wheat varieties resistant to Hessian fly have been released since 2000. The variety “Arrihane” of bread wheat occupies more than 200,000 ha and the variety “Faraj” of durum wheat is grown on more than 5% of durum wheat acreage. However, there is a need to revise the process of access to the new varieties and of seed increase to ensure larger adoption of the newly released varieties.
- More than 12 students have conducted their MSc. and PhD research on Hessian fly related areas and more than 30 joint peer-reviewed publications have been produced.



- Because of these great achievements, the Hessian fly team (INRA-ICARDA-KSU-CIMMYT) received in 2014 the Award of Merit for Research on Hessian fly Resistance in Wheat from the International Plant Resistance to Insects Working Group (IPRI).

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## **AB26: Contribution of the wild relative species in the Algerian wheat breeding programme**

*Abdelkader Benbelkacem*

*Institut National de la Recherche Agronomique d'Algérie.*

*Email:* Abdelkader Benbelkacem ([benbelkacem@mail.com](mailto:benbelkacem@mail.com))

### **Introduction**

Algeria is the largest country in Africa, covering more than two million square kilometers. With 238 million hectares, only 3.4% (8.6 million hectares) is arable land used for agricultural production. Most agricultural activities are in the north of the country. Cereals are the predominant crops grown by Algerian farmers, covering annually 3 to 3.5 million hectares, nearly 40% of Algeria's total agricultural land. On average, annual cereal production in Algeria is about 3.2 million tons with a range varying from a minimum in bad season of 1.7million tons to a maximum of 6.25million tons. Most of the arable land is situated in semi-arid areas (61%) where wheat is predominating. Only 4% of this area receives around 500 mm of precipitation, all the rest has an amount of rainfall ranging from 300 to 400 mm. Yields are low due to several abiotic and biotic stresses. Scarcity and poor quality of underground water resources, low and erratic rainfall, drought recurrence, high and low temperatures and salinity are the key constraints to agricultural production. Cereal diseases and insect pests are also causing serious problem in epidemic situation. Algeria is one of the world's leading importers and consumers of durum wheat which is used for making couscous, pasta, local bread or "galette" and frike-made from immature green seed that is dried, grilled and broken and different sorts of traditional cookies. Bread wheat is largely used for bread (baguette) and for cookies.

### **Breeding approaches**

The Algerian wheat program has a close bilateral relationship with CIMMYT/ICARDA wheat improvement programmes which aim at enhancing wheat production, in order to reduce imports. It emphasizes the use of landraces and wild relatives in breeding to develop germplasm that is better adapted to Algerian environmental conditions. As a result, most of the released varieties until the mid 90's were derived from CIMMYT/ICARDA germplasm.

The breeding programme has developed an efficient approach to germplasm enhancement for stress-prone environments. Improved screening techniques for drought, heat, cold and common diseases and pests are used along with multi-location testing and with decentralized breeding entailing the provision of varieties with specific adaptation to local environments. This requires the use of a range of genotypes, which permits retention of increased genetic diversity in the field, and thus reduces the risk from diseases, pests and uncertain weather conditions. This approach is better suited for harsh environments than is the contrasting approach of developing a broad adaptation targeted for more uniform and less stressful environments.

Most of breeding efforts are concentrated on maximizing the yield potential under favorable environment, in addition to breeding for tolerance to major biotic and abiotic stresses. In low





rainfall areas, the priority is given to drought tolerance and resistance to biotic and abiotic stresses such as cold and frost. Nowadays, more than 50% of the improved genetic material are derived from our national wheat improvement breeding program involving multi-disciplinary team.

### Examples in pre-breeding efforts

Genetic gains in wheat improvement need to deal with potentially complex and difficult biotic and abiotic stresses that likely require increased use of genetic resources including the wild relatives more distantly related to cultivated wheat and belonging to different gene pools. Wheat wild relatives still represent a vast untapped reservoir of genes for further wheat improvement. There are several reviews which summarize the traits successfully transferred from wild relatives into cultivated wheats (Rajaram, 2000; Lage et al., 2003; Gill et al., 2006; Maxted and Kell, 2009). Traits include resistance/tolerance to major biotic and abiotic stresses and yield and quality improvement. Worldwide, the development of synthetic hexaploid wheat from crosses between durum wheat (the cultivated form of the ancestral tetraploid species) and *Aegilops tauschii* (D-genome donor to bread wheat, and one of its ancestral species) has allowed the enrichment of wheat gene pool with elite and improved germplasm having novel sources of resistance/tolerance to major biotic and abiotic stresses (Monneveux et al., 2000; Mujeeb-Kazi et al., 1993, 2008; Gill et al., 2006; van Ginkel and Ogonnaya, 2007; Ogonnaya et al., 2008; Trethowan and van Ginkel, 2009).

In our wheat breeding program, we have used germplasm of mainly cultivated species to develop elite breeding lines. *Aegilops* and wild *Triticum* species that potentially hold a rich reservoir of genes needed to overcome existing and future biotic and abiotic challenges have been used in several crossing programs at NARS and some Algerian universities (Constantine and Elharrach mainly) to contribute to this effort in developing new cultivars possessing different attributes such as drought tolerance, disease resistance and good quality. Pre-breeding activities using promising landraces, wild relatives and popular cultivars have been initiated in the early sixties where the variety T.Pol x Z.B was released and developed since there. However, the narrow genetic base of cultivars coupled with low utilization of these genetic resources is the major factor limiting wheat production and productivity globally.

In our experiments, we used most *aegilops* species encountered in Algeria and some wild *triticum* species such as *T. dicocoides* and *T. polonicum* that were crossed to some local varieties. Despite several physiological barriers we could have some products that were studied. Inter specific crosses concerned also three *Aegilops* species (*Aegilops geniculata* Roth, *Aegilops triuncialis* L. and *Aegilops ventricosa* Tausch) with four durum wheat *Triticum durum* Desf varieties (Waha, Montpellier, Hoggar and Oued Zenati). Three hybrids were obtained without embryo rescue. Pollination of *Aegilops geniculata* by the wheat was not so successful in comparison to the *Aegilops triuncialis/durum* wheat cross. Hoggar and Oued Zenati 368 presented a good crossing ability with *Aegilops triuncialis* than with *Aegilops geniculata*, they regenerated several embryos, however the cross with Waha could not regenerate any embryo. Cytogenetic and molecular studies have been also done (OZenati x *Ae. geniculata*). We also tried fifteen *Aegilops* accessions (*Ae. geniculata*, *Ae. ventricosa*, *Ae. triuncialis*, *Ae. comosa* and *Ae. umbellulata*) and four durum wheat varieties (Montpellier, Oued Zenati, Vitron et Waha). Results confirmed a cytoplasmic heredity. Global physiologic analysis of the inter specific hybrids obtained showed a lot of controversy responses with a large heterosis level for different drought stress physiological traits.



## **AB28: Engaging farmers in sorghum improvement: Insights from prior activities and current breeding with wild relatives in Mali and Kenya**

*Eva Weltzien<sup>1</sup>, Colette Ouedraogo<sup>2,3</sup>, Boubacar Aly<sup>4</sup>, Evan Ouma, Baloua Nebie<sup>2</sup>, Fred Rattunde<sup>1</sup>*

*<sup>1</sup>University of Wisconsin-Madison; <sup>2</sup>International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Bamako/Mali; <sup>3</sup>Université Ouaga I Pr Joseph Ki-Zerbo Ouagadougou Burkina Faso; <sup>4</sup>Institut d'Economie Rurale (IER)*

*Corresponding author: Eva Weltzien (eva.weltzient@gmail.com)*

The successful improvement of sorghum in sub-Saharan Africa is not easy considering the complex and ever-changing environments in which it is produced and the limited availability of research stations of questionable representativeness for farmers' conditions. However, by enabling farmers' engagement in the breeding process their willingness to contribute knowledge and capacity for well targeted large-scale collaboration with breeders can be tapped. We reflect on the ways that farmers contribute to the first three stages of the breeding cycle and give examples from the crop wild relative breeding (CWRB) underway in Mali and Kenya. The first stage – *Setting Breeding Objectives* – typically focusses on the choice of which traits to target and their relative importance. Prior experiences reveal farmers' preoccupation with increasing grain yield while retaining the adaptation and quality of their own varieties. Farmers engaged in CWRB in Mali and Kenya highlight specific traits (e.g. maturity and stem strength) for adaptation. The farmers own varieties make priceless contributions to the second stage – *Generating Diversity* - as was the case with creation of backcross progenies with wild relatives introgressed into the Malian variety "Jakumbé". Both Kenyan and Malian farmers have shown high motivation and capacity to test and observe the CWR progenies, and thus contributing to the third stage – *Selecting in Segregating Materials*. The methods and feasibility of farmer engagement in yield evaluations and preference-scoring are described from long-term farmer-participatory sorghum activities in West Africa. The methods for testing the CWR backcross progenies in Mali (n=155) and Kenya, with subsets tested by individual farmers, will be detailed.



## **AB31: Plant genetic resources management and pre-breeding in genomics era**

*Kuldeep Singh, Kavita Gupta and Pratiba Brahma*

*ICAR- National Bureau of Plant Genetic Resources, New Delhi, India*

*Corresponding author: Kuldeep Singh ([kuldeep.singh4@icar.gov.in](mailto:kuldeep.singh4@icar.gov.in))*

Plant genetic resources (PGR) are the foundation of agriculture as well as food and nutritional security. The ICAR-National Bureau of Plant Genetic Resources is the nodal institution at national level for management of PGR in India under the umbrella of Indian Council of Agricultural Research (ICAR), New Delhi. India being one of the gene-rich countries faces a unique challenge of protecting its natural heritage while evolving mutually beneficial strategies for germplasm exchange with other countries. The Bureaus activities include PGR exploration, collection, exchange, characterization, evaluation, conservation documentation and distribution. The multifarious activities are carried out from ICAR-NBPGR headquarters and its 10 regional stations located in different agro-climatic zones of the country and has linkages with international organizations of the Consultative Group on International Agricultural Research (CGIAR) and national crop-based institutes to accomplish its mandated activities. NBPGR collects and acquires germplasm from various sources, conserves it in the national Genebank, characterizes and evaluates it for different traits and provides ready material for breeders to develop varieties for farmers and has collection of 438922 seed samples. NBPGR works in service-mode for effective utilization of PGR in crop improvement programmes which depends mainly on its systematic characterization and evaluation, and identification of potentially useful germplasm. NBPGR is responsible for identifying trait-specific pre-adapted climate resilient germplasm, promising material with disease resistance and quality traits which the breeders use for various crop improvement programmes. The system has contributed immensely towards safeguarding the indigenous and introducing useful exotic PGR for enhancing the agricultural production. Presently, our focus is on collection of crop wild relatives; characterization of ex situ conserved germplasm and detailed evaluation of prioritized crops for enhanced utilization; assessment of impact of on-farm conservation practices on genetic diversity; genome-wide association mapping for identification of novel genes and alleles for enhanced utilization of PGR; identification and deployment of germplasm/ landraces using climate analog data; validation of trait-specific introduced germplasm for enhanced utilization.

Gene banks have often, through necessity, focused mainly on the immediate but long-term conservation of plant genetic resource activities. There is currently a major gulf between the operations of PGR collections and modern plant breeding. The conservation of genetic resources must be linked to their increased and sustainable use if they are to play a key role in climate change adaptation. This could be achieved through active engagement with all stakeholders to assure the functionality of the entire “Genetic resource-chain”. The recent progress in genomics has opened enormous possibilities, both for introgression of specific traits and for base broadening in pre-breeding. Development of molecular markers since 1980s has seen a striding development from RFLPs to SNPs because of progress in high throughput genomics at very low costs. A few case studies on alien introgression in rice, wheat, lentil, and Brassica involving use of genomics tools for rapid introgression of desirable variability from un-adapted germplasm to high yielding varieties in India and their commercialization will be presented.



# POSTERS



## PP01: Developing strategies for genetic conservation of crop wild relatives in North Africa

Sami Lala<sup>1</sup>, Ahmed Amri<sup>2</sup> and Nigel Maxted<sup>1</sup>

<sup>1</sup>School of Biosciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK; <sup>2</sup> Genetic Resources Section, ICARDA, Morocco

Corresponding author: Sami Lala ([samilalah2012@gmail.com](mailto:samilalah2012@gmail.com))

Crop wild relatives (CWR) are wild species that are genetically related to crops that can be used to introgress useful genes for improvement of productivity, resistance to biotic and abiotic stresses and quality of cultivated crop. These resources are increasingly threatened in their natural habitats through over-exploitation and land reclamation and degradation. Therefore, their conservation would be valuable and will contribute to maintaining and promoting the sustainability of crop diversity, facilitating agricultural production and supporting the increasing demand for food, feed and natural resources. The aim of this study is to identify CWR diversity in North Africa and establish *ex situ* and *in situ* conservation priorities. The methodology proposed by Maxted et al., (2007) for the development of national CWR conservation strategies was adopted. A checklist of 5,854 Crop Wild Relatives (CWR) taxa from North Africa was obtained using the CWR Catalogue for Europe and the Mediterranean (PGR Forum). The checklist contains 5,662 (~97%) native taxa and 192 introduced taxa. About 9% (502) CWR taxa from North Africa were identified as priority taxa for urgent conservation, using four criteria: the economic value of the crop, the relatedness degree to the crop, threat status using IUCN red list assessment and center of origin and/or diversity of the crop. Approximately 2% (119 taxa) of the CWR in the region assessed as threatened. The eco-geographic survey, and *in situ* and *ex situ* gap analysis was undertaken to assess the current conservation status and identification gaps; hotspots of diversity and genetic reserve sites were identified. Genetic information is being included whenever possible to help ensure efficient and effective conservation. Furthermore, climate change assessment was conducted to predict the impact of climate change on the distribution of CWR in the region, and therefore conservation priorities were identified. The outcomes will contribute to establishing conservation priorities to help lay the foundations for future *ex situ* and *in situ* conservation, and subsequent use in crop breeding.





## **PP02: *Ex-situ* conservation of the crop wild relatives in the Moroccan genebank: present status and future prospect**

*Ali Sahri, and Hassan Ouabbou*

*Institut National de la Recherche Agronomique, Morocco*

Morocco is considered as one of the "hotspots" regions of biodiversity in the Mediterranean basin. It is home to a very diverse flora (4,200 species representing 940 genera and 135 families). For these reasons, Morocco opted for the creation of a central genebank to federate and organize the conservation activities of the important plant genetic resources present in the country.

The Moroccan genebank, located at INRA of Settat, started its activities in 2003 with the mandate to coordinate and promote the conservation and sustainable use of plants genetic resources. It has a capacity for *ex-situ* conservation of 80,000 entries. Currently, the genebank conserves more than 66,000 accessions, including a large diversity of crop species such as cereal crops, legumes and forages that have been historically staple crops in North Africa.

Crop wild relatives (CWRs) conserved at the Moroccan genebank are represented by almost 7,000 accessions originated from a wide range of geographical regions of Morocco. These accessions are contributing to the breeding of more resilient varieties, because CWRs harbor genes for stress tolerance, yield and nutritional traits.

The most represented families are Fabaceae (111 taxa with 4,093 accessions), Poaceae (51 taxa with 2749 accessions) and Brassicaceae (9 taxa with 22 accessions); while the genera *Medicago*, *Trifolium*, *Aegilops* and *Avena* are represented by the highest number of taxa and number of accessions.

Since 2003, the Moroccan genebank has repatriated 4260 accessions of CWRs from international institutions and has distributed 400 accessions for research purposes. Furthermore, strategic and targeted collecting missions are conducted by the Moroccan genebank, to assemble a very large number of wild relatives and endangered species.



### **PP03: Use of wild cross, doubled haploid production and Marker-assisted selection to develop a new varieties of bread wheat in Morocco**

*El Haddoury J<sup>1</sup>, S.M. Udupa<sup>2</sup> and Ahmed Amri<sup>2</sup>*

<sup>1</sup>*Institut National de la Recherche Agronomique (INRA), Settat, Morocco;* <sup>2</sup>*ICARDA, P.O. Box 6299, Rabat, Morocco*

Hessian fly, leaf rust and stripe rust are the most destructive pests of wheat production in Morocco especially in dry areas. Biotechnology tools such as interspecific crosses with *in vitro* embryo rescue, doubled haploid production (DH) and marker-assisted selection had been integrated to develop new resistant varieties. Crosses with *Aegilops tauschii*, have enabled the development of interspecific lines and synthetic hexaploid wheat that include resistance to Hessian fly, leaf rust and stripe rust. Some of these lines have been deployed in bread wheat breeding program of Morocco. Crossing of synthetic hexaploid wheat germplasm with bread wheat cultivars, followed by several backcrossing and DH production has resulted in the development of improved lines with a new source of resistance to Hessian fly from *Ae. tauschii*. Two of these lines were registered as spring as new varieties: Khadija in 2012 and Malika in 2016. Khadija variety is issued from interspecific cross with *Ae. tauschii* following doubled haploid and combines resistance to leaf rust and Hessian fly, tolerance to drought with high yield potential. Khadija is commercialized through a private company 'Agri-Maroc' since 2014. Malika was selected from the doubled haploids derived lines from the cross 'Achtar3\*//Kanz'/Ks85-8-4. The use of interspecific crosses, doubled haploid production and markers assisted selections allowed speedy and efficient breeding efforts which led to the development of new varieties combining resistance to Hessian fly, leaf rust, stripe rust and high yielding capacity.

**Key words:** Bread wheat, *Aegilops tauschii*, interspecific cross doubled haploid, resistance, Hessian fly, leaf rust, stripe rust, markers assisted selection.



## PP04: Exploration of wild relatives - seed storage proteins diversity for improvement of gluten wheat quality

M. Taghouti<sup>1</sup>, S. Bennani<sup>1</sup>, G. Diria and A. Bouzidi<sup>1</sup>

<sup>1</sup>National Institute for Agricultural Research (INRA), Rabat, Morocco.

### Introduction

Wheat has accompanied humans since ancient times, evolving through both natural and human selection from the primitive form; emmer wheat toward the current cultivated species; hexaploid bread wheat (*Triticum aestivum* L.) and tetraploid durum wheat (*T. turgidum* L. var. *durum*). Wheat's wide adaptation to diverse environmental conditions, along with its unique characteristic of possessing a viscoelastic storage protein complex called gluten, are the main factors making wheat among the basic staple food of major civilizations in the world. Wheat storage proteins play a crucial role in forming strong, cohesive dough that will retain gas and produce baked products. These properties make wheat alone suitable for the preparation of a great diversity of food products; breads, noodles, pasta, cookies, cakes and many other foods.

Wheat flour quality is mainly characterized by gluten strength which represents about 78-85% of total wheat proteins. The monomeric gliadins and polymeric glutenins constitute the main components of gluten and are associated with quality differences among wheat cultivars (Horvat et al. 2006; Pena et al. 2005). High-molecular weight glutenin subunits (HMW-GS) play a critical role in determining dough elasticity (Shewry et al., 2002), mainly by affecting the size distribution of glutenin polymers, while gliadins, act as a plasticizer affecting mainly dough viscosity and extensibility (Khatkar et al., 2002). Gliadins are encoded by Gli-1 loci (Gli-A1, Gli-B1, Gli-D1) and Gli-2 loci (Gli-A2, Gli-B2, Gli-D2) (Payne et al. 1982). Glutenins are classified into high-molecular-weight glutenin subunits (HMW-GSs) and low-molecular-weight glutenin subunits (LMW-GSs) (Payne et al. 1984). HMW-GSs are encoded by unique Glu-1 loci (Glu-A1, Glu-B1, Glu-D1) (Payn et al. 1982). LMW-GSs consist of subunits encoded by a multigene family, Glu-3 loci (Glu-A3, Glu-B3, Glu-D3) consisting of 30–40 genes and linked to Gli-1 loci (D'Ovidio and Masci, 2004). Glutenins subunits differ in terms of molecular weight, varying from 12 to 60 kDa for LMW-GS and from 65 to 130 kDa for HMW-GS. Molecular weight of gliadins subunits is around 25 to 70 kDa (Autran, 1987). The proportion of glutenins and gliadins, the composition of HMW-GS and LMW GS, and the amount of soluble and insoluble glutenins, all have close relationships with dough rheological properties and bread baking quality (Carrillo et al. 1991; Martinez et al. 2005). Furthermore, variations in dough rheological properties are largely determined by the genotype (G), the environment (E) and the GXE interaction which play an important role in the expression of the quality traits of a genotype (Taghouti et al., 2010; Subira et al. 2014; Taghouti et al. 2017). For each HMW-GS and LMW-GS coding loci, a high degree of polymorphism was revealed by SDS-PAGE for bread and durum wheat (Payne and Lawrence, 1983), due to the presence of many alleles at each locus. They are used as a tool in selection by biochemical markers in breeding programs for improving quality of wheat end-products.

Genetic diversity in the wild relatives of wheat gene pool can provide much information in understanding the variation of glutenin subunits. This could be very useful for expanding the narrow allelic diversity at some glutenins loci particularly at Glu-A1 locus in both bread wheat and



durum wheat where only a limited number of x-type and virtually no y-type subunits are expressed. Breeding for wheat quality using the existing genetic diversity in the bread and durum wheat gene pools may be insufficient to access current and future breeder's goals (Rejesus et al., 1996). Wild relatives of wheat, being a rich genetically gene pools, could be the best hope for crop improvement at future (Feldman and Sears, 1981; Nevo, 1986). Wild relatives, *Triticum L.* and *Aegilops L.* provide a useful source of new accessible genetic variation for wheat improvement since they maintain rich genetic resources for good quality proteins (Nevo, 1998). In this perspective, the main objective of our research is to evaluate the genetic diversity of glutenins in *Aegilops* and *Triticum* species to identify specific bands to each group that may be of interest for improving the gluten quality of wheat.

## Methodology

Genetic diversity analysis using SDS-PAGE was carried out on 71 accessions including different species of *Aegilops* and *Triticum* provided from gene-bank of Settat-INRA, Morocco. Moroccan bread and durum wheat varieties were used as checks. Proteins were extracted from meal flour following the procedure described by Singh et al. (1991). Electrophoresis of reduced and alkylated proteins were performed on 14% sodium dodecyl sulphate polyacrylamide gels (SDS-PAGE) according to Laemmli (1970). For each accession, electrophoregram was scored and the presence (1) or absence (0) of each band was noted and bi-variate 1-0 data matrix was generated. Genetic diversity parameters, principal coordinate analysis (PCoA) and genetic distances of Nei were led with GenAlex Software (6.501).

## Results

Results pointed out the existence of wide polymorphism and allelic variation ranging from 81% to 100%, *Aegilops taushii* being most genetically heterogeneous. Specific bands belonging to HMW-GS and LMW-GS were detected for each species. Besides, the assessment of inter-specific variability showed larger number of bands and wide polymorphism rate in *Aegilops* and *Triticum* species compared to wheat varieties. In addition, the largest number of private bands (34) was found in *Aegilops* species, 14 bands (41.2%) corresponding to LMW-GS. Moreover, wheat varieties were genetically heterogeneous ( $He = 0.276$  and  $He = 0.263$  respectively) indicating different genetic background of the released varieties. The principal component analysis based on Nei genetic distances showed the close relationship between *Aegilops* and *Triticum* (0.021), while durum wheat varieties are genetically distant from bread wheat (0.281).

Results evidenced the existence of wide polymorphism. Private subunits were detected in wheat progenitor (*Triticum* and *Aegilops*). Extensive allelic variation revealed in wild relatives is related to the amount of very large glutenin polymers that should be characterized for their technological interest to be used in breeding program for wheat quality improvement.

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## PP05: Association mapping for drought tolerance trait in spring bread wheat under different drought scenarios in Morocco

Sahar Bennani<sup>1,2</sup>, Wuletaw Tadesse<sup>3</sup>, Jlibene Mohammed<sup>1</sup>, Naserlhaq Nsarellah<sup>4</sup>, and Ahmed Birouk<sup>2</sup>

<sup>1</sup>National Institute of Agricultural Research, INRA Morocco, P.B. 415, Rabat, Morocco; <sup>2</sup> Agronomy and Veterinary Hassan II Institute, P.B. 6202, Rabat, Morocco; <sup>3</sup> International Center for Agricultural Research in the Dry Areas, P.B. 6299, Rabat, Morocco; <sup>4</sup> National Federation of milling, Casablanca, Morocco

Corresponding Author: sahar.bennani@gmail.com

Given the importance of bread wheat as a staple crop worldwide and the current climate change threats, the adoption of appropriate preventive approaches is of paramount importance for increasing food security, especially in developing countries.

Morocco is already recognized as an arid- semi-arid country over 93% of its area. More than 85% of Morocco's arable land is dominated by rainfed system, where yields depend mainly on rainfall fluctuations over regions and years (Jlibene and Balaghi, 2009). Drought is a structural phenomenon in Morocco affecting, at different intensities and magnitude, all its agro-ecological zones. The frequency of dry years ( $<400 \text{ mm year}^{-1}$ ) is estimated to happen every third year during the last three decades. Climate forecasts indicate the increase of aridity due to lower rainfall amount (-20% predicted by 2050) and higher temperatures ( $+3^{\circ}\text{C}$  by 2080). The release of high yielding and resilient varieties to drought stress may help improving wheat productivity and limiting farmers risk for yield losses under climate change conditions.

While conventional plant breeding plays key roles in developing high yielding and stress tolerant varieties, molecular tools are playing important roles to undertake gene mining, introgression, pyramiding and increasing genetic gain by improving selection efficiency. Association mapping is an approach for the identification of marker-trait associations (MTAs), for potential application in Marker Assisted Selection process.

In this perspective, the current study was performed using a collection of 197 spring bread wheat genotypes from ICARDA to identify quantitative trait loci (QTL) for drought tolerance under 4 Moroccan drought scenarios. The genetic material was evaluated for grain yield under well-watered and semi-arid conditions during 2015 and 2016. The association mapping analysis was performed using 15k SNPs with TASSEL 5 software.

From linkage disequilibrium (LD) analysis, 65% of inter-chromosome locus pairs showed a significant LD ( $P < 0.01$ ) from which 54% have  $r^2 > 0.20$ . The genome B have the highest percentage of significant pairs of markers (68%), followed closely by genome A (63%) and to a lesser degree by the genome D (54%). The LD decay at  $r^2 > 0.20$  varied from 1 cM for chromosome 4D and 6D to 7 cM for the chromosome 1D. The genome A and B present a LD decay at 3cM; while the genome D showed a wide range of LD of 7cM. This result is in line with Chao et al. (2010) and Edae et al. (2013) findings.

Based on 102 unlinked markers, the population structure analysis identified three main sub-populations. The variation is explained mainly by inter-sub-populations differences (84%). The



importance of inter-population variation demonstrates the impact of selection for maintaining an important allelic diversity in populations and elite lines. Under this panel, many lines share one or many parents recognized for their agronomic performances and used frequently in breeding crosses, namely SERI, KAUZ, Attila and also wild types like *Aegilops Squarrosa*, and therefore induce population structuring based on genetic basis (Edae et al., 2013).

Using 10568 polymorphic markers and Mixed Linear Model, the association mapping analysis identified 14 significant associations for grain yield under the chromosomes 5B (4), 4B (3), 3B (2), 5A (2), 1B, 2B and 7B (1) and 3 associations with unknown markers positions. However, the strength of the QTLs found in the present study remains limited by both drought scenario and genetic population. Two positions (3B and 5B) were identified at two environments.

From this study, the lines "SERI.1B//KAUZ/HEVO/3/AMAD/4/PFAU/MILAN", "HUBARA-5/ANGI-1", "KAUZ'S/SERI/3/KAUZ//KAUZ/STAR" and "BOUSHODA-1/5/CHEN/AEGILOPSSQUARROSA(TAUS)//BCN/3/VEE#7/BOW/4/PASTOR" presented important yield performances under the different drought scenarios with average yield levels of 3.30 ( $\pm 0.31$ ), 4.42 ( $\pm 0.93$ ), 3.89 ( $\pm 0.67$ ) and 4.01 t/ha ( $\pm 0.50$ ), respectively.

**Key Words:** Bread wheat, drought tolerance, association mapping, QTL

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## PP06: Oat National Research Program – INRA, Morocco

Saidi N<sup>1</sup>., N. Shaimi<sup>1</sup>, A. Souihka<sup>1</sup>, A. Salih Idrissi<sup>1</sup>, M. Bouksaim<sup>1</sup>, M. Taghouti<sup>1</sup>, A. Benali<sup>1</sup>, A. Douaik<sup>1</sup>, F. Gaboun<sup>1</sup>, A. Zouahri<sup>1</sup>, R. Manzali<sup>2</sup>, S. Boutoumate<sup>3</sup>, H. Bouselham<sup>4</sup>, C. Al Faiz<sup>1</sup> and G. Ladizinsky

National Institute for Agricultural Research-CRRA-Rabat, Rabat, Morocco; Hassan 1st University, Settat, Morocco; Ibn Tofail University, Kénitra, Morocco; Sultan My Slimane University, Beni Mellal, Morocco; Principal Investigator, USAID, Project No. TAMOU 97-053, Washington, DC 20523, U.S.A

Oat is an important forage crop in Morocco. Cultivation of this cereal was introduced to Morocco by French Protectorate during the 1920s. The main cultivated oat is the hexaploid species *Avena sativa* ( $2n=6x=42$ ) which occupy an area of around 90 000 ha, essentially in rainfed regions. Due to its economic importance, INRA-Morocco has launched a national breeding programme in 1980s aiming at selection of oat varieties from introduced material from Canada and USA. Twenty hexaploid oat varieties were registered in the national official catalogue. These varieties have high dry matter yield up to 14 t/ha and good grain yield reaching 5.3 t/ha in addition to good tolerance to oat diseases mainly powdery mildew, helminthosporiose, Barley Yellow Dwarf Virus (BYDV) and crown rust.

Recently, oat is considered as a healing plant since it has beneficial nutritional and physiological effects engendering a good impact on blood cholesterol, diabetes, hypertension as well as obesity. Due to its economic importance, there is an increased demand for oats for human consumption during the last decades. Hence, most of the breeding programmes are currently directed towards grain yield improvement rather than forage yield to satisfy these demands. A breeding programme was initiated by INRA in early 2000s, aiming the development of new varieties conceived for human consumption. This programme aims the use of the two tetraploid oat species *A. magna* Murph and Terrell., endemic of Morocco, and *A. murphyi* Ladiz. endemic of Southern Spain and North of Morocco, found to be of great nutritive value since their groat protein content exceed that of common oat *A. sativa* by 30 to 40%. The transfer of the tetraploid species' high groat protein content to the Moroccan hexaploid oat varieties was achieved through interspecific crosses to enlarge their genetic pool. Derivative varieties from crosses with *A. murphyi* have shown a good resistance to diseases and a good grain yield exceeding by 8 to 21 Q/ha that of their hexaploid parents. As for the material derivative from the crosses using *A. magna*, it also has a good resistance/tolerance to diseases mainly BYDV and recorded high dry matter yield exceeding by 3 to 6 t/ha that of their hexaploid parents.

Analysis of groat protein content of derivative lines from crosses involving *A. magna* has revealed that this trait was increased by 4 to 56 % compared to that of their hexaploid parents. However, for the crosses using *A. murphyi*, the groat protein content of derivative lines was improved by 1 to 18 %. Among the yielded lines, two varieties were recently registered (Al Fawz and Abtah) in the official catalogue conceived for human consumption.

**Keywords:** Oats, *A. sativa*, tetraploid oat species, *A. magna*, *A. murphyi*, forage feed, human consumption



## PP07: Introgression of wild sorghum to enhance resilience to climate variability in the Sahel of West Africa

*Quedraogo OC<sup>1,2\*</sup>, Nebie B<sup>1</sup>, Toure A<sup>1</sup>, Weltzien E<sup>3</sup>, Sissoko I<sup>1</sup>, Toure AK<sup>1</sup>, Rattunde F<sup>3</sup>, Boubacar A<sup>5</sup>, Deshpande S<sup>4</sup>, and Sawadogo M.<sup>2</sup>*

<sup>1</sup>International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Bamako/Mali; <sup>2</sup>Université Ouaga I Pr Joseph Ki-Zerbo Ouagadougou Burkina Faso; <sup>3</sup>University of Wisconsin-Madison; <sup>4</sup>International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Patancheru/India, <sup>5</sup>Institut d'Economie Rurale (IER)

Corresponding author: *Collette Quedraogo* ([O.Collette@cgiar.org](mailto:O.Collette@cgiar.org))

Unpredictable water availability is a pervasive constraint for sorghum production in the Sahel of West Africa. Sorghum breeders require useful genetic diversity to develop improved varieties that offer farmers new options for resilience in the Sahel. The goal of the present study is to help develop drought tolerant varieties with farmer-preferred traits through introgression of novel sources of diversity. The specific objectives were i) to assess the genetic variability for grain yield and associated agronomic traits of introgressed BC1F5 backcross progenies, and ii) examine the possibility of obtaining new introgressed sorghum progenies that surpass the performance of current farmer varieties for grain yield and drought relevant traits under drought prone environments. Two wild sorghum (BBISS-08 and BBISS-09) were crossed with a cultivar IS16044 from Cameroon (CSM63E cv. Jakumbe) to develop backcross nested association mapping populations. Jakumbe, a local variety largely grown in Mali and Burkina Faso for its early maturity, grain yield, and quality was used as recurrent parent. The advanced populations BC1F5 along with the recurrent and donor parents were evaluated in the Sahel of West Africa, at two research stations in Mali, at Cinzana, with 881mm rainfall, and at Bema, with 418mm rainfall in 2017. Data were collected for grain yield, flowering time and physiological traits such as relative chlorophyll content (SPAD), stay green on visual score (STG), lodging and survival rate of each line. The two populations derived from wild parents consisting of 155 progenies exhibited large variation for grain yield, flowering time, stay green and chlorophyll content at maturity in both environments compared to 99 progenies derived from the cultivated variety IS16044. The results showed a high significant interaction between genotype and environment for grain yield and flowering time. The genetic coefficients of variation among genotypes varied 5.10 to 65.30. High heritability ( $H^2$ ) was found for flowering time for both Bema and Cinzana with respectively 0.54 and 0.85. Delay of flowering time and yield reduction were observed at Bema comparatively to Cinzana. About 50% of progenies showed a grain yield greater than that of the recurrent parent Jakumbe. Twenty progenies derived from wild parents exhibited a grain yield over 2.5 t/ha. More than 50% of the total progenies showed a greater chlorophyll content at maturity than Jakumbe.

Forty-seven progenies at Cinzana and 79 progenies at Bema were found to be better than Jakumbe for grain yield and drought tolerant traits. Hundred nine progenies derived from wild parents were evaluated on farm trials and on stations in 2018. These progenies will be further evaluated for a second year in Mali and Burkina using farmer participatory approach to enhance farmers' resilience to climate variability. Furthermore, activities are ongoing to map genes responsible to drought tolerant traits and to develop markers for improving future breeding for drought tolerant in the region.



## **PP08: Evaluation and utilization of lentil crop wild relatives for breeding in Morocco: towards development of drought and herbicide tolerant varieties**

Omar Idrissi<sup>1</sup>, Said Ben Ghoulam<sup>2</sup>, Abdellah El Aissaoui<sup>1</sup>, Ali Sahri<sup>1</sup>, and Sripada Udupa<sup>3</sup>

<sup>1</sup>National Institute for Agronomic Research of Morocco, Settat, Morocco, <sup>2</sup>FST Hassan the 1<sup>st</sup> University, Settat, Morocco, <sup>3</sup>International Center for Agricultural Research in Dry Areas, Rabat, Morocco

Corresponding author: Omar Idrissi ([o.idrissi@yahoo.fr](mailto:o.idrissi@yahoo.fr))

Lentil is an important food legume worldwide. It contributes to enhance human nutrition thanks to its nutritionally rich-grains consumed as staple food, in vegetarian dishes and in various healthy salads. Also, the ability of lentil plants to fix atmospheric nitrogen in soils, thus reducing the need for N fertilizers, helps to enhance farming sustainability. However, a number of challenging constraints, for instance drought and plant susceptibility to herbicides, are limiting its production and profitability. The use of crop wild relatives could provide interesting resistance/tolerance alleles that could be incorporated into breeding programs to develop improved varieties. A set of *Lens orientalis* accessions originated from different countries was evaluated under progressive drought stress. Drought tolerant accessions showing pubescent leaves, high values for cell-membrane stability, root-shoot ratio, leaves' relative water content; and low values for wilting severity, leaves' water losing rate and canopy temperature (measured using thermal infrared imaging) were identified. These wild accessions were crossed with adapted varieties and elite lines of the Moroccan national breeding program. Confirmed interspecific F<sub>1</sub> were obtained and are currently under segregation and advancement for trait fixation. On the other hand, interspecific populations involving *Lens ervoides* accessions introduced from Saskatchewan University, Canada, are under use and evaluation for herbicide tolerance using locally available commercial products. Important tolerance/resistance sources for these two recalcitrant constraints are expected from this genetic material that could help to deliver interesting varieties with such added-value traits. These pre-breeding results could help increase the productivity and climate change resilience of lentil in Morocco in the perspective of enhancing food security.



## PP09: Moroccan lentil landraces and wild relatives - potential in enhancing breeding efficiency

Nadia Benbrahim<sup>1</sup>, A. Beggar<sup>1,2</sup>, J. Mbasani-Mansi<sup>1,3</sup>, F. Gaboun<sup>1</sup>, R. Mentag<sup>1</sup>, G. Diria<sup>1</sup> and M. Taghouti<sup>1</sup>

<sup>1</sup>National Institute of Agricultural Research, Rabat, Morocco; <sup>2</sup>Faculty of Sciences and Techniques of Mohammedia, Hassan II University, Casablanca, Morocco; <sup>3</sup>Mohammed V University, Rabat, Morocco

Corresponding author: Nadia Benbrahim ([nadiabenbrahim@gmail.com](mailto:nadiabenbrahim@gmail.com))

Lentil (*Lens culinaris Medikus Subsp. culinaris*) provides environmental and ecological services towards a sustainable farming system. In addition, it is an important dietary source of essential macro and micro-nutrients for human health. In Morocco, lentil is produced in rainfall areas mainly in low potential lands through traditional farming system using landraces to insure home-consumption. However, because of climatic constraints, lentil productivity remains below its potential, leading to the need of regular import to meet domestic needs. Consequently, local sources of genetic diversity are threatened of valuable gene losses.

Drought and heat during flowering and pods filling are the major abiotic stresses for lentil crop causing significant yield losses. Breeding for drought tolerance during critical growing stages is based on developing early high yielding varieties to escape the terminal drought and heat stresses. The prevalence of malnutrition and micronutrient deficiency has increased in Morocco leading to adverse effects on human health and productivity particularly in low-income rural households. The healthcare costs and productivity losses due to nutrients deficiency account for about 5% of national GDP. Iron deficiency is among the most common micronutrient deficiency affecting 50% of children aged between 7 and 9 old; and 10% of women of childbearing age. Lentil, as dietary valuable source of nutrient, might be a key component to improve consumer's nutritional status and health. Breeding is the promising and cost effective approach in long-terms to manage iron deficiencies and promote therefore health and nutritional status of vulnerable people. Hence, the need of developing resilient varieties to climate stresses with an adding value of nutritional quality becomes a priority.

Lentil landraces have been on-farm conserved for their wide adaptation under adverse climatic conditions and lentil wild crop relatives have evolved under constraint conditions (drought, heat, poor soils, etc..) that might be genetic resources representing optional source of adaptive alleles or of promoted genotypes/genes candidate with nutritional beneficial traits. Current study aims to evaluate landraces collected from the most cropped lentil areas in Morocco and *Lens* wild relative accessions provided by ICARDA gene-bank unit for climate stresses as well as for nutritional quality traits. Screening these basic genetic resources of lentil crop for climate stresses tolerance and seed iron concentrations is essential to identify valuable alleles to use for developing novel varieties with desirable traits. Trials were carried out under both field conditions and controlled conditions in greenhouse or laboratories. Genetic diversity within these genetic cores was assessed using agro-morphological traits, biochemical and molecular markers. Nutritional contents were determined compared to improved varieties. Multi-environmental trials were undertaken to analyze effects of genotype, environment and genotype x environment interaction on genotype performances and stability under climate stresses. Measured traits are associated with plant water use efficiency. The main drought tolerance related traits are root system development and biomass





development compared to no-stress condition. We also recorded roots size and weight, total pods and filled pods number and seed yield per plant, total biomass and chlorophyll content recorded using SPAD chlorophyll meter. In addition, heat tolerance was evaluated using pollen viability criterion.

Results indicated a wide polymorphism rate within landraces, drought tolerance variation potential among studied genotypes and high and large range of nutrients content mainly for protein, iron and zinc. Accessions of lentil crop wild relatives are being evaluated and the preliminary results are promising. Current study provides important basic information related to yield potential and nutritional content of lentil landraces useful for improving productivity and nutrient traits through genetic improvement. Genotypes combining high yield potential and high concentration for iron will be selected to be used in breeding program for developing high yielding and nutritious lentil varieties. This study provides useful preliminary information in lentil pre-breeding program.

In conclusion, lentil landraces represent the primary genetic resource for yield stability in climate stresses and for micronutrient enhancement. Further work will be applied in breeding program related to interspecific hybridization using classical crossing of wild relatives with advanced cultivated lines/back cross/segregation or modern methods using molecular tools.



## PP10: An overview of chickpea improvement program in Morocco

Houasli Chafika<sup>1</sup>, O Idrissi<sup>1</sup>, Saker bouazza<sup>1</sup>, A Hamwih<sup>2</sup>, M Amri<sup>2</sup> and SK Bencheqroun<sup>1</sup>

<sup>1</sup>INRA, Institut National de la Recherche Agronomique, Settat, Morocco; <sup>2</sup>International Center for Agricultural Research in the Dry Areas (ICARDA), Rabat, Morocco

Corresponding Author: Houasli Chafika ([houaslichafika@gmail.com](mailto:houaslichafika@gmail.com))

Chickpea is the second most important food legume crop in Morocco after faba bean. It plays an important role in diet and often referred to as 'poor man's meat'. It is an important source of protein, fiber and micronutrients, and play a vital role in cropping cycles due to their ability to fix atmospheric nitrogen. The national chickpea breeding program in Morocco aims to develop and release varieties that are well adapted to different agro-climatic conditions and farming systems with high yield potential, acceptable grain quality and resistance/tolerance to key abiotic and biotic stresses prevalent in the target area. To this end, conventional breeding methods have been used. However, DNA markers have been developed recently in Morocco breeding program aiming at molecular tagging of agronomic traits and towards marker-assisted breeding.

Continuous breeding efforts have resulted in the release of 7 improved chickpea varieties with improved yield, seed quality, disease resistance and better adaptation to different production zones within Morocco. In recent years, cultivars resistant to *Ascochyta* blight (*Ascochyta rabiei* [Pass.] Lab.) with large seed size have been released and registered in the National official catalogue. However, seed increase and commercialization are still needed to make those varieties available to farmers. Also, several chickpea breeding lines appear to be promising candidates for future release as improved germplasm or new cultivars based on their performance in advanced yield trials. Currently, breeding efforts are focused on broadening of the genetic base by bringing genes from wild *Cicer* species and landraces to develop suitable plant types with multiple and durable resistance to major biotic and abiotic stresses. Also, marker-assisted backcrossing (MABC) is being used to combine super-early maturity and high seed carotenoid levels.



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