

# Wheat Breeding Handbook at ICARDA

W. Tadesse, M. Sanchez-Garcia, A.S. Thabet, S. Tawkaz,  
S. El Hanafi, P. Skaf A. El-Baouchi, K. Eddakir,  
K. El-Shamaa, S. Assefa, M. Baum



Science for resilient livelihoods in dry areas

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**Citation:**

W. Tadesse, M. Sanchez-Garcia, A.S. Thabet, S. Tawkaz, S. El Hanafi, P. Skaf A. El-Baouchi, K. Eddakir, K. El-Shamaa, S. Assefa, M. Baum

2019. Wheat breeding Handbook at ICARDA. Pp-, Beirut, Lebanon.

**ISBN (10 digit):** 92-9127-5360

**ISBN (13 digit):** 978-92-9127-5366

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

P.O.Box 114/5055, Beirut, Lebanon

Tel: +963 21 2213433, 2213477, 2225012

E-mail: [icarda@cgiar.org](mailto:icarda@cgiar.org)

Web site: [www.icarda.org](http://www.icarda.org)

\*Corresponding author: Email: [w.tadesse@cgiar.org](mailto:w.tadesse@cgiar.org)

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## **Foreword**

Wheat is the most widely grown and consumed food crop in the world with current annual production level of more than 620 million tons on a total production area of 217 million hectares. In the year 2050, the world population is estimated to be 9.8 billion and the demand for wheat reaches more than 900 million tons. The Central and West Asia and North Africa (CWANA) region with an average wheat demand of about 191 kg/capita/year, accounts more than 50% of the wheat production area in the developing world. During the periods from 1961-2013, wheat production in the region has increased from 22 to 126 million tons, mainly due to the adoption of modern wheat varieties of CIMMYT/ICARDA origin, utilization of inputs, better agronomic practices and favourable policies. However, compared to the global average yield (3 t/ha), the productivity of wheat in the CWANA region remains still low (2.5 t/ha) principally due to abiotic and biotic constraints. The current annual production level of about 126 million tons of wheat on a total area of 54 million hectares is far below the regional demand of about 164 million tons. Such imbalance between demand and production has led to the importation of 44 million tons of wheat at a cost of 15 billion dollars during the 2011 season alone. In the year 2050, the CWANA population is expected to increase from the current 0.9 billion to 1.4 billion, and the demand for wheat reaches 268 million tons. Fulfilling this demand is very challenging in the face of climate change, increasing drought, heat stress and emergence of new virulent diseases and pests. Offsetting these challenges requires designing an effective wheat breeding strategy with the application of new technologies and tools in order to develop varieties with high yield potential and resistance/tolerance to abiotic and biotic stresses with acceptable end use qualities. This wheat breeding handbook summarizes wheat origin and evolution, challenges and opportunities of wheat production in CWANA, and current strategies of ICARDA's wheat breeding program in developing better wheat varieties for the CWANA and SSA regions

using both conventional and molecular approaches in partnership with CIMMYT, NARS and other Advanced Research Institutes (ARIs). As this handbook will be freely available in printed and on-line versions, it will serve as an important reference for wheat breeders, geneticists, pathologists and others who are working in the wheat breeding programs of the CWANA and SSA regions and beyond.



Prof. Jacques Wery

Deputy Director General for Research, Cairo, ICARDA

A handwritten signature in blue ink, consisting of several overlapping horizontal and vertical strokes, positioned below the text of Prof. Jacques Wery's title.

## List of staff in the bread wheat improvement program at ICARDA

| Name         | Position   | Email  | Photo  |
|--------------|--|--|--|
| A.Amri       | Head of genetic resources                                  | <a href="mailto:a.amri@cgiar.org">a.amri@cgiar.org</a>               |    |
| A.Abdellat   | Molecular geneticist                                       | <a href="mailto:a.al-abdallat@cgiar.org">a.al-abdallat@cgiar.org</a> |    |
| A.El baouchi | Senior research assistant –<br>Cereal & legume quality lab | <a href="mailto:a.el-baouchi@cgiar.org">a.el-baouchi@cgiar.org</a>   |    |
| A.Hamweih    | Biotechnologist  | <a href="mailto:a.hamwieh@cgiar.org">a.hamwieh@cgiar.org</a>         |  |
| A.Niane      | International nursery scientist                            | <a href="mailto:a.niane@cgiar.org">a.niane@cgiar.org</a>             |  |

|              |                       |  |  |
|--------------|-----------------------|--|--|
| A.Sherif     | Breeder               | <a href="mailto:alisherif875@yahoo.com">alisherif875@yahoo.com</a> |    |
| H.Halila     | Food security project | <a href="mailto:h.halila@cgiar.org">h.halila@cgiar.org</a>         |    |
| H.El Ali     | Research Technician   |  |    |
| I.Tahir      | Breeder               | <a href="mailto:i.tahir@cgiar.org">i.tahir@cgiar.org</a>           |   |
| K.Nazari     | Rust pathologist      | <a href="mailto:k.nazari@cgiar.org">k.nazari@cgiar.org</a>         |  |
| K. Al-Shamaa | Database manager      | <a href="mailto:k.el-shamaa@cgiar.org">k.el-shamaa@cgiar.org</a>   |  |

|                  |   |  |  |
|------------------|---|--|--|
| K.Eddakir        | PhD student - bread wheat program                   | <a href="mailto:kenza.eddakir@hotmail.fr">kenza.eddakir@hotmail.fr</a>     |    |
| M.Baum           | Director, Biodiversity and crop improvement program | <a href="mailto:m.baum@cgiar.org">m.baum@cgiar.org</a>                     |    |
| M.EL Bouhssini   | Entomologist  | <a href="mailto:m.bohssini@cgiar.org">m.bohssini@cgiar.org</a>             |    |
| M.Sanchez-Garcia | Winter wheat breeder and quality lab coordinator    | <a href="mailto:m.sanchez-garcia@cgiar.org">m.sanchez-garcia@cgiar.org</a> |    |
| P.Skaf           | Senior research assistant – Bread wheat Program     | <a href="mailto:p.skaf@cgiar.org">p.skaf@cgiar.org</a>                     |   |
| R.Sharma         | Breeder   | <a href="mailto:r.c.sharma@cgiar.org">r.c.sharma@cgiar.org</a>             |  |

|             |   |  |  |
|-------------|---|--|--|
| S.Assefa    | Breeder – SARD-SC, TAAT   | <a href="mailto:s.assefa@cgiar.org">s.assefa@cgiar.org</a>       |    |
| S.Tawkaz    | DH specialist   | <a href="mailto:s.tawkaz@cgiar.org">s.tawkaz@cgiar.org</a>       |    |
| S.El hanafi | Senior research assistant –<br>Bread wheat Program                | <a href="mailto:s.el-hanafi@cgiar.org">s.el-hanafi@cgiar.org</a> |    |
| S.Rehman    | Associate Scientist - Cereal<br>Pathology                         | <a href="mailto:s.rehman@cgiar.org">s.rehman@cgiar.org</a>       |    |
| S.Kumari    | Head Seed lab   | <a href="mailto:s.kumari@cgiar.org">s.kumari@cgiar.org</a>       |   |
| W.Tadesse   | Principal wheat breeder and<br>head of the bread wheat<br>program | <a href="mailto:w.tadesse@cgiar.org">w.tadesse@cgiar.org</a>     |  |

|            |  |  |  |
|------------|--|--|--|
| Z.Kehel    | Biometrician                             | <a href="mailto:z.kehel@cgiar.org">z.kehel@cgiar.org</a>       |    |
| Z.Bishaw   | Seed systems and international nurseries | <a href="mailto:z.bishaw@cgiar.org">z.bishaw@cgiar.org</a>     |    |
| R.Taiq     | Senior research technician               | <a href="mailto:r.taiq@cgiar.org">r.taiq@cgiar.org</a>         |    |
| H.Rachid   | Research technician                      |  |    |
| M.Tahiri   | Research technician                      |  |   |
| W.Shiferaw | Research Assistant - Wheat Breeding      | <a href="mailto:w.shiferaw@cgiar.org">w.shiferaw@cgiar.org</a> |  |

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## 1. INTRODUCTION

Wheat is the most widely adapted crop, growing in diverse environments ranging from sea level to regions as high as 4570 m.a.s.l. in Tibet (Percival, 1921) and from the Arctic Circle to the equator, but most suitably at the latitude range of 30° and 60°N and 27° and 40°S (Nuttonson, 1955) enabling wheat harvest somewhere in the world during every month of the year (Briggle and Curtis, 1987). Based on ploidy levels (number of chromosomes sets in a cell), cultivated wheats could be diploids ( $2n = 2x = 14$ , AA), tetraploids ( $2n = 4x = 28$ , BBAA) and hexaploids ( $2n = 6x = 42$ , BBAAADD) (Kihara, 1924). The chromosome sets in the tetraploids and hexaploid wheats are duplications of different genomes, and hence bread wheat and durum wheats are allopolyploids or to be exact allotetraploids and allohexaploids, respectively. World-wide, bread wheat accounts for 95% of all the wheat produced. Based on growth habit, wheat is classified into spring wheat and facultative/winter wheat, covering about 65 and 35% of the total global wheat production area, respectively (Braun et al., 2010; Braun and Saulescu, 2002).

Wheat has played a fundamental role in human civilization and improving food security at global and regional levels. The flour of bread wheat is used to make french bread, arabic bread, chapatti, biscuits, pastry products and the production of commercial starch and gluten while the flour from durum wheat is used for the production of semolina for use in pasta and macaroni products. In North Africa, durum wheat is preferred for the preparation of couscous and bulgur. It is also widely used to prepare a special bread by mixing both bread and durum flours. According to Braun et al. (2010), wheat provides about 19% of the calories and 21% of protein needs of daily human requirements. It is a staple food for 40% of the world's population mainly in Europe, North America, and the western and north parts of Asia. The demand for wheat is growing rapidly in new wheat growing regions of the world such as Eastern and Southern Africa (5.8%), West and Central Africa (4.7%) and South Asia and

Pacific (4.3%). Demand is also growing in the traditional wheat growing regions of Central Asia (5.6%), Australia (2.2%) and North Africa (2.2%) (Shiferaw et al, 2013). Worldwide, wheat is the most traded agricultural commodity with a trade volume of 144 MT, with a total value of 36 billion US dollars (2010 data; Shiferaw et al, 2013). Many of the developing countries that depend on wheat as a staple crop are not self-sufficient in wheat production, and accordingly, wheat is their single most important imported commodity. Wheat also accounts for the largest share of emergency food aid (Dixon et al., 2009).

Wheat production at global level has significantly increased through the years (Figure 1). According to FAO (2018), about 749.5 million tons of wheat were produced on average of 220 million ha with a productivity level of 3.4 t ha<sup>-1</sup>, a highly significant increase from 1961, which stood at 222 million tons with a productivity level of only 1.2 t/ha (Figure 1). The accelerated increase in wheat production is attributed to the adoption of technology packages, in particular improved high yielding and disease resistant varieties with better response to inputs (e.g. fertilizers, water), improved irrigation systems, machineries and pesticides as well as better management practices, coupled with conducive policies and strong institutions (Baum et al., 2013).

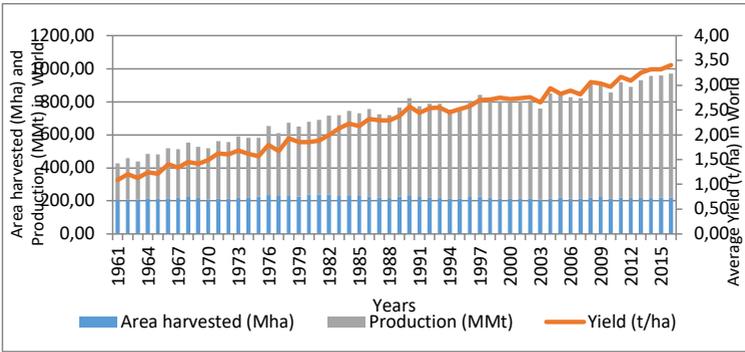


Figure 1: World wheat yield, production and area from 1961 to 2016 (FAO, 2018)

## 2. ORIGIN AND EVOLUTION OF WHEAT

Identification of the wild progenitors and its present and past distribution facilitates the study of the origin and evolution of a cultivated plant as it indicates the changes that led to domestication as well as the site of the initial cultivation. However, when such a wild progenitor is not found, or is extinct, understanding the complete history of that cultivated plant is greatly impaired (Feldman, 2001).

Archaeological and botanical studies of both wild and cultivated forms of wheat indicated that the Fertile Crescent (Figure 2) is the birth place of cultivated wheats about 8000 to 10000 years ago (Gill and Friebe, 2001; Feldman, 2001).

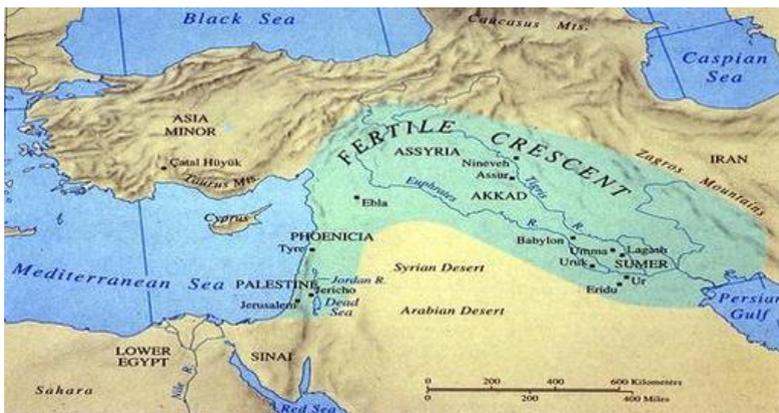


Figure 2: Geographic origin of Triticum (adapted from: Valkoun et al., 1998)

All cultivated wheats in the genus *Triticum* belong to three groups: einkorn (*T. monococcum* L.,  $2n = 2x = 14$ , AA), emmer (*T. turgidum* L.  $2n = 4x = 28$ , AABB) and dinkel (*T. aestivum*,  $2n = 6x = 42$ , AABBDD) Schultz, 1913; Kihara, 1924).

The diploid einkorn wheat, *Triticum monococcum* var. *monococcum* ( $2n = 2x = 14$ , AmAm), was domesticated directly from its wild form, *T. monococcum* var. *aegilopoides* ( $2n = 2x = 14$ , AmAm) in the Fertile Crescent, probably in the Karacadag mountain range in the

southeast Turkey (Heun et al., 1997) approximately 10,000 years ago. Currently, it is grown only in few Mediterranean countries for animal feed purposes (Nesbitt and Samuel, 1996).

The cultivated emmer wheat, *Triticum dicoccum* ( $2n = 2x = 28$ , BB AuAu) is one of the ancient cultivated wheats. It was domesticated approximately 9000 years ago in southeast Turkey from the wild emmer, *Triticum dicoccoides* ( $2n = 2x = 28$ , BB AuAu), which is an allopolyploid that arose by amphiploidy between *Triticum urartu* ( $2n = 2x = 14$ , AuAu) and the B genome ancestor, *Aegilops speltoides* ( $2n = 2x = 14$ , SS) 300,000-500,000 before present (Dvorak and Akhunov, 2005; Feldman and Levy, 2005; Johnson and Dhaliwal, 1976). The remains of the cultivated emmer (*Triticum turgidum* ssp. *dicoccum*) have been discovered at several archaeological sites in Syria dating to 7500 BC (Zohary and Hopf, 1993; Zohary, 1999). Emmer cultivation has declined through time and currently it is found only in limited areas in Ethiopia and Russia. The other cultivated tetraploid wheat, *Triticum timopheevii* ( $2n = 4x = 28$ , GGAtAt) is believed to be domesticated from the wild emmer wheat, *T. dicoccoides* ssp. *armeniacum* (Feldman, 2001). However, its cultivation is very limited and of little economic importance. Among the tetraploid wheats, the free-threshing macaroni or durum wheat ( $2n=4x=28$ , AABB), which arose by few mutations from primitive emmer wheats 8,500 years ago, is currently under cultivation in relatively large areas (Gill and Friebe, 2001).

The hexaploid species, *T. aestivum* ( $2n = 6x = 42$ , BBAADD) and *T. zhukovsky* ( $2n = 6x = 42$ , BBAAGG) have no wild progenitors, and are only found in cultivated forms in farmers' fields by hybridization between cultivated tetraploid wheat and wild diploid species (Feldman, 2001). Common wheat (*T. aestivum*) is currently the dominant wheat worldwide while *T. zhukovsky* is of limited economic importance.

Common wheat, *Triticum aestivum* ( $2n = 6x = 42$ , BBAADD), was formed from a hybrid between the cultivated tetraploid wheat species *T. turgidum* ( $2n = 4x = 28$ , BBAA) and the wild diploid species *Aegilops tauchii* var. *strangulata* ( $2n = 2x = 14$ , DD) (Dvorak et al., 1998, McFadden and Sears, 1946). Genome analyses by Kihara (1919) and Sax (1922) on the pairing behaviour of interspecific hybrids between  $2x/4x$  and  $4x/6x$  wheats indicated that *T. monococcum* and *T. turgidum* share one genome in common, and *T. turgidum* and *T. aestivum* have two genomes in common. However, the cytological data did not discriminate between *T. monococcum* ( $2n = 2x = 14$ , AmAm) and *T. urartu* ( $2n = 2x = 14$ , AA) genomes (Johnson and Dhaliwal, 1976). However, molecular evidence showed that *T. urartu* is actually the A genome donor of both tetraploid and hexaploid wheats (Dvorak et al., 1993). The other hexaploid wheat, *T. zhukovsky* ( $2n = 6x = 42$ , AtAt AmAm GG), arose from the hybridization of *Triticum timopheevii* ( $2n = 4x = 28$ , AtAtGG) with *T. monococcum* ( $2n = 2x = 14$ , AmAm) (Upadhyya and Swaminathan, 1963). Based on recent molecular evidence, *Ae. speltoides* is reportedly the donor for both the B and G genomes of polyploid wheats (Petersen et al., 2006).

Chromosome pairing in polyploid *Triticum* species occurs in a diploid-like fashion between homologous chromosomes and not between homoeologues (partially homologous chromosomes of the different genomes). This is due to the suppressor Ph1 (Riley and Chapman, 1958; Sears, 1976) and Ph2 (Dong et al., 2002) genes. Therefore, in plants lacking these genes, particularly the Ph1 gene, multivalents were observed during meiosis due to pairing among the homoeologous chromosomes, resulting in partial sterility of plants, indicating the crucial role of the Ph1 gene for diploid-like chromosome pairing and for the evolution of polyploid wheats and their domestication (Riley and Chapman, 1958; Sears, 1976).

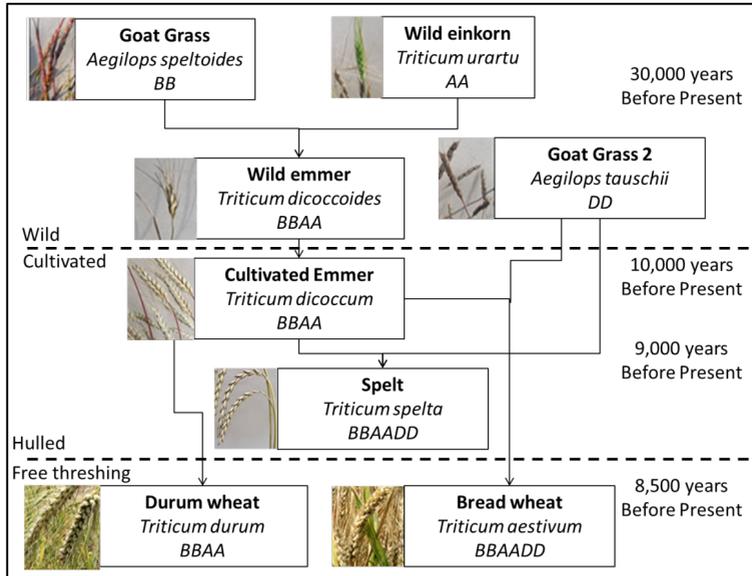


Figure 3: Adapted from New Hall Mill: The Evolution of Wheat.

*T. urartu*, though never cultivated, occurs in parts of the Fertile Crescent, and has played a significant role in the wheat evolution by contributing the AuAu genome to all tetraploid and hexaploid wheats (Dvorak et al., 1993). The wild forms of both tetraploid wheats, *Triticum turgidum* ssp. *dicocoides* and *T. timopheevii* ssp. *armeniacum*, are widely distributed in the Fertile Crescent (Figure 3). *T. dicocoides* is found exclusively in Lebanon, Israel, Palestine and Syria, while *T. armeniacum* is dominantly found in Azerbaijan and Armenia, and yet both progenitors overlap in Turkey, northern Iraq and possibly Iran (Feldman et al., 2001; Gill and Friebe, 2001).

Bread wheat arose farther northwest, away from the Fertile Crescent, in the corridor extending from Armenia in Transcaucasia to the south west coastal areas of the Caspian Sea in Iran. In this region, *Ae. tauschii* var. *strangulata* is predominant, which evidently hybridized with cultivated emmer to produce *T. aestivum*. This is supported by results from a recent study (Wang et al. 2013) in which they genotyped 477 *Ae. tauschii* and wheat

accessions collected from Eastern Turkey to China using Single Nucleotide Polymorphism (SNP) markers. Their results conclusively showed that the Aegilops populations growing nowadays in the Southern part of the Caspian Sea are the most genetically similar to the D genome of modern wheats.

According to Miller (1987) five subspecies of *T. aestivum* ( $2n = 6X = 42$ , BBAADD) namely: *T. aestivum* ssp. *aestivum* (QQ cc S1S1), *T. aestivum* ssp. *compactum* (QQ CC S1S1), *T. aestivum* ssp. *spelta* (qq cc S1S1), *T. aestivum* ssp. *macha* (qq CC S1S1) and *T. aestivum* ssp. *sphaerococcum* (QQ CC s1s1) have been identified. They differ principally due to allelic variations of single major genes: q (the speltoid gene) and its dominant allele Q (which confers free-threshing grain and tough rachis) on chromosome 5A and 2D; c and its dominant compact-ear producing allele C on chromosome 2D; S and its recessive spherical-grain producing s allele on chromosome 3D. *T. aestivum* ssp. *spelta* found growing in Iran is believed to be the first bread wheat from which free-threshing types were derived by mutation (McFadden and Sears, 1946). According to Ohtsuka (1998) and Yan et al. (2003), the European spelt wheats may have been derived secondarily from a hybridization involving *T. compactum* and emmer wheat. More recently, Matsuoka and Nasuda (2004) on the other hand, suggested durum wheat (*T. durum* ssp. *durum*) as a candidate for the female progenitor (BBAA) genome of bread wheat after embryo rescue-free crossing of the durum wheat cultivar Langdon with *Ae. tauschii* line and successfully producing fertile triploid F1 hybrids which spontaneously (without colchicine treatment) set hexaploid F2 seeds at average selfed seed rate of 51.5%. Currently, common wheat (*T. aestivum*,  $2n = 6X = 42$ , BBAADD), is the world's most widely cultivated crop grown in all temperate and in most subtropical countries with altitude levels ranging from below sea level near the Dead Sea and the Imperial Valley of California to more than 4500 m in Tibet (Stoskopf, 1985).

### 3. WHEAT GENETIC RESOURCES AND GENE POOLS

The wheat genetic resources are composed of landraces, obsolete cultivars, wild relatives, and elite breeding lines and modern cultivars. The concept of the gene pools was proposed by Harlan and de Wet (1971) and later on the base of evolutionary distance from each other and their genomic constitution (Jiang et al., 1994), they gave the idea of the three gene pools i.e. primary, secondary and the tertiary gene pools. These gene pools are usually in relation to the cultivated species (Table 1). The knowledge of the ancestry cultivated wheats is important for understanding variation and genetic diversity in their primary and secondary gene pools and the potential for exploiting the valuable genes responsible for disease resistance or stress tolerance, into new varieties (Smale, 1996).

Table 1: Number of wheat genetic resources accessions held by gene banks

| Triticum and related species | Number of accessions |
|------------------------------|----------------------|
| Diploid                      | 13659                |
| Tetraploid                   | 106398               |
| Hexaploid                    | 444648               |
| Octaploid                    | 38                   |
| Other Triticum hybrid        | 367                  |
| Unspecified Triticum         | 167133               |
| Aegilops                     | 42026                |
| Triticale                    | 37439                |
| <b>Total</b>                 | <b>811708</b>        |

Adapted from Knüpfper, 2009

The primary gene pool of wheat is composed of wheat landraces, early domesticates and wild species that hybridize directly with the cultivated and the diploid donors of the A and D genome to bread wheat and durum wheat. Bread wheat arose recently (6,000–8,000 years ago) from the hybridization of tetraploid (*T. turgidum*) and diploid, *Ae. tauschii* Coss. So, these two species constitute the primary gene pool (Qi et al., 2007). The primary gene pool is often preferred due the easiness of its cross-ability with wheat (Mujeeb-Kazi, 2003). The chromosomes of these species are homologous to the cultivated types and can be utilized

easily by breeding methods (Feuillet et al., 2007). During the last decades many useful genes may have been lost due to crop improvement for specific environments, some of these lost genes can somehow be recovered from the primary gene pool. The primary gene pool of wheat carries a highly diverse, geographically widespread and sexually compatible germplasm (Feuillet et al., 2007). It is important to note that only a small proportion of the existing genetic diversity of the primary gene pool for most crop species has been utilized for crop improvement (Tanksley and McCouch, 1997).

The secondary gene pool of wheat contains polyploid species that share at least one homologous genome with the cultivated types, such as polyploid Triticum and Aegilops species. These species share only one genome with wheat (Feuillet et al. 2007). Transferring genetic material is comparatively more complex, there are usually problems of hybrid seed death, female sterility of F1 hybrids, reduced recombination (Ogbonnaya et al. 2013) and often to obtain F1 hybrids embryo rescue is required. This gene pool includes, for instance, *T. timopheevii* (AAGG) and the diploid S-genome (similar to the B genome) species from Aegilops (Curtis et al. 2002, Qi et al. 2007).

The tertiary gene pool is composed of more distantly related diploids and polyploids with non-homologous genomes. They have no genome constitutions of the cultivated species and transfer of genetic material is highly complex (Feuillet et al. 2007). Usually special techniques such as irradiation or gametocidal chromosomes are needed for gene transfer and embryo rescue is necessary in these cases (Jiang et al. 1994, Mujeeb-Kazi and Hettel 1995). This group includes mostly germplasm of Triticeae that are not within the primary or secondary gene pools. Most of germplasm in this group are perennials (Feuillet et al. 2007, Mujeeb-Kazi 2003). Although tertiary gene pool resources are highly complex to utilize, they have the potential of becoming important means to develop new diverse wheat germplasm (Mujeeb-Kazi, 2006).

The World Information and Early Warning System (WIEWS) database compiled by FAO (FAO, 2009) possesses 856,167 wheat genetic resources accessions, including 25,242 of *Aegilops* and 6015 wild *Triticum* species. In the 'Crop Strategy for Wheat' conducted in 2007 by the GCDT, CGIAR centres and other partners reported around 750,000 wheat accessions held in over 80 gene banks, of which 4% were wild relatives, although no detailed information on *Aegilops* and wild *Triticum* was included (CIMMYT, 2007 <http://www.croptrust.org/documents/cropstrategies/WheatStrategy.pdf>).

According to FAO (2009), 95% of wheat landraces and more than 60% of wild relatives have been collected and conserved in gene banks of international centres such as CIMMYT and ICARDA; and other national gene banks (Table 2). ICARDA gene bank holds a total of 41,331 accessions of wheat genetic resources representing 28% of the total holdings of ICARDA gene bank, placing it in the top six gene banks in the world.

Collecting missions and conservation of large number wheat germplasm accessions have been carried out at international and national gene banks as indicated above. Management of such accessions for characterization, regeneration, evaluation and distribution is costly. The key questions associated to gene bank management and germplasm evaluation are:

1. How unique are these accessions within the gene bank and among gene banks at international and national levels? Crop registry was developed for wheat to identify the duplicates among all accessions held in different gene banks and the results showed that there are a lot of duplication among the main gene banks mainly for the germplasm collected by Vavilov Institute of Russia or the germplasm maintained by USDA-Gene bank. Most of the wheat genetic resources held at ICARDA are unique as they are predominately landraces, primitive wheats, *Aegilops* and wild *Triticum*, most of which are issued from collecting missions organized jointly by ICARDA in CWANA region.

2. Are we confident that the global wheat gene pool has been adequately captured and conserved?

The gap analysis combines the collecting locations of all available accessions with known natural range of each species to determine areas requiring further collecting. Also, future collecting missions can be based on priority levels (below) as outlined in Maxted et al. (2008), based on the number of accessions available. Based on this, there are 10 ‘high-priority’ *Aegilops* species (*Ae. bicornis*, *Ae. columnaris*, *Ae. juvenalis*, *Ae. kotschyi*, *Ae. longissima*, *Ae. searsii*, *Ae. sharonensis*, *Ae. uniaristata*, *Ae. vavilovii*, *Ae. ventricosa*) and two ‘high-priority’ wild *Triticum* species (*T. timopheevii* subsp. *armeniacum*, *T. turgidum* subsp. *dicoccoides*) which have fewer than 200 accessions in total, in global collections. In addition, new collecting missions could be justified if targeting adaptive traits such as drought, heat, salinity tolerance. The wheat genetic resources conservation strategy (CIMMYT, 2007) identified priority regions and certain ‘target’ wild wheat relatives on which to base future collection missions, including:

- Albania, Greece and Former Yugoslavia, which have 13 species including *Ae. uniaristata* known to possess tolerance to heavy metals.
- Iran, Jordan, Pakistan and Syria, very dry areas, for heat- and drought-tolerant *Ae. searsii*, *Ae. tauschii* and *Ae. vavilovii*.
- Iran, in the mountains near Shiraz and near Esfahan, along the Zagros mountains and in the desert and salt affected areas, all wild *Triticum* and *Aegilops* species, for drought- and salt-tolerance.
- Algeria, Cyprus, Egypt, Greece, Iran, Israel, Libya, Pakistan, Palestine, Spain, Syria, Tajikistan, Tunisia, Turkey, Turkmenistan, Uzbekistan, targeting *Ae. bicornis*, *Ae.*

comosa, *Ae. juvenalis*, *Ae. kotschyi*, *Ae. peregrina*, *Ae. sharonensis*, *Ae. speltoides*, *Ae. uniaristata* and *Ae. vavilovii* (Maxted et al. 2008)

- Aegean region, western Mediterranean region (France, Portugal, Spain), North Africa, Iran and Syria, (Skovmand et al. 2006).

3. How much of the conserved germplasm has been well characterized both at phenotypic and molecular levels?. Most of the conserved germplasm is characterized for major descriptors and for some agronomic traits. However, few were characterized using molecular markers techniques and CIMMYT is recently engaged in this activity using genotyping by sequencing technique and around 34,000 accessions of ICARDA will be genotyped in 2015.

4. Is the conserved germplasm accessible and useful? For the international collections access is facilitated by the use of the Standard Material Transfer Agreement (SMTA) and the same is done for some collections from national gene banks. However, accessibility to accessions in some national gene banks is still not guaranteed.

The ultimate goal of collection, conservation, characterization and evaluation of germplasm is to identify accessions with traits of interest to breeders to be utilized in breeding to develop high yielding varieties with resistance/tolerance to major biotic and abiotic constraints with acceptable level of end use quality. In this regard, the main question is how much of the gene bank diversity has been utilized by breeders? To what extent are gaps in collected accessions tied to value for end-users? Though there are few attempts to estimate the contribution of genetic resources for wheat improvement, available reports indicate that only limited amount (10%) of the genetic resources (land races and wild relatives) have been utilized in crosses for pre-breeding and breeding purposes (Chapman, 1986). The t probable reasons for low utilization of genetic resources by breeders include (1) gene bank materials are too wild, obsolete and difficult to breed (2) the characterization and evaluation data is poor and often it

is done for traits which are not of high priority to the target of breeding programs (3) breeders may have traits of interest from their elite breeding materials and cultivars (4) gene bank accession's information might not be accessible and too technical for non-curators. (5) transferring some traits/genes from the wild relatives to modern wheat is often time-consuming and success is not always granted. Even when traits are successfully transferred, there may be linkage drag associated with the transferred traits.

Table 2: Germplasm collections of Triticum, Aegilops and Triticale species.

| Genus    | Gene bank /Institution   | Country   | Number proportion of accessions |                                  |                                     |       |
|----------|--|-----------|---------------------------------|----------------------------------|-------------------------------------|-------|
|          |  |           | Total                           | Wild landraces and old cultivars | Breeding lines and modern cultivars | Other |
| Triticum | Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT)                  | Mexico    | 110281                          | 37%                              | 57%                                 | 6%    |
|          | National Small Grains Germplasm Research Facility (NSGC)                       | USA       | 57348                           | 61%                              | 38%                                 | <1%   |
|          | Institute of Crop Germplasm Resources (ICGR-CAAS)                              | China     | 43039                           | 5%                               | 0%                                  | 95%   |
|          | National Bureau of Plant Genetic Resources (NBPGR)                             | India     | 35889                           | 6%                               | 10%                                 | 84%   |
|          | International Center for Agricultural Research in the Dry Areas (ICARDA)       | Syria     | 34951                           | 80%                              | <1%                                 | 21%   |
|          | National Institute of Agrobiological Sciences (NIAS)                           | Japan     | 34652                           | 7%                               | 31%                                 | 61%   |
|          | N.I. Vavilov All-Russian Scientific Research Institute of Plant Industry (VIR) | Russia    | 34253                           | 44%                              | 55%                                 | <1%   |
|          | Istituto di Genetica Vegetale (IGV)  | Italy     | 32751                           | 100%                             | 0%                                  |       |
|          | Leibniz Institute of Plant Genetics and Crop Plant Research (IPK)              | Germany   | 26842                           | 53%                              | 44%                                 | 4%    |
|          | Australian Winter Cereals Collection (TAMAWC)                                  | Australia | 23811                           | 3%                               | 82%                                 | 16%   |
|          | Others   |           | 424123                          | 16%                              | 32%                                 | 52%   |
|          | Total  |           | 857940                          | 28%                              | 33%                                 | 39%   |
| Aegilops | Lieberman Germplasm Bank (ICCL-TELAVUN)  | Israel    | 9146                            | 100%                             |                                     | <1%   |
|          | International Center for Agricultural Research in the Dry Areas (ICARDA)       | Syria     | 3847                            | 100%                             |                                     | <1%   |
|          | National Plant Gene Bank of Iran (NPGBI-SPII)                                  | Iran      | 2653                            | 99%                              |                                     | 1%    |
|          | National Institute of Agrobiological Sciences (NIAS)                           | Japan     | 2433                            | 5%                               |                                     | 95%   |
|          | N.I. Vavilov All-Russian Scientific Research Institute of Plant Industry (VIR) | Russia    | 2248                            |                                  |                                     | 100%  |
|          | National Small Grains Germplasm Research Facility (NSGC)                       | USA       | 2207                            | 100%                             |                                     |       |
|          | Laboratory of Plants Gene Pool and Breeding (LPGPB)                            | Armenia   | 1827                            | 100%                             | <1%                                 |       |
|          | Leibniz Institute of Plant Genetics and Crop Plant Research (IPK)              | Germany   | 1526                            | 100%                             |                                     | <1%   |
|          | Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT)                  | Mexico    | 1326                            | 99%                              | <1%                                 | <1%   |
|          | Cereal Research Centre, Agriculture and Agri-Food (WRS)                        | Canada    | 1100                            | 100%                             |                                     |       |
|          | Others   |           | 13713                           | 77%                              | 2%                                  | 21%   |
|          | Total  |           | 42026                           | 82%                              | 1%                                  | 18%   |

Adapted from FAO (2009)

### **3.1. Role of genetic resources in wheat breeding**

The ultimate goal of collection, conservation, characterization and evaluation of germplasm is to identify accessions with traits of interest to breeders to be utilized in breeding to develop high yielding varieties with resistance/tolerance to major biotic and abiotic constraints with acceptable level of end use quality. In this regard, the main question is how much of the gene bank diversity has been utilized by breeders? To what extent are gaps in collected accessions tied to value for end-users? Though there are few attempts to estimate the contribution of genetic resources for wheat improvement, available reports indicate that only limited amount (10%) of the genetic resources (land races and wild relatives) have been utilized in crosses for pre-breeding and breeding purposes (Chapman, 1986). The most probable reasons for low utilization of genetic resources by breeders include (1) gene bank materials are too wild, obsolete and difficult to breed (2) the characterization and evaluation data is poor and often it is done for traits which are not of high priority to the target of breeding programs (3) breeders may have traits of interest from their elite breeding materials and cultivars (4) gene bank accession's information might not be accessible and too technical for non-curators. (5) transferring some traits/genes from the wild relatives to modern wheat is often time-consuming and success is not always granted. Even when traits are successfully transferred, there may be linkage drag associated with the transferred traits. Nevertheless, genetic resources have played significant role in wheat breeding in the following major areas.

#### **3.1.1. Increasing yield potential**

The first scientific breeders started in the 19th century selecting and crossing landraces and cultivars from different origins and genetic pools to obtain the combinations that led to superior varieties. The Italian breeder, Nazareno Strampelli, used the Japanese variety Akakomugi in his crossing block and made crosses with Italian landraces and breeding lines

in the first decades of the 20th century (Salvi et al., 2013). Such crosses resulted in successful varieties such as Ardito and Mentana possessing Ppd-D1 alleles for photoperiod insensitivity and Rht8c for short straw. These varieties later became the backbone of most of the new varieties developed in the Mediterranean countries, USSR, China and some South American countries (Dalrymple 1986, Lupton 1987, Yang and Smale 1996).

The Nobel laureate Norman E. Borlaug in the 1950s made the Norin-10/Brevor cross that introduced the Rht-B1 and Rht-D1 dwarfing alleles which led to the development and release of input responsive semi-dwarf varieties of “The Green Revolution” (Borlaug 2008). The dwarfing genes Rht-B1 and Rht-D1 originated from the Japanese landrace Shiro Daruma from which they were successfully introgressed into Norin-10 (Kihara, 1983). The introgression of these genes into modern wheat cultivars, however, was tedious and time consuming and required persistent efforts (Borlaug, 1988). Crosses between winter and spring varieties – two gene pools that used to remain isolated one from another due to geographical and physiological barriers – have been widely used at CIMMYT and ICARDA to obtain new high yielding widely adapted varieties. Similarly, 1B/1R rye-wheat translocation lines also contributed significantly to the development of mega-varieties around the world (Tadesse et al 2016).

### 3.1.2. Drought, heat and salinity tolerance

Wheat landraces have been exploited for many abiotic and biotic stress tolerances and are the sources of many important genes including genes for drought tolerance (Reynolds et al. 2007). Wild relatives, synthetic hybrid wheat (SHW), synthetic derivatives (SDW) or modern lines with alien translocations have shown better tolerance compared to their modern counterparts under heat and drought stress conditions. At CIMMYT and in other studies, several SHW and SDW with superior adaptation to drought and heat conditions have been

identified (Reynolds et al., 2005; Trethowan and van Ginkel, 2009; Lopes and Reynolds, 2011; Jafarzadeh et al. 2016; Afzal et al. 2017; Tang et al. 2017; Aziz et al 2018). Wheat lines carrying 7DL/7A translocation from *Agropyron elongatum* showed improved water stress adaptation and root biomass at deeper soil levels under drought stress (Placido et al., 2013). Yield advantage of synthetic backcrossed derived wheat under rain-fed environments such as northern and southern Australia was reported to be 11 and 30% higher yielding, respectively compared to elite cultivars of bread wheat (Gororo et al. 2002; Dreccer et al. 2007; Ogonnaya et al. 2007). Similarly, Lage and Trethowan (2008) reported that synthetic derived wheat germplasm, “Vorobey” (Croc\_1/Ae. squarrosa (224)//Opata/3/Pastor) was the highest yielding line in 48 out of 52 trial sites worldwide. Synthetic derived wheats have also been reported to possess resistance/tolerance to salinity, and boron toxicity (Dreccer et al., 2003, Emebiri and Ogonnaya 2015) indicating the importance of wild relatives in improving common wheat.

### 3.1.3. Resistance to diseases and insects

Some of the important genes for resistance to diseases and insects identified from genetic resources are summarized in Table 3. Most of these genes originated from wild relatives (Roelfs, 1988, Hajjar and Hodgkin, 2007) or from landrace cultivars (McIntosh et al 1995). The Sr2 gene, which continue to provide durable resistance to stem rust of wheat, was originally transferred to hexaploid wheat from Yaroslav emmer by McFadden in 1923 (Stakman and Harrar, 1957). This gene in combination with other major and minor genes has provided resistance to stem rust successfully across many countries. Successful resistance genes such as Sr24 from *Thinopyrum ponticum* (IRS.1AL); Sr38 from *Triticum ventricosior* and Sr36 from *Triticum timopheevi* (McIntosh et al., 1995) originated from genetic resources. Currently, most of the effective resistance genes such as Sr22, Sr25, Sr26, Sr39, Sr42, Sr45 which provide resistance against the Ug99 stem rust race are derived from translocations of

wheat wild relatives (Singh et al. 2007; Hajjar and Hodgkin, 2007). Rouse et al. (2011) reported screening of 456 non-duplicated *Ae. tauschii* accession for resistance to stem rust Ug99 races as an important step for subsequent identification of novel genes and their transfer to bread wheat. The results suggested that 22 % of the *Ae. tauschii* accessions screened were resistant to Ug99 races. Similarly, Zegeye et al. (2014) evaluated 181 primary SHWs at seedling and adult plant stages in Ethiopia using virulent Kusba/Attila isolates. The SHWs were genotyped with 9K infinium SNP array and used in GWAS to identify loci linked to stripe rust resistance. They identified nine genomic regions associated with stripe rust resistance with a novel QTL on 6DS. Periyannan et al. (2013; 2014) identified two genes Sr33 and Sr45 from *Ae. tauschii* and developed diagnostic markers that are widely used and deployed in wheat breeding. Sources of resistance for diverse diseases and insect pests such as Septoria, tan spot, nematodes, and Hessian fly and aphids have been reported from synthetic wheats (Villareal et al., 1992; Tadesse et al., 2007; El Bouhssini et al., 2012; Ogbonnaya et al., 2013).

Table 3: Example of genes identified from wheat gene pools

| Traits             | Locus | Source                 | Reference                     |
|--------------------|-------|------------------------|-------------------------------|
| Disease resistance |       |                        |                               |
| Leaf rust          | Lr9   | <i>Ae. umbellulata</i> | Soliman et al., 1963          |
|                    | Lr18  | <i>T. timopheevii</i>  | Dyck and Samborski, 1968      |
|                    | Lr19  | <i>Th. elongatum</i>   | Browder 1972                  |
|                    | Lr23  | <i>T. turgidum</i>     | McIntosh and Dyck, 1975       |
|                    | Lr24  | <i>Ag. elongatum</i>   | McIntosh et al., 1976         |
|                    | Lr32  | <i>Ae. tauschii</i>    | Kerber, 1987                  |
|                    | Lr35  | <i>Ae. speltooides</i> | Kerber and Dyck, 1990         |
|                    | Lr37  | <i>Ae. ventricosa</i>  | Bariana et al., 1991          |
|                    | Lr47  | <i>Ae. speltooides</i> | Dubcovsky et al., 1998        |
|                    | Lr57  | <i>Ae. geniculata</i>  | Kuruparthi et al. 2007        |
| Stem rust          | Sr2   | <i>T. turgidum</i>     | Ausemus et al., 1946          |
|                    | Sr5   | <i>T. aestivum</i>     | Ausemus et al., 1946          |
|                    | Sr24  | <i>Th. ponticum</i>    | McIntosh et al., 1976         |
|                    | Sr25  | <i>Th. ponticum</i>    | McIntosh et al., 1976         |
|                    | Sr26  | <i>Th. ponticum</i>    | McIntosh et al., 1976         |
|                    | Sr38  | <i>Ae. ventricosa</i>  | Bariana et al., 1991          |
| Stripe rust        | Yr5   | <i>T. spelta</i>       | Macer, 1966                   |
|                    | Yr10  | <i>T. spelta</i>       | Macer, 1975                   |
|                    | Yr9   | <i>S. cereale</i>      | Macer, 1975                   |
|                    | Yr15  | <i>T. dicoccoides</i>  | Gerechter-Amitai et al., 1989 |
|                    | Yr17  | <i>Ae. ventricosa</i>  | Bariana et al., 1991          |
|                    | Yr40  | <i>Ae. geniculata</i>  | Kuruparthi et al. 2007        |
| Powdery mildew     | Pm1   | -                      | Sears and Briggie, 1969       |

|                            |            |                          |                             |
|----------------------------|------------|--------------------------|-----------------------------|
|                            | Pm12       | <i>Ae. speltoides</i>    | Jia et al., 1994            |
|                            | Pm21       | <i>Haynaldia villosa</i> | Qi et al., 1995             |
|                            | Pm25       | <i>T. monococcum</i>     | Shi et al., 1998            |
| Wheat streak mosaic virus  | Wsm1       | <i>Ag. elongatum</i>     | Talbert et al., 1996        |
| Karnal bunt                | QTL        | <i>T. turgidum</i>       | Nelson et al., 1998         |
| Barley Yellow Dwarf Virus  | Bdv2       | <i>Th. intermedium</i>   | Chen et al., 1998           |
|                            | Bdv3       | <i>Th. intermedium</i>   | Sharma et al., 1995         |
| Tan spot                   | tsn3a,b,c  | <i>Ae. tauschii</i>      | Tadesse et al., 2007        |
| Pest resistance            |            |                          |                             |
| Hessian fly                | H21        | <i>S. cereale</i>        | Friebe et al., 1990         |
|                            | H23, H24   | <i>Ae. tauschii</i>      | Gill et al., 1986           |
|                            | H27        | <i>Ae. ventricosa</i>    | Delibes et al., 1997        |
|                            | H30        | <i>Ae. triuncialis</i>   | Martin-Sanchez et al., 2003 |
|                            | H32        | <i>Ae. tauschii</i>      | Sardesai et al., 2005       |
| Cereal cyst nematode       | Cre3, Cre4 | <i>Ae. tauschii</i>      | Eastwood et al., 1991       |
|                            | Cre6       | <i>Ae. ventricosa</i>    | Ogbonnaya et al., 2001      |
| Abiotic stress             |            |                          |                             |
| Drought tolerance          | QTL        | <i>Ag. elongatum</i>     | Placido et al., 2013        |
| Salinity tolerance         | Kna1       | <i>T. aestivum</i>       | Dvorak and Gorham, 1992     |
| Quality                    |            |                          |                             |
| Pre-harvest sprouting      | QTL        | <i>T. aestivum</i>       | Anderson et al., 1993       |
| Protein, Zn and Fe content | QTL        | <i>T. turgidum</i>       | Uauy et al., 2006           |

#### 3.1.4. Improving grain quality

Genetic resources such as land races, wild relatives, and synthetic wheats have been reported as novel sources for improving wheat grain quality (Ogbonnaya et al., 2013). Several studies assessing the diversity across wheat gene pools for Zn and Fe grain content have been carried out and candidates with improved Zn and Fe allocation to the grain were found (Ortiz-Monasterio et al., 2007). Currently, efforts are underway at international centers and national programs to develop bio-fortified varieties and advanced lines with high Zn and Fe content from emmer wheat.

## **4. WHEAT PRODUCTION AND MAJOR ENVIRONMENTS IN CWANA**

### **4.1. Trends in wheat production**

Wheat is the principal staple food in most countries of the Central and West Asia and North Africa (CWANA) region, accounting for 45% of the region's per capita calorie intake with an average wheat consumption of about 200 kg/capita/year, which is the highest in the world. The CWANA region is a vast geographic area extending west to east from the Atlas Mountains in Morocco to the fertile irrigated Indus valley in Pakistan, and from the highland, high-rainfall areas of Ethiopia in the south to the temperate and dry northern Kazakhstan. As expected, this vast geographic area is characterized by large variations in agro-ecology, farming systems, moisture, temperature, soil types and cultural practices. Accordingly, the region harbors all kinds of wild and cultivated wheat types of different growth habits. During the period 1961-2017, wheat production area has increased from 26.9 to 54 million ha, while total production and yield (t/ha) increased from 22 to 122 million tons and from 1.1 to 2.6 t/ha, respectively (Figure 4).

The increase in production is mainly due to the adoption of improved wheat varieties originated from the International Maize and Wheat Improvement Center (CIMMYT) and the International Center for Agricultural Research in the Dry Areas (ICARDA), utilization of inputs, better agronomic practices, increased area of production, and favorable policies.

The most important wheat growing countries in the region (Table 4) are in decreasing order: Kazakhstan (13.7 Mha), Pakistan (8.9 Mha), Turkey (7.9 Mha), Iran (6.8Mha), Morocco (3.0 Mha), Afghanistan (2.4 Mha), Algeria (1.8 Mha), Ethiopia (1.6 Mha), Syria (1.5 Mha), Uzbekistan (1.4 Mha) and Egypt (1.3 Mha).

Table 4: Average area, production and yield of wheat from 2009-2017 in wheat growing countries of the CWANA region; FAO (2018)

| Country                 | Region                          | Area (Million ha) | Production (Million tons) | Yield (t ha <sup>-1</sup> ) |
|-------------------------|---------------------------------|-------------------|---------------------------|-----------------------------|
| Afghanistan             | Central Asia                    | 2.45              | 4.64                      | 1.89                        |
| Algeria                 | North Africa                    | 1.79              | 2.97                      | 1.66                        |
| Egypt                   | North Africa                    | 1.33              | 8.47                      | 6.35                        |
| Ethiopia                | North Africa/Sub Saharan Africa | 1.60              | 3.26                      | 2.03                        |
| Iran (Islamic Republic) | West Asia                       | 6.82              | 13.42                     | 1.97                        |
| Iraq                    | West Asia                       | 1.36              | 2.90                      | 2.11                        |
| Jordan                  | West Asia                       | 0.02              | 0.02                      | 1.16                        |
| Kazakhstan              | Central Asia                    | 13.70             | 14.64                     | 1.07                        |
| Kyrgyzstan              | Central Asia                    | 0.36              | 0.81                      | 2.20                        |
| Lebanon                 | West Asia                       | 0.04              | 0.12                      | 3.38                        |
| Libya                   | North Africa                    | 0.15              | 0.16                      | 1.03                        |
| Morocco                 | North Africa                    | 3.05              | 5.62                      | 1.84                        |
| Pakistan                | Central Asia                    | 8.88              | 24.05                     | 2.71                        |
| Saudi Arabia            | West Asia                       | 0.18              | 1.01                      | 5.60                        |
| Sudan                   | North Africa/Sub Saharan Africa | 0.23              | 0.39                      | 1.74                        |
| Syrian Arab Republic    | West Asia                       | 1.51              | 3.49                      | 2.32                        |
| Tajikistan              | Central Asia                    | 0.33              | 0.89                      | 2.70                        |
| Tunisia                 | North Africa                    | 0.65              | 1.32                      | 2.00                        |
| Turkey                  | West Asia                       | 7.92              | 20.84                     | 2.63                        |
| Turkmenistan            | Central Asia                    | 0.72              | 1.59                      | 2.20                        |
| Uzbekistan              | Central Asia                    | 1.42              | 6.67                      | 4.70                        |
| Yemen                   | West Asia                       | 0.13              | 0.24                      | 1.81                        |
|                         | North Africa                    | 8.80              | 22.2                      | 2.57                        |
|                         | West Asia                       | 18.00             | 42.0                      | 2.62                        |
|                         | Central Asia                    | 27.86             | 53.3                      | 2.50                        |
|                         | CWANA                           | 54.52             | 117.5                     | 2.55                        |
|                         | World                           | 219.5             | 684.0                     | 3.12                        |

As indicated in Figure 4, there is a huge difference between production and consumption in the CWANA region. There is not sufficient production at regional level to cope with the increasing demand for wheat consumption.

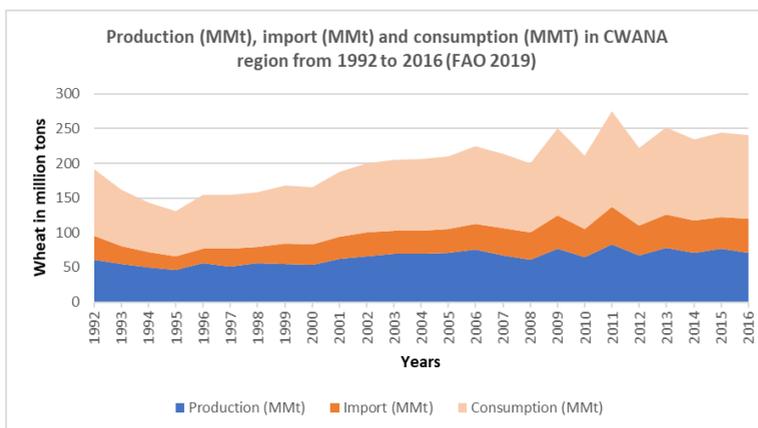


Figure 4: Annual wheat production deficit in CWANA from 1961 to 2011; FAO, 2018.

Most of the countries in the CWANA region except Kazakhstan, Syria, Pakistan and Turkey are not self-sufficient in wheat production, and accordingly, wheat is their single most important imported commodity. Among North African countries, Egypt is the largest importer with 9 million tons of wheat imported every year. According to Shiferaw et al. (2013), demand for wheat is growing at 5.6 % and 2.2 % /year in Central Asia and North Africa, respectively. In the year 2050, the population in CWANA is expected to increase from the current 0.9 billion to 1.4 billion, and the demand for wheat will rise from the present 164 million tons to 268 million tons, calling for more research and development efforts to increase wheat productivity at the regional and global levels to meet the needs of the increasing population.

In Africa, wheat is grown on a total of 10 million hectares with annual production level of about 25 million tons. Sub Saharan Africa (SSA) region, produced a total of 7.5 MT on a total

area of 2.9 Mha accounting for 40 and 1.4% of the wheat production in Africa and at global levels, respectively (FAO, 2017). Bread wheat, which accounts 95% of the wheat production at global level, is also the dominant wheat type produced in SSA. Trend analysis of wheat production in SSA from 1970-2014 (Figure 5) indicates that the total wheat production area showed slight reduction while the total production has increased from 2.8 to 7.5 MT due to the increase in productivity of wheat from 1.3 t/ha in 1970 to 2.1 t/ha in 2014.

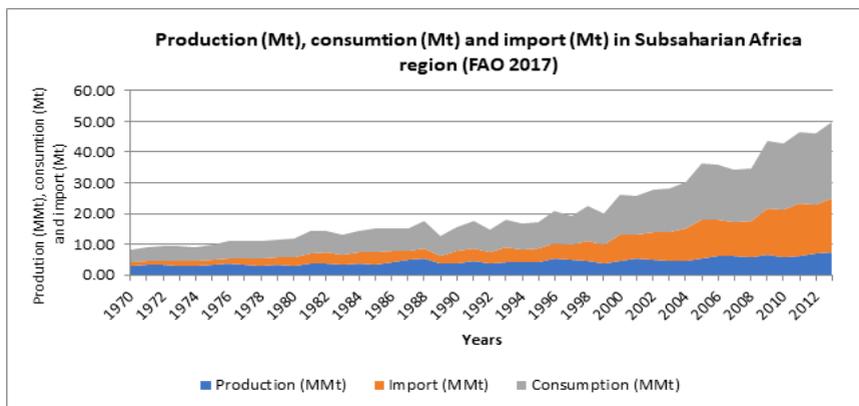


Figure 5: Wheat production, import and consumption in SSA, 1970-2014 (Tadesse et al 2018)

The most important wheat producing countries in SSA are Ethiopia, South Africa, Sudan, Kenya, Tanzania, Nigeria, Zimbabwe and Zambia in descending order Ethiopia accounts the largest production area (1.7Mha) followed by South Africa (0.5 Mha).

Though traditionally wheat was not the leading staple crop in SSA, it is becoming important through time especially in the urban areas. The rapid population growth coupled with increased urbanization and change in food habits has resulted in the surge for wheat demand in SSA. On average, from 2011-2013, SSA countries imported 17 million tons of wheat per year at a cost of 6 billion US dollar/year which of course depletes the meagre foreign currency reserve of the respective countries.

#### 4.2. Wheat mega environments in the CWANA and SSA regions

CWANA as a region has the largest wheat areas (more than 50 million hectares) in the world compared to other geographic locations such as China, Indian Subcontinent, North America, and European Union (EU) (FAO, 2018). The area is not only vast in geographical spread, but also presents large variation in moisture regimes, temperatures extremes, soil types, cultural practices, and farming systems. The areas extend from the irrigated Indus Valley in Pakistan in the east to Atlas Mountains range in Morocco in west; and from highland high rainfall areas of Ethiopia in south to very temperate and very dry northern Kazakhstan, a large area of summer rainfall dryland wheat area similar in climate to Russian Siberia (Figure 6). This geographic spread in itself is indicative that the CWANA region harbors all kinds of wheat from spring to facultative to winter growth habits. It also represents well high rainfall and irrigated environment to supplementary irrigation condition to very semiarid environment.

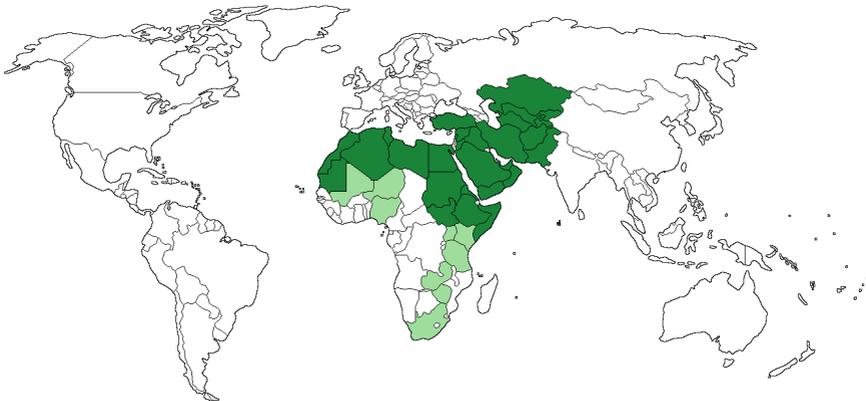


Figure 6: Central and West Asia and North Africa region (Dark green) and Sub Sahana Africa region (light green + Ethiopia and Somalia).

Thus, based on moisture availability, cropping systems and temperature regimes, the wheat production area in the CWANA region can be classified under 5 distinct large agro-climatic zones (mega-environments) namely: Favorable Irrigated and High Rainfall Spring Wheat

Environment (E1), Semiarid (Mediterranean Rainfed) Spring Wheat Environment (E2), Favorable Irrigated Winter/Facultative Wheat Environment (E3), Semiarid Rainfed Winter/Facultative Wheat Environment (E4) and High Latitude (Spring Planted) Spring Wheat Environment (E5) each covering 14, 12, 6, 13 and 8 million ha, respectively. A mega environment is a region with similar climate, moisture regimes, soil types, growth habit, plant performance, prevalent diseases and insect pests. This classification would not necessarily be contiguous in geography and political country boundary (Rajaram et al 1995).

Similarly, the wheat production area in SSA can be divided into two major mega-environments: (i) Rain-fed and (ii) Irrigated. The rain-fed production system exists dominantly during the summer season in the highlands of eastern Africa (Ethiopia, Eritrea, Kenya, Uganda, Rwanda, Burundi and Tanzania) and South Africa. The irrigated systems, on the other hand, are commonly practiced during the dry winter season in the lowlands of southern Africa (Zambia, Zimbabwe, Malawi, Madagascar, and Mozambique), western Africa (Nigeria, Senegal, and Mali) and in the low lands of Sudan. In South Africa, irrigated wheat is grown during the summer season. Though we have divided the environment in SSA into two major mega environments, it is important to note that in each country and at regional level, there is high degree of diversity and environmental variability. There is a huge potential for the expansion of irrigated wheat production in the region particularly in Sudan, Zimbabwe, Zambia, Nigeria, Somalia, and the lowlands of Ethiopia if irrigation facilities are to be installed. Spring bread wheat cultivars are cultivated dominantly in the SSA region except in South Africa where the spring wheat is grown during the winter season under irrigation while the winter/facultative wheat types are dominantly grown during the summer rainfall season accounting for about 20% of production.

### 4.3. Challenges to wheat production

#### 4.3.1. Abiotic and biotic stresses

Though wheat is the dominant crop in the CWANA region, its productivity is very low (1-2.5 t/ha) and highly variable due to abiotic stresses (drought, cold, heat, salinity) and biotic stresses (yellow rust, leaf rust, stem rust, root rots, Russian Wheat Aphid, Barley Yellow Dwarf Virus, Sunn pest, and Hessian Fly). Drought and yellow rust are principally the most important wheat yield limiting factors in the CWANA region. Similarly, in the SSA region, the most important abiotic stresses are drought, soil acidity, erosion, poor soil fertility, water-logging, and pre-harvest sprouting. Such constraints are most common in the East African highlands of Ethiopia, Eritrea, Kenya, Tanzania, Uganda, Rwanda and Burundi. Similarly, the rainfed environments in the mid altitude areas of South Africa, Angola, Zambia, Malawi and Madagascar face these challenges. Heat and lack of water for irrigation are the most important abiotic constraints in the irrigated environments across the CWANA and SSA regions.

The most important biotic constraints which affect wheat production in SSA include diseases, insects and weeds. Rusts (*Puccinia* spp.), *Helminthosporium*, septoria (*Septoria tritici*), tan spot (*Pyrenophora tritici repentis*), fusarium (*Fusarium* spp.), the bunts and smuts, take-all, and root rots are important wheat diseases common in in the CWANA and SSA regions. Yellow rust of wheat is the most devastating disease across the region. Yield losses caused by yellow rust epidemic was estimated up to 100,000 tons of wheat in Morocco when the dominant cultivar was susceptible in 2010. Similarly, yield losses of up to 70% were reported in Syria, Turkey and Ethiopia (Figure 7).

## Re-occurrence of Yellow rust in Morocco, Turkey, Syria, Ethiopia

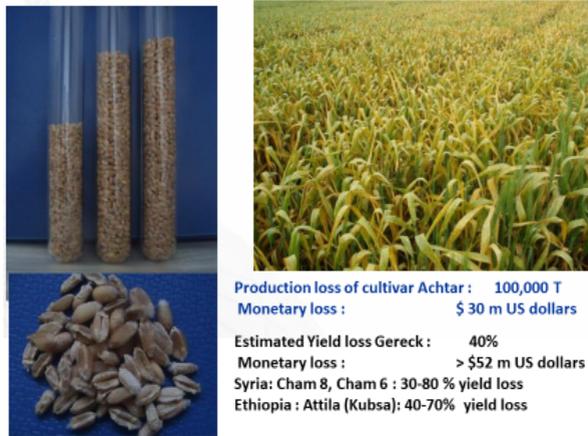


Figure 7: Yield losses due to yellow rust

Stem rust caused by *Puccinia graminis* f. sp. *tritici* is prevalent in the low and mid altitude areas of Ethiopia and Kenya with warmer temperature. In Ethiopia, stem rust epidemics has knocked out major cultivars such as Enkoy with Sr36 gene in 1994; and Digalu with SrTmp+ gene in 2013 and 2014 causing 100% yield loss. The Digalu race (TKTTF) which is different from the Ug99 race (TKTTSK) is dominant across the major wheat growing regions of Ethiopia and becomes a major threat to wheat production in the country (Tadesse et al 2018).

Of all the insects attacking wheat crop, Hessian fly, Sunn pest, wheat stem sawfly and Russian wheat aphids cause significant economic losses. Hessian fly (*Mayetiola destructor*) damage can result in total loss of the crop if high infestations occur in the early stages of crop development. In Morocco, bread wheat and durum wheat yield losses due to Hessian fly (Figure 8) have been estimated annually at 36% and 32%, respectively, amounting to about US\$200 million/annum (Lhaloui et al. 1992). Sunn pest (*Eurygaster integriceps Puton*) affects some 15 million ha of wheat in West and Central Asia.



Figure 8: Hessian fly effect on early and late planting

With the current climate change effects, it is anticipated that new pests and diseases will emerge as already exemplified in the recent epidemics of stripe/yellow rust across the Central & West Asia and North Africa (CWANA) region and the Ug99 stem rust epidemic in East African countries (Solh et al., 2012). The effect of climate change is also affecting the quality of wheat as increasing CO<sub>2</sub> may negatively affect N content and protein quality and content and increasing temperatures can negatively affect grain size. Modelling results indicate that wheat production will be more affected by climate change in the developing countries than the developed countries (Braun et al., 2010).

#### 4.3.2. Limited availability and high price of inputs

As has been clearly witnessed as a result of the Green Revolution, increasing yield per unit area has been achieved mainly through the application and utilization of inputs such as improved varieties, irrigation, fertilizers and pesticides. However, the costs of these inputs are also rising and are sometimes becoming unaffordable to poor farmers in developing countries. Rising energy costs for example have contributed to higher fertilizer prices, because the costs of natural gas used to produce ammonia is increasing, and indirectly through higher transportation costs (Figure 9). Such rapid increases in input costs offset wheat production and pose a disincentive to wheat producers.

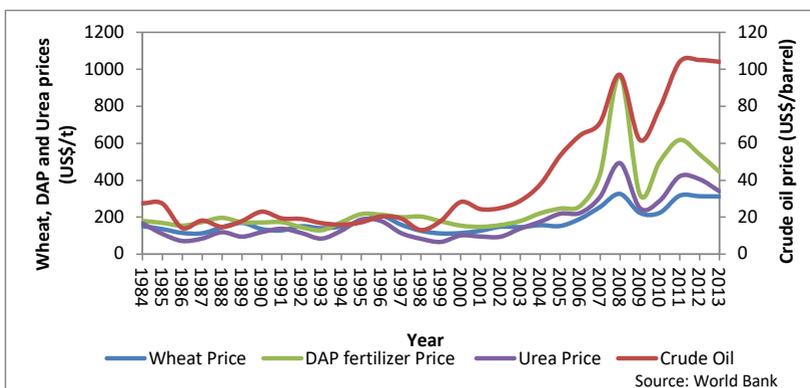


Figure 9: Association among wheat, DAP, urea prices with crude oil prices

#### 4.3.3. Yield gap, stagnating yield and increasing population

After the quantum leap in wheat production attributed to the Green Revolution, wheat yields have recently been rising by only 1.1% per year, a level that falls far short of the demand of a global population that is growing at 1.5% or more, annually. According to some estimates global wheat production must increase at least by 1.6% annually to meet a projected yearly wheat demand of 760 million tons by 2020. In the year 2050, the world population is estimated to be 9 billion, while the demand for wheat is estimated to reach more than 900 million tons (Dixon et al., 2009). The current annual production level of about 126 million tons of wheat on a total area of 54 million hectares is far below the regional demand of about 164 million tons. Such imbalance between demand and production has led to the importation of 44 million tons of wheat at a cost of 15 billion dollars during the 2011 season alone. Occasionally, a price hike in wheat can also cause political instability as has been seen with the price hikes in 2008. In the year 2050, the CWANA population is expected to increase from the current 0.9 billion to 1.4 billion, and the demand for wheat will reach 268 million tons.

Fulfilling this demand is challenging amid reports of yield stagnation in major wheat growing regions of the world and in the face of the expected adverse effects of climate change. Yield stagnation is a complex issue, which might be the result of a combination of factors, such as approaching a genetic ceiling in wheat improvement, declining soil fertility, unfavourable policies and marketing, biotic and abiotic stresses associated with climate change and other factors. Some authors have attributed yield stagnation to the genetic ceiling in India and Europe (Nagarajan, 2005) while others have reported the presence of genetic gain in both spring wheat (Manes et al., 2012; Sharma et al., 2012) and facultative winter wheat (Tadesse et al., 2013). However, it is evident that the potential of new cultivars has not been fully utilized in most of the developing countries due to poor agronomic management, application of incomplete packages of inputs, reduced incentives and unstable market prices. Recent yield gap analysis at global level in wheat indicated that the range in the difference between potential yield and farm yield in most countries although narrowing ranges between 26% to 69% with an average of 48% (Fisher et al., 2014). However, at regional level, especially in most developing countries of the CWANA region, there is still a huge yield gap (Figure 10).

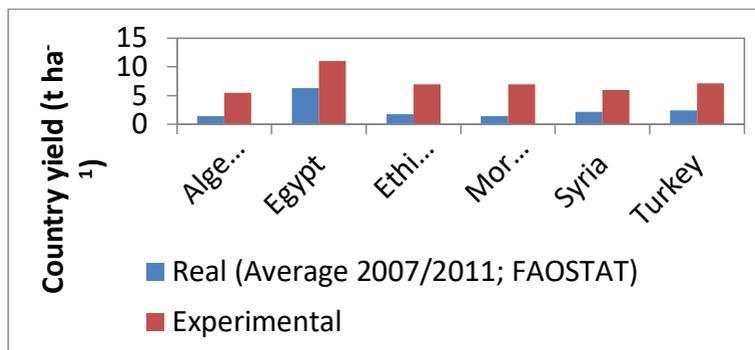


Figure 10: Yield gap analysis in selected countries

According to Pala et al. (2011), average yield gaps in Morocco ranged from 98-207% in rainfed areas and from 51-89% in irrigated areas. Similarly yield gaps of 82-125% and 61-201% have been reported in Syria and Turkey, respectively.

## **5. WHEAT BREEDING AT ICARDA**

### **5.1. History**

The International Center for Agricultural Research in the Dry Areas (ICARDA) was established at Aleppo, Syria, in 1977 to undertake multidisciplinary agricultural research in order to generate agricultural technologies for the non-tropical dry areas of the developing world. As it is located in the heart of the Fertile Crescent, where wheat originated, the Center started its wheat (spring bread wheat, facultative/winter bread wheat and durum wheat) improvement program since its inception, targeting the West Asia and North Africa (WANA) region, and more recently, including Central Asia and the Caucus countries (CAC), collectively known as the CWANA region. The spring bread wheat and durum wheat improvement program was managed as a joint activity between the International Center for the Improvement of Maize and Wheat (CIMMYT) as the CIMMYT/ICARDA wheat improvement program from the 1970's until 2003, with major emphasis on the semi-arid rain-fed environments of the CWANA region. In 2005 a joint program was re-established as the ICARDA/CIMMYT Wheat Improvement Program (ICWIP), with ICARDA taking the lead for the CWANA region. ICARDA then began to address the irrigated spring wheat areas through partnership initiatives with Iran in 2005 and with Egypt in 2009. ICARDA has had a facultative winter wheat program operating from Aleppo, Syria, since its establishment. In 1986, an International Winter Wheat Improvement Program (IWWIP) was established in Ankara, Turkey between CIMMYT and Turkey. The review of the winter wheat program in Turkey and Syria in 1990 recommended merging the two programs, which materialized in 1991. Since then IWWIP operates as a three-party entity: Turkey-CIMMYT-ICARDA (TCI) with breeding activities both at Syria and Turkey, but with one international nursery composition and distribution system. The international Winter Wheat Improvement Program (IWWIP) was reviewed in 2012 by a team of internationally renowned wheat scientists. The

review panel recommended the importance of better integration and coordination of the winter wheat improvement activities with the application of modern genomic and doubled haploid technologies, and joint research facilities. Starting from 2011, CIMMYT and ICARDA are implementing the WHEAT CGIAR Research Program (CRP3.1), which is part of a concerted effort of the CGIAR to implement a new, results-oriented strategy through a series of CRPs that fully exploit the potential of international agricultural research-for-development to enhance global food security and environmental sustainability. WHEAT draws on and empowers the capacities and commitment of the two leading international wheat research centers (CIMMYT and ICARDA), in partnership with farming communities, national research systems, advanced research institutes, private companies, policy makers, and diverse development organizations.

## **5.2. Breeding Objectives**

The general objective of the wheat breeding program at ICARDA is to enhance the productivity, yield stability, and end-use quality-based production systems in CWANA.

Specific objectives:

- Develop high yielding and disease and pest resistant wheat genotypes with acceptable grain quality for irrigated production systems of the region.
- Develop high yielding, drought tolerant, broadly adapted, disease and pest resistant wheat genotypes with good grain quality for the rain fed production systems.
- Identification of germplasm for heat and salt tolerance.
- Identification, mapping and pyramiding of major genes and QTLs for durable disease resistance and drought tolerance.

- Capacity building of NARS through long term and short-term training in wheat breeding/genetics.

### **5.3. Breeding Methods and Strategies**

The CWANA region is vast in geographical spread, and presents large variation in moisture regimes, temperatures extremes, soil types, cultural practices, and farming systems. This geographic spread in itself is indicative that the region harbours all kinds of wheat from spring to facultative to winter growth habits. The wheat breeding program at ICARDA applies both conventional and molecular breeding approaches and techniques in order to develop widely adapted and high yielding germplasm with resistance to abiotic and biotic stresses for the CWANA region and beyond. Some of these strategies and techniques include targeted crossing blocks, shuttle breeding, utilization of doubled haploids (DH) and marker assisted selection (MAS), key location yield trials, distribution of germplasm to NARS through international nurseries, and partnership & capacity building of NARS. As water is becoming scarce even in the irrigated areas of the CWANA region, ICARDA's germplasm development approach is to identify genotypes with disease resistance, high yield potential and water use efficiency so that wheat genotypes targeted for irrigated areas can cope with temporary drought periods. Similarly, this approach enables to minimize and maximize yield gains during drought and good seasons, respectively, for the rain fed production system. The General wheat breeding scheme and germplasm flow is indicated below (Figure 11).

## Breeding Methods and Germplasm Flow

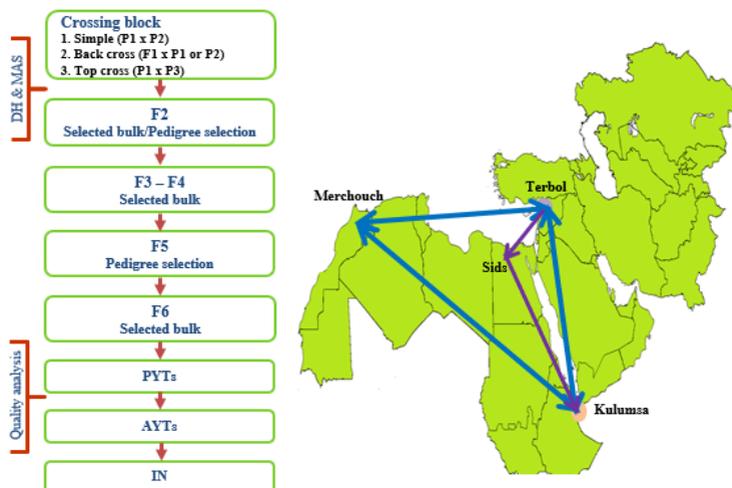


Figure 11: Breeding Methods and germplasm flow

### 5.3.1. Targeted Crossing Blocks

A crossing block is a collection of material, mostly elite, that will be used as parental lines in a breeding program. Selecting varieties that will set up a breeder's crossing block is probably the most important step of every breeding program. The proverb says the apple does not fall far from the tree. Accordingly, a breeder cannot expect excellent segregating populations from mediocre parental lines. Principally, high yielding and adapted CWANA hall mark wheat cultivars, synthetic wheats and elite lines from ICARDA are used as parents. Newly released varieties and other elite sources from the national programs are introduced and evaluated in observation nursery previously at Tel Hadya (Syria) and currently at Terbol (Lebanon) and Merchouch (Morocco) stations before fully composed into the crossing block. The wheat pre-breeding and pathology/entomology sections at ICARDA do also contribute germplasm for parental purposes. The parents need to be characterized for adaptation zones, quality, resistance to biotic and abiotic stresses, photoperiod sensitivity, Rht genes,

vernalization requirements, physiological traits, and other important traits both at phenotypic and molecular levels.

The dwarfing genes such as Rht1, Rht2, Rht3 and Rht8 can be used to reduce plant height and increase lodging tolerance. However, mostly it is Rht1 and Rht2 genes which contribute significantly to increase yield. The most commonly used dwarfing genes in the ICARDA's bread wheat breeding program are Rht1 or Rht2. These dwarfing genes also affect pleiotropically yield since they will help more tillers to survive and thereby increase biomass. Rht genes may increase yield through the increase of harvest index (HI). However, not all genotypes with high HI are high yielding indicating the presence of other factors such as photoperiod insensitive genes (Ppd1, Ppd2) which contributes to high yield. Ppd1 and Ppd2 genes have noticeable individual effects on flowering. The presence of only one of these genes results in an intermediate flowering effect. Together, the effects of these genes are great, making wheat mature very early. Let us consider two classes of maturity: early (120 days) and intermediate (140 days). The best combinations are either Rht1 + Ppd1, Rht1 + Ppd2, Rht2 + Ppd1 and Rht2 + Ppd2. When both dominant alleles of photoperiod insensitivity are combined, yields are generally low. Most current high-yielding lines have only one Ppd gene and either Rht1 or Rht2. The Ppd gene establishes a proper balance between the vegetative phase and the reproductive phase, including the grain filling period. Without this optimum balance, the source-sink relationship is somehow biased, and the plant's resources are not proportioned properly to produce high yield. Utilization of the winter wheat gene pool through a spring x winter wheat crosses is very important to develop widely adapted and high yielding genotypes as witnessed through the veery crosses. It is evident that 1B/1R translocated segment from rye present in Veery and derived from Russian winter wheat cultivar 'Kavkaz' has contributed significantly to the high yield potential and wide adaptation of the of the wheat varieties resulted from the very crosses. The Veery

crosses develop vigorous populations, robust plants, healthy stay-green leaves, many spikes/m<sup>2</sup> and/or bigger spikes to produce a plant type that could be called a Veery ideotype. Lines developed from such crosses are also very good in drought and heat tolerance and in improved nutrient (N and P) use efficiencies. Primary synthetics have been used extensively for the fact that they tend to be better adapted than modern cultivars to extreme abiotic stress conditions (Trethowan and Van Ginkel 2009). The potential of the wild relatives to provide new sources of adaptation, yield potential and disease resistance to modern wheat varieties is confirmed by the high proportion of wild relative's genes in modern wheat backgrounds. About 20% of the new CIMMYT and up to 24% of ICARDA material include synthetic background (Ogbonnaya et al., 2013).

The CB is planted in plastic houses in plots of 1m length with 2 rows of 0.2m apart each at 0.4m spacing between plots. Under field condition, the CB is planted in plots of 2.5m length with 6 rows of 20cm apart and 40 cm spacing between plots. Usually, three planting dates are carried out in the field to capture the variability in heading dates and to increase success as it will be difficult to undertake all the planned crosses in a single planting date. The F1 is planted in the field using plots of 1m length with 2 rows of 0.2m apart each at 0.4m spacing between plots in one planting date as there will not be enough F0 seeds to undertake 2-3 planting dates. Vernalization is required for the facultative/winter wheat genotypes. And hence we germinate seeds in petridishes in refrigerator at 4oc for minimum of 4 weeks before transplanting into the plastic house. Under field condition, there is no problem as both Tel Hadya and Terbol environments are conducive to grow both winter and spring wheat using the November/December planting dates.

Simple, top and back crosses are carried out in our wheat breeding program. The simple crosses are mostly spring x spring; spring x winter; and winter x winter crosses. The top crosses are crosses of the F1 with another parent to increase the genetic diversity. The

backcrosses on the other hand are crosses of the F1 with either of the parents (recurrent parent) to increase the frequency of the desirable alleles. Mostly a single/limited backcross is carried out. In most of the cases, the spring x winter F1 cross is either top crossed/backcrossed to a spring wheat if it is targeted to spring wheat environments and to winter wheat genotype if it is targeted to facultative/winter wheat environments. The simple crosses are carried out both in the plastic house and under field conditions while the top crosses and back crosses are carried out under field conditions.

The crosses need to be managed under optimum condition both under plastic house and field conditions. Under field conditions the F1 fields are surrounded by a rust susceptible spreader row and inoculated artificially with mixture of yellow rust races. Weak and susceptible F1 crosses are automatically discarded while vigorous F1 crosses with desirable traits but susceptible to yellow rust could be backcrossed/top crossed to other resistant parents. For ICARDA's spring bread wheat breeding program, 2000 crosses (simple, top and back crosses) are made at Terbol station and about 1000 crosses are made at Guich in Rabat, Morocco, on annual basis.

To assemble the crossing block (CB), the following points are worse to consider.

- Target regions and objectives
- Number of individuals and target traits in each crossing block
- Number of planting dates and management of crossing blocks
- Location of crossing block: In the field or plastic-house or both
- The type of crosses to be made: simple; double; top or back crosses
- The number of spikes to be pollinated per cross depending of the type of crosses

- Future resource demands for evaluation of the subsequent generations

### 5.3.2. Making the crosses

Hybridization or making crosses involves two major steps: Emasculation and Pollination (Figure 12). Emasculation is the removal of the three anthers at green stage in each floret from the spike of the wheat plant without damaging the stigma. It involves some important steps: Selection of the plant and the spike, removal of the central florets, cutting of the glumes, lemma, and palea just above the top of the stigma using scissors, removal of the three anthers from each floret with the forceps, and labelling of tags and bagging of the emasculated spikes. For proper bagging, fold the bottom edge of the bag over and staple or place a paper clip on fold so that it is tight against the peduncle or stem to prevent wind from blowing off the bag. It is also important to keep record of which plants have been emasculated in your crossing block book. In general, emasculation is very tedious process and an experienced breeder/technician can emasculate 10-15 spikes/hour.

On the other hand, pollination is the dusting of pollen from the selected spikes into the stigma of the emasculated spike after 3-4 days when the stigma is feathery and receptive. Pollination is much faster than emasculation.

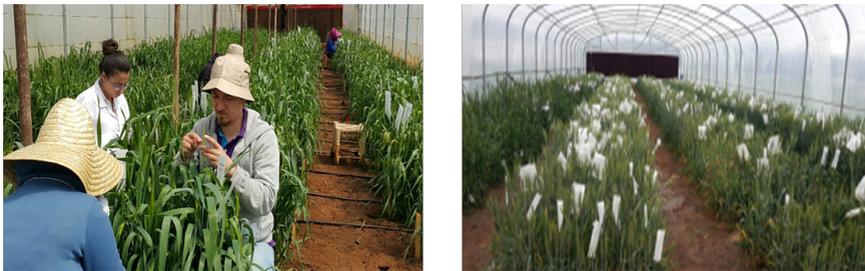


Figure 12: Crossing in plastic-houses at Terbol (Lebanon), and Guich, Rabat.

It involves identification of the right stage of the spike for pollination; pulling of the spikes from the stem, fastening of the spikes together with a rubber band and tag labelling with the male parent plot number. Place them all in a bottle with water. Cut the lemma and palea just above the anthers and clip the spikes for each female together and place the peduncles in the ground in a sunny place protected from the wind to enable anther extrusion. As soon as we see the extrusion of anthers, we need to cut the top of the glysine bag from the female plot, and then twirl the corresponding male spike (pollen source) around the female several times till it completely dusts off its pollen. In general, one male spike is enough to pollinate the female with just two or three good anthers extruded. After pollination, close the bag and place the tag under the paper clip, at the bottom of the glassine bag with side showing dates of emasculation and pollination out.

For crossing purpose, breeders need small, sharp, fine quality scissors; forceps or pincers with somewhat blunted ends; 3x5 cm tags with an attached string; 5x15 cm glassine bags; a wax pencil; a permanent marker; staples; paper clips; rubber bands; and a crossing block book. The crossing block book contains the list and pedigree of the CB genotypes, date of planting, date of emasculation and date of pollination and number of seeds harvested per cross.

#### 5.3.2.1. Recording Pedigrees

Pedigrees provide the parentage or the sequence through which a cultivar was obtained and are important sources of information. The pedigree for each line is generally included in the field books, crossing books, and on crossing tags. At present, ICARDA & CIMMYT use the United States Department of Agriculture (USDA) system of designating pedigrees. This system is widely used around the world, making it easier to trade data. This system consists of just a few basic rules:

1. The female parent is written first and is separated from the male parent by:

a. A single slash “/” in the first cross. Example: Cham6/Dashen

b. A double slash “//” in the second cross. Example: Cham6/Dashen//Imam/Hubara

c. Two slashes with the number of the cross between them: /3/, /4/, /5/, etc... in the subsequent crosses. Example: Cham6/Dashen//Imam/3/KBG-01/Attila-7//Qafza/Atlas

2. The parental material involved in any cross includes all that is listed on either side of the highest number of crosses in the pedigree. In the example indicated above in “c”, the pedigree of parent 1 = CHAM6/DASHEN //IMAM; and the pedigree of parent 2 = KBG-01/ATTILA-7//QAFZA/ ATLAS.

3. Simple and top crosses are formatted as A/B, and A/B//C, respectively.

4. Backcrosses are indicated with an asterisk (\*) and a number indicating the number of times the recurrent parent was used. The asterisk and the number are placed next to the crossing symbol that divides the recurrent parent and donor parents. Examples of pedigree formats for backcrosses are indicated below.

CHAM-6\*2/DASHEN; CHAM-6 is the recurrent parent

CHAM-6/3\*DASHEN; DASHEN is the recurrent parent

KBG-01/ATTILA-7\*2//QAFZA/ATLAS; KBG-01/ATTILA-7 is the recurrent parent

CHAM-6/DASHEN //4\*MISIR-1; MISIR-1 is the recurrent parent

#### 5.3.2.2. Selection History

Every F<sub>1</sub>, segregating line or advanced line in the program is assigned a so-called breeder’s cross ID (BCID) and a selection history. These codes record the process of selection, which

describes where and how the initial cross was made and where and how subsequent selection took place for each generation selection.

Each BCID begins with a letter designation of the cross origin (e.g. ISBW = ICARD Spring Bread Wheat). This is followed by an indication of the kind of cross (e.g. SS = spring x spring wheat; SW= spring x winter wheat), the abbreviation of the year when the cross was made (e.g. 00 = 2000), and the location (e.g. AP= Aleppo; TR= Terbol; MR = Merchouch) and finally a sequential number representing the order in which the cross was made within the crossing cycle (e.g. 01). The BCID is followed by the selection history; where the numbers indicate the individual plant selected and the letter indicates the location where the selection took place and/or the condition under which the selection has taken place. The zero-letter combinations (e.g. 0TR, 0AP, 0SD, 0KU, 0MR etc.) indicates that the entire plot was cut, and bulk harvested and threshed in that generation at the specified location. On the other hand, a zero followed by a number and then by letters (e.g. 010TR; 020SD, 030KU, 040MR etc.) indicates the number of spikes selected and harvested in bulk.

Example: ISBW12-TR-1120-0SD-0KU-0DZ-5TR-0TR indicates the cross is originated from ISBW (ICARDA Spring Bread Wheat); the cross was made at Terbol in 2012 with a cross ID of 1120. The F<sub>2</sub> population was bulk harvested at Sids station, and the F<sub>3</sub> and F<sub>4</sub> populations were bulk harvested at Kulumsa and Debrezeit, respectively. The F<sub>5</sub> population was grown at Terbol, and plant number 5 was selected and harvested individually which was then grown as plant/head rows in the F<sub>6</sub> at Terbol and bulk harvested. It is important to maintain the pedigree information along with the selection history. Automated generation of F<sub>1</sub> crosses and F<sub>1+n</sub> crosses has been designed using excel macro program (Shaama and Tadesse, 2012, 2017). This program is available at and can be used by any breeder freely (<http://geoagro.icarda.org/flcross/>).

### 5.3.3. Breeding schemes following crosses

Following successful crosses, the  $F_1$  through  $F_{1+n}$  generations, can be evaluated using different selection schemes such as pedigree, single seed descent (SSD), modified pedigree-bulk, and selected bulk methods (Figure 13).

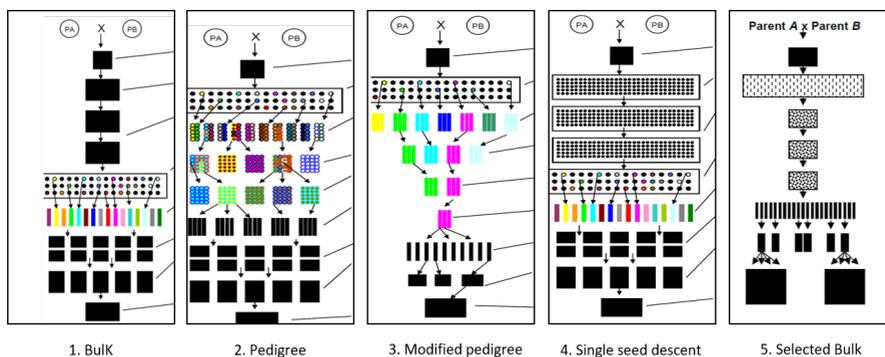


Figure 13: Selection methods in wheat breeding (Adapted from J.M Poehlman, 1987)

At ICARDA, the pedigree and modified pedigree methods have been used in the past. However, individual plant selection in the  $F_2$  in both methods lead to big number of  $F_3$  plots which becomes resource intensive. Currently, as indicated in Figure 13, segregating populations from  $F_2$  to  $F_4$  are selected using the selected-bulk method, whereby on average 1500-2000  $F_2$  plants are space-planted on a plot of 8 rows of 10m length and individual plants are selected and bulk harvested, and then planted in the  $F_3$ - $F_4$  as entries by planting 40 gm seed from the bulk harvest in a plot of 4 rows with 2.5m length at 0.2m spacing between rows. In the  $F_5$ , 5-10 selected plants or heads are harvested separately, and grown in the  $F_6$  as head-rows and with selected plants harvested in bulk. Head-rows could be made from  $F_4$  populations. It all depends on how the population is looking stable and uniform in terms of segregation. This being the general scheme, practically modifications on plot sizes, population size, number of populations and number of generations to advance are always there depending on the locations, crosses, and resource availability. Susceptible and weak

populations are discarded at the  $F_1$ ,  $F_2$  and  $F_3$  stages. Summer and winter season nurseries are carried out at Terbol, Kulumsa, and Merchouch stations. The winter x spring crosses at Terbol during the winter cycle will be advanced to  $F_1$  in the summer cycle, and the harvested  $F_1$  seeds will be shipped to Sids (Egypt) to be grown as  $F_2$  populations. The selected  $F_2$  populations are harvested in April and sent to Kulumsa (Ethiopia) to be grown as  $F_3$  populations in the summer (July-October). The selected  $F_3$  populations are bulk harvested and sent to Merchouch (Morocco) to be grown as  $F_4$  populations during the winter cycle. From the selected populations, 5-10 spikes are harvested individually and planted in the next winter season as head-rows. Such a multiple shuttle approach enables the identification of disease resistant, photoperiod insensitive, high-yielding, and widely adapted genotypes. It also shortens the breeding cycle by half, as two generations can be grown per year. In the early days of spring wheat breeding at ICARDA, the pedigree selection method was used almost exclusively. In this method individual plants are selected at each segregating generation and promoted as individual entries. This has been the most widely used method for wheat breeding worldwide and has some advantages such as the possibility of using previous years plant-based data in selection. However, because of the enormous number of entries to handle, record keeping, and nursery preparation becomes vary laborious. In general, pedigree or modified pedigree or selected bulk selection schemes are still applied in most breeding programs. In addition to cost factor, the heritability of the desired traits and the population size are important points to be considered while choosing the selection method.

#### 5.3.4. Doubled Haploid (DH)

In vitro haploid production followed by chromosome doubling reduces the time needed for the production of completely homozygous lines in a single generation and increases the precision and efficiency of the selection process in wheat breeding (Tadesse et al 2013). It also facilitates the detection of linkage and gene interactions, estimation of genetic variance

and the number of genes for quantitative characteristics, produces genetic translocations, substitutions and chromosome addition lines, and facilitates genetic transformation and mutation studies. Wheat varieties developed from doubled haploids using anther-culture or maize induction systems have been released for cultivation in both developed and developing countries. At ICARDA, DH lines are produced using anther-isolated microspore culture (Figure 14). Between 1000 and 2000 DH lines are produced each year. We undertake DH production from F<sub>1</sub> plants originating from elite x elite crosses instead of F<sub>2</sub> plants to increase the gain in time rather than the increase in genetic gain, which could be obtained by having one more generation of recombination. This is specifically important for winter wheat as only one field cycle of generation advancement per year is possible compared to two generations per year for spring wheat using shuttle breeding under diverse irrigated and rain-fed schemes in winter and summer seasons or using the Single Seed Descent method (SSD) under greenhouse conditions.

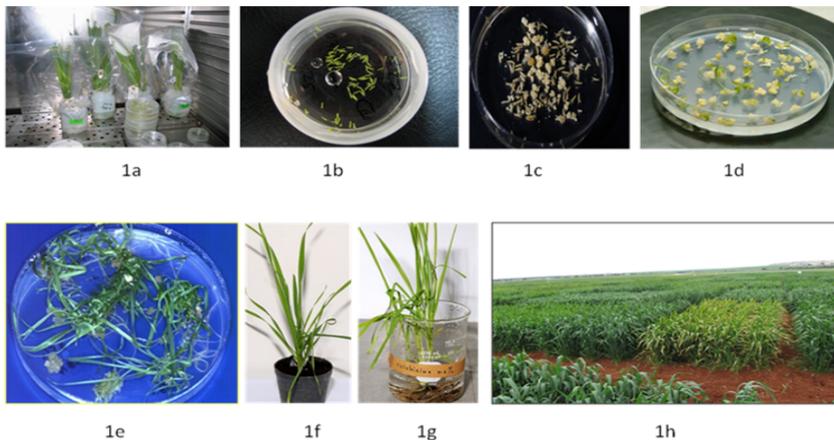


Figure 14: Procedures of anther-culture for doubled haploid wheat production. 1a = pre-treatment of the donor plants at 40C; 1b = anthers in liquid induction medium; 1c = developing of embryos in liquid induction medium; 1d=embryo converting to green on solid regeneration medium; 1e= green plants in regeneration medium; 1f= haploid plants at acclimatization stage; 1g= haploid plants under Colchicine treatment (0.2%); 1h= doubled haploid lines in the field , showing uniformity within lines (Tadesse et al 2014).

The ICARDA bread wheat program, follows MAS-F<sub>2</sub>-derived DH production strategy to increase genetic gain. Every year the genotypes in the crossing block are genotyped for main functional markers for adaptation, phenology, biotic and abiotic stress resistance and end-use quality. High-yielding parents with contrasting combinations of the markers are selected for crossing at the ICARDA research station in Terbol (Lebanon) and F<sub>2</sub> plants derived from the crosses are genotyped for the segregating markers. The number of F<sub>2</sub> plants genotyped per cross will depend on the number of markers segregating. The F<sub>2</sub> plants carrying the most positive alleles are then selected for DH production. Additionally, one spike per selected plant is used for recurrent crossing for further gene pyramiding (Figure 15).

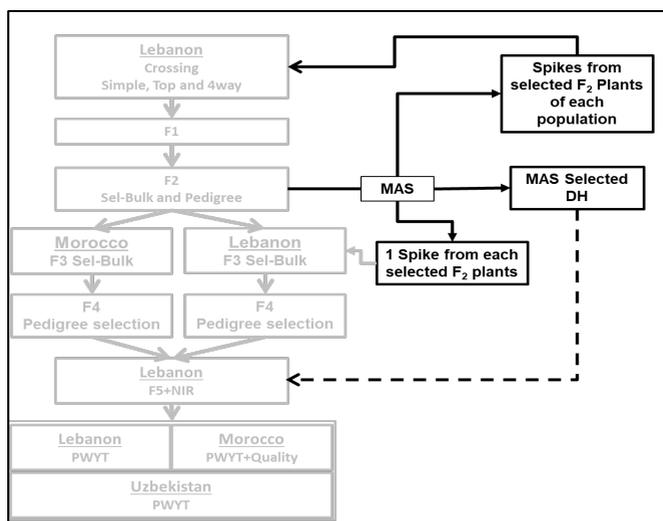


Figure 15: Diagram of the use of MAS-DH at the ICARDA-IWWIP Facultative and Winter Wheat breeding hub

After crossing, it generally takes at least six generations before a sufficiently homozygous population is obtained to undertake screening and preliminary yield trials at the F<sub>8</sub> generation. In the case of shuttle breeding where the nurseries are evaluated in winter and summer seasons, the time required to obtain fixed lines for preliminary yield trials evaluation

across locations could be achieved in a matter of four years from the F1 (Figure 16; Tadesse et al 2016). In addition to the cycle shortening, additional advantages of shuttle breeding involve additional recombination events, selection of transgressive segregants and selection for resistance against biotic and abiotic stresses with broad adaptation. However, such shuttle breeding under field conditions is possible only for spring bread wheat whereas for winter wheat it requires a previous step of vernalization which in field conditions can only be achieved in the winter season. The use of doubled haploid in winter wheat can reduce the breeding cycle by 37.5% (from 8 to 5 years) for F1 derived DH and by 25% (from 8 to 6 years) in F2 derived strategies. The advantage of F2-derived DH relies in the potential genetic gain that could be obtained by having one extra generation of recombination and the possibility of applying selection at F2 plant level. DH production from F1 helps shortening the cycle length at the expense of selection accuracy. Thus, in this strategy no selection can be applied and the resulting DH expresses the full recombination spectra between the parents.

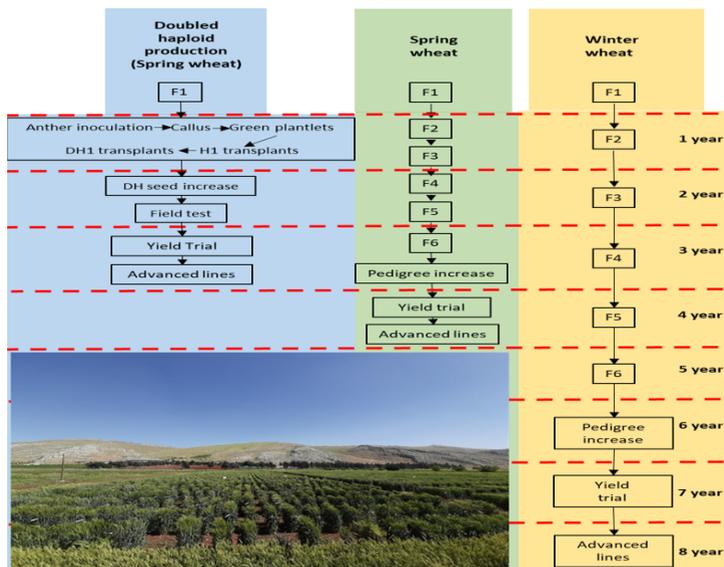


Figure 16: Comparison of conventional and anther culture breeding methods: Adapted from Li et al. (2013)

DH is similar to SSD method in that both methods provide rapid generation advancement for producing homozygous lines (Grafius 1965). However, in the DH breeding there is only one opportunity for recombination if F1 plants are used as donors – while in SSD, recombination can occur in every generation of inbreeding (Grafius 1965).

#### 5.3.5. Marker Assisted Selection (MAS)

The wheat breeding program at ICARDA uses physiological and molecular screening techniques in order to increase rates of genetic gain through (a) strategic trait-based crossing to combine complementary traits in the progeny, (b) high-throughput phenotyping to identify and enrich desirable alleles in intermediate generations and (c) exploration of genetic resources to broaden the genetic base for hybridization (Reynolds and Tuberosa, 2008). MAS using recommended diagnostic markers are used to characterize new parental materials for disease resistance genes (stripe rust, leaf rust, stem rust, nematodes); insect resistance (Hessian fly and Russian Wheat Aphid), phenological traits such as photoperiodism (Ppd), vernalization requirement (Vrn); plant height (Rht), grain hardness and other desired genes. Diagnostic markers are also used for gene pyramiding in the F2, F1top, and BC1F1 populations (William et al., 2007).

Doubled haploid technology has great potential to improve marker-assisted selection efficiency for gene combination. Thus, besides the gain in cycle shortening, MAS coupled with DH production can increase gene combination efficiency from 6 to 17.7 times when combining 2-8 markers respectively as compared to F4 derived lines obtained through conventional breeding (Fig. 17; Howes et al., 1998). This is due to the only partial homozygosity of F4 plants (93.75%) as compared to DH plants and the need for testing more plants derived from the selected F4 to confirm that the markers are fixed in the genotype.

Additional breeding strategies combining DH and MAS can be followed to optimize gene pyramiding. For instance, MAS-F2-derived DH can dramatically increase the efficiency of gene pyramiding. For instance, MAS-F2-derived DH can dramatically increase the efficiency of gene pyramiding. Although this strategy extends the cycle by one generation (from F1 derived to F2 derived DH), it requires less DH production and individuals subjected to MAS to produce and identify the lines carrying the markers to be combined especially when the number of markers to combine is high (Figure 17, Howes et al., 1998). In this strategy, marker assisted selection is applied at F2 level and only F2 plants carrying all alleles of interest (both in homozygosis or heterozygosis) are selected to produce DH. As a result, if the objective is to combine 8 markers in a single individual, following this strategy 1 every 50 DH plants would carry all 8 alleles of interest whilst in a pure MAS-DH approach only 1 every 256 lines would. Additional breeding strategies for gene pyramiding such as DH production from a recombinant obtained from the cross of two F2 plants selected through MAS (Recombinant F2 Selections DH production) or from two DH plants selected through MAS (Recurrent DH selection) can also increase dramatically pyramiding efficiency at the expense of cycle extension (Howes et al., 1998).

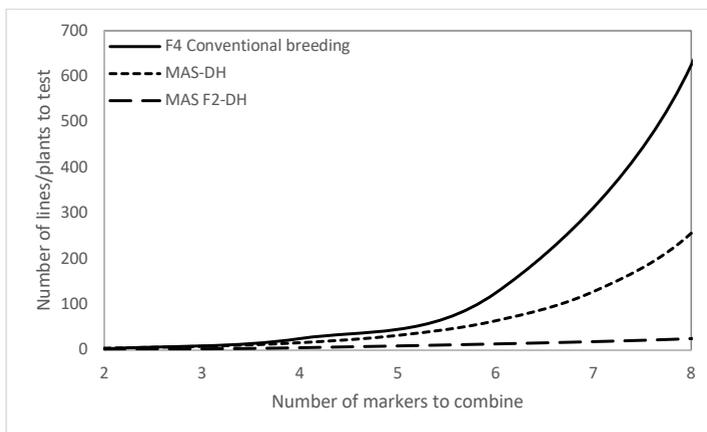


Figure 17: Number of genotypes needed to be tested to identify one that combines  $n$  markers in one single line according to the breeding strategy; adapted from Howes et al., (1998).

### 5.3.6. Modified speed breeding

Speed breeding' technology is the production of up to six generations of wheat per year involving extended duration of light and early harvesting of wheat under glasshouse conditions. This enables effectively to shorten the breeding cycle through acceleration of generation advancement. However, it might be limited in its scope to screen the population for different biotic and abiotic stresses in addition to the difficulty of handling of large number of populations required at F<sub>2</sub> stage. At ICARDA, we have developed a modified speed breeding for elite x elite crosses whereby we made crosses in the plastic house and harvested in March then the F<sub>1</sub> is planted in the greenhouse in pots and harvested in June. The F<sub>2</sub> populations are planted at Merchouch in July using irrigation and harvested end of October. The F<sub>3</sub> population is planted in December and selected spikes are harvested individually in May to be planted as head rows in July at Merchouch. The selected head-rows will be harvested in October and planted as PYT in December across locations. Selected PYTs (about 700) will be harvested in May/June and planted as advanced Yield Trials (AYTs) across locations in alpha lattice design using two replications. At the same time the, AWYTs are planted in plots of 20 m length x 6 rows of 0.2m apart each for seed multiplication for distribution of international nurseries in August/September. Using this scheme, it takes a total of 4 years from crossing to the distribution of international nurseries. Using doubled haploids and shuttle breeding, it takes minimum of 5 years from crossing to the distribution of international nurseries as indicated in figure 18. It should be noted that in this modified speed breeding scheme, we are using elite x elite crosses, selection of spikes for head-rows is carried out in the F<sub>3</sub> and it requires rouging at the head row, PYT and AYT stages. This method is rapid, efficient and enables to attain fast genetic gain.

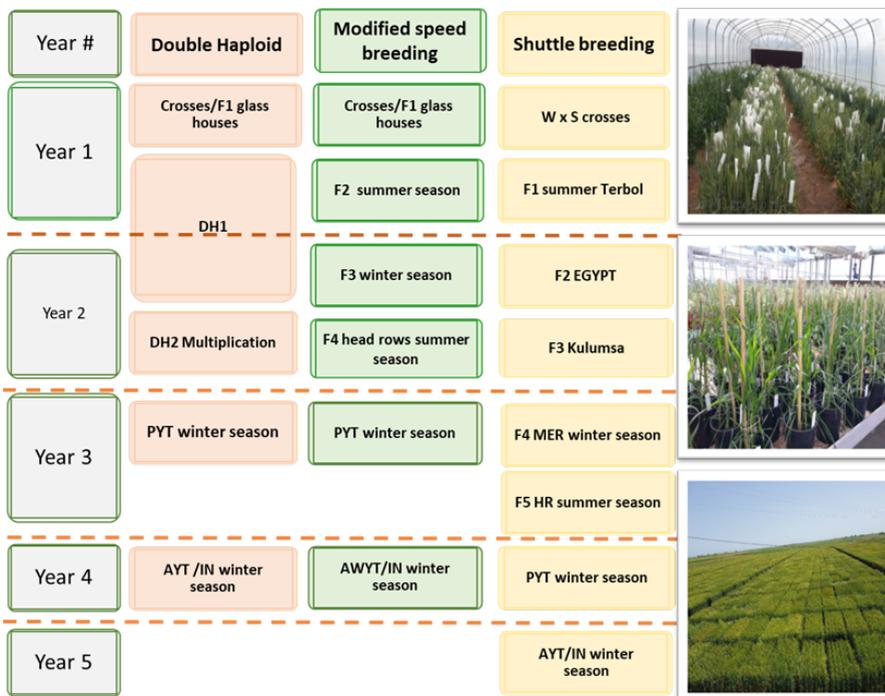


Figure 18: Comparison of doubled haploid, modified speed breeding and shuttle breeding schemes at ICARDA

### 5.3.7. Genomic selection (GS)

GS is the utilization of genome-wide marker data to predict and select for better multi-genetic traits early in the breeding cycle in order to increase genetic gain by reducing the breeding cycle while increasing breeding efficiency and reducing costs. For the GS to be implemented in any breeding program, the following three populations are required.

1. The training population (TP): This is a diverse set of population which is phenotyped for different traits and genotyped across the entire genome. The phenotypic and genotypic data of the TP is used to train the model and used to predict the performance of other non-phenotyped but genotyped populations.

2. The breeding population (BP): This is the set of breeding lines which are not phenotyped but genotyped, and hence their performance is predicted using the model developed by using the training population. The model estimates the genomic estimated breeding value (GEBV) for each of the BP lines using their genotypic data by undertaking allelic identity test with the loci that were correlated with the phenotypic data of the training population. BP lines with intensive phenotypic and genotypic data could be utilized to effectively re-train and calibre the model. The important question from practical point of view is at which stage of the BP (F2-F6, or Preliminary yield trial or advanced yield trials stage) should we apply GS for maximum genetic gain and resource use efficiency? It is possible to apply at any stage and promote only those selected BP with the highest GEBV. The most plausible approach is to integrate GS with doubled haploid breeding. In this case DH lines could be produced from F1 or F2 and the DH lines will be the BP which are genotyped but not phenotyped. The model predicts the GEBV of each DH (BP) lines and those with the highest GEBV are used as parents, and for yield trials across locations. With their phenotypic and genotypic data these DH lines serve also to retrain and re-calibrate the model. The integration of GS with DH is especially practical and effective for winter wheat breeding programs. For the spring bread wheat breeding program, it is possible to use the shuttle breeding and modified speed breeding program to advance the generation cycle and hence GS can be applied at preliminary yield trials or 1<sup>st</sup> year advanced yield trials stage and predict the performance of the lines across locations based on their corresponding GEBV.

3. Validation population (VP): This set of lines are both phenotyped and genotyped. Using these two data sets, the GEBV is determined for each of the VP lines. Correlation analysis is carried out between the GEBV and the actual phenotypic values of the VP lines to validate the efficiency of the model for genomic selection.

4. The expected genetic gain through time by the implementation of GS is defined as:  $\Delta G = ir\sigma_A/T$  where  $I$  is the selection intensity,  $r$  is the selection accuracy,  $\sigma_A$  is the square root of the additive genetic variance, and  $T$  is the length of time to complete one breeding cycle (Falconer and Mackay, 1996). GS can be advantageous over PS for those traits such as grain quality, insect resistance and predict the performance of genotypes in other locations which are not easily accessible to undertake trials. The cost-benefit analysis of GS over phenotypic selection varies across breeding programs in different countries and the correlation of the GEBV with the field-based selections, and accordingly the decision to apply GS should be made by the respective programs. However, it should be noted that GS is not a replacement over PS. Currently, the cost of genotyping become cheaper, and it is attractive to include the GEBV as one along with agronomic data and breeders scores across locations to increase breeding efficiency and increase genetic gains.

#### 5.3.8. Exploring Hybrid wheat

In the past, hybrid wheat production was discouraged for its poor economic return, because of very limited heterotic advantage (10%); lack of clear advantage in terms of agronomic, disease and quality traits; expensive seed production costs; and due to the argument that yield increment can be obtained using conventional/line varieties and consequently hybrids would have no biological advantage over inbred lines (Pickett and Galwey, 91). However, with time, it became apparent that hybrid wheats can provide higher and stable grain yield, better genetic gains, higher thousand grain weight, more tillers, higher biomass, deeper roots and better resistance to both biotic and abiotic stresses as compared to their parents. Hybrid wheats can be produced using the chemical hybridizing agent, cytoplasmic male sterility and biotechnological systems.

Currently, hybrid wheat is predominantly produced in Europe using the chemical hybridization agent (CHA) CROISOR® 100 (Hybrid wheat, 2013). The CHA system involves planting of the maternal line in strips alternating with the pollen donor (male) parent line. Only the maternal lines are sprayed at booting stage. Paternal lines with excellent anther extrusion are highly preferred for successful pollination and production of F<sub>1</sub> seeds. Genotype, environment, time of application and their interaction can influence the effectiveness of the CHA though in general CHA functions for a broad spectrum of genotypes with relatively very low toxicity in wheat. The Cytoplasmic Male Sterility (CMS) method is based on the application of three parent systems involving the A, B, and R parents where A and B are identical except that B is not cytoplasmic sterile and hence it is used to maintain the A parent which is cytoplasmic sterile. R is the fertility restorer parent and the F<sub>1</sub> is produced by crossing A with R parent ( $A \times R = F_1$ ). This system is used in USA, China, India and Australia. It is also anticipated that the application of biotechnological methods will help capture increased heterosis by direct selection of epistatic alleles and the development of new genetically based systems to control male sterility such as the recessive split-gene transgene system which utilizes complimentary fragments of barnase to induce male-sterility in maternal plants while retaining pollen fertility and enables production of F<sub>1</sub> hybrid seeds. Hybrid wheats are expected to adapt more on marginal areas and produce more stable and higher yields as compared to the conventional varieties. Currently, Bayer, Syngenta and Dow DuPont are working to develop their respective hybrid wheat varieties to Europe, USA, and Australian markets (<https://www.futurefarming.com>).

In all the systems, it is important to predict hybrid performance based on general combining ability (GCA) and specific combining ability (SCA). Recent studies have indicated that application of genomic selection has a great potential in predicting hybrid performance. We hope that further identification of a stable and effective CHA, development of heterotic pools,

and integration of molecular markers and genomic selection in the hybrid wheat breeding program will enable develop high yielding, stable and profitable wheat hybrids by improving the system efficiency, increasing heterosis, and reduction of the cost of hybrid seed production.

#### **5.4. Key Location Yield Trials**

A total of 2000-3000 F7 generation genotypes selected from the F6 nursery are tested annually in Preliminary Yield Trials (PYT) using non-replicated augmented designs at the Terbol and Kheferdan (Lebanon), Sids (Egypt), Merchouch (Morocco), Wadmedani (Sudan) and Kulumsa (Ethiopia) research stations (Figure 19, Tables 5 & 6). The Tel Hadya and Breda sites in Syria were also used up until 2012, and then had to be abandoned due to security concerns. On an annual basis, about 400-500 lines are selected from the PYTs based on yield potential data from the Sids and Terbol stations, disease (rusts, fusarium, septoria and tan spot) resistance data from the Kulumsa station, heat tolerance data from the Wadmedani, and Terbol and Marchouch (late planting) stations, drought tolerance data from Kheferdan and Marchouch stations, and insect resistance (Hessian fly and sun pest) data. These are then yield tested in Advanced Yield Trials (AYTs) using alpha-lattice designs with two replications. They are grown at the same locations as the PYT, both under irrigation and rain-fed conditions to screen for phenological traits, disease and insect pest resistance, yield and yield components, and grain quality traits in order to identify genotypes combining yield potential and wide adaptation with resistance to biotic and abiotic stresses.

**Marchouch + Jemaat Shaim:** Yellow rust, Septoria, drought tolerance, HF resistance

**Sids:** Yield potential

**Izmir:** Screening for rusts (Lab +field)

**Kulumsa /Dz:** Stem rust, yellow rust, septoria, fusarium

**Wadmedani:** Heat tolerance

**Terbol:** CB, adaptation, yield potential, rusts, cold, drought (at Kheferdan)



Figure 19: Key locations for ICARDA’s spring bread wheat breeding program

Table 5: Agro-ecology and other characteristics of the ICARDA’s key locations

| Location              | Altitude | Latitude / longitude | Min-Max Temp | Total rain fall | Soil type | PH      | ME                       |
|-----------------------|----------|----------------------|--------------|-----------------|-----------|---------|--------------------------|
| Terbol                | 900      | 34°N; 36° E          | -12 - 40.5   | 500-600         | nitosol   | 6.7     | Supplementary irrigation |
| Kulumsa               | 2200     | 08° N; 39° E         | 10-22.8      | 500-1000        | vertisol  | 5.5-6.5 | rainfed                  |
| Wadmedani             | 410      | 14°N;33° E           | 20-42        | 0               | vertisol  | 8.5     | irrigated                |
| Sids                  | 32.2     | 29°N;31° E           | 20-35        | 0               | alluvial  | 7.8     | irrigated                |
| Merchouch             | 430      | 33.6°N-6.7° W        | 10-35        | 350-500         | cambisol  | 6.5     | Rainfed (ME4A)           |
| Sidi Al-Aydi (Settat) | 406      | 33.6°N-7.6°W         | 10-40        | 300             | vertisol  | 7.5     | Rainfed (ME4A)           |

Table 6: Designs and number of genotypes across generations and trial stages

| Stage             | No. entries   | Plot size                      | No. locations | remarks                       |
|-------------------|---------------|--------------------------------|---------------|-------------------------------|
| parents           | 300-400       | 1m x 2rows                     | 2             | Non-replicated                |
| crosses           | 2000-3000     | 2-3 spikes/cross               | 2             | Non-replicated                |
| F1                | 1500-2500     | 1m x 1 row                     | 2             | Non-replicated                |
| F2                | 1500-2000     | 10m x 8 rows;<br>2.5m x 4 rows | 2             | Non-replicated                |
| F3                | 800-1200      | 2.5 x 4 rows                   | 2             | Non-replicated                |
| F4                | 500-700       | 2.5m x 4 rows                  | 2             | Non-replicated                |
| F5                | 10,000-20,000 | 1m x 1 row                     | 2             | Non-replicated                |
| PYT               | 1000-3000     | 2.5m x 2 rows                  | 3-5           | Augmented                     |
| 1st Year AYT's    | 500-900       | 2.5m x 6 rows                  | 2-3           | Replicated, alpha lattice     |
| 2nd year AWYT's * | 250-600       | m x 6 rows,                    | 2-3           | Replicated;<br>non replicated |
| Strip plot (IN)   |               | 10m x 6 rows                   | 1             |                               |
|                   |               | 20m x 6 rows                   | 1             | Non-replicated                |

\*The second year AWYT's could be optional. Instead, it is possible to undertake the first year AWYT's and the strip plot increase at the same year. This will enable to reduce the breeding cycle though the number of genotypes in the strip plot might increase relatively. However, the cost of doing so is minimum as compared to undertaking 2<sup>nd</sup> year AWYT's. It is to be noted that the materials from the international nursery will be used at the observation nursery

or preliminary yield trial stages by the national programs. Hence, it is important to shorten the breeding cycle at the CGIAR breeding program levels.

### **5.5. Field books, data collection, management and storage**

Breeding trials should be planted following standard designs and lay outs. The type of design, number of rows, total plot size and replication depends on the stage of the nurseries and trials. The spring bread wheat breeding program at ICARDA uses the stages and designs indicated in Table 6 above.

The field books for F1, Fn+1 with BCID, pedigrees and selection history are generated using the automated system in Macro, <http://geoagro.icarda.org/f1cross/> (El-Shamaa and Tadesse, 2017). Currently, we are also using BMS software to generate field books, design crosses, advance generations, and upload both phenotypic and molecular data sets. Efficient data management and analysis is one of the important components for a successful breeding program. As much as possible, automation of data collection and uploading systems will help to minimize error and increase efficiency. In this regard, it is important to collect data of most of the agronomic traits such as days to heading and maturity, canopy temperature, plant height, disease scores, agronomic notes etc using Tablets and upload to the BMS data base. Field plots can be barcoded to facilitate such operations.

For all the yield trials, we have developed a barcode program called GENO. Barcodes with the important information (trial name, variety number, replication, plot number, location) is printed and attached to each of the envelopes and distributed to each plots of the yield trials at the time of harvesting. The barcode scanner, scale, and the computer are connected as indicated below in figure 20.

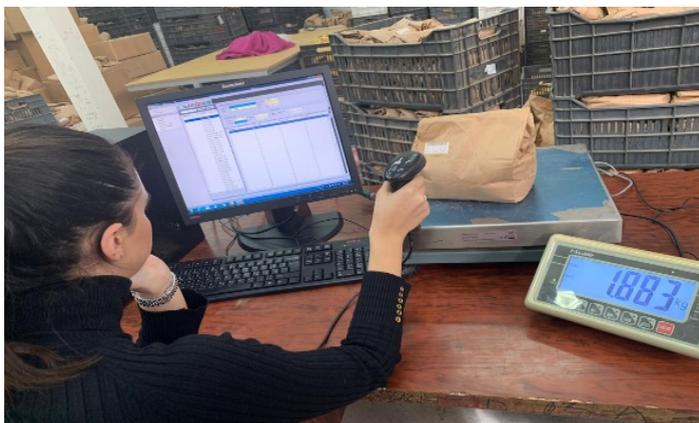


Figure 20: ICARDA's Geno program for automated yield measurement at Terbol station

A moderate size store is very important to undertake seed preparation, seed selection, and storage. It is advisable to store at least the copy of the materials (F1-F4 generations), selected head rows, PYTs, AWYTs, and elite genotypes for a season until the harvesting time. If not, you may lose all the materials in case of natural hazards. Genotypes at the last cycle of the breeding process (elite genotypes for international nursery) should be kept in long-term storage. At ICARDA, we do store 5-10 gm of such materials in the gene bank along with their passport data.

### 5.6. Data Analysis

Adaptation to the different target environments will determine the success of a variety in terms of farmer's adoption. From an agronomical point of view, yield is the most important phenotypic trait and is also the ultimate expression of a variety's adaptation. The total phenotypic variance ( $\sigma^2P$ ) of a variety is a function of the environment effect ( $\sigma^2E$ ), the genotypic effect ( $\sigma^2G$ ) and the differential phenotypic response of the genotypes to changes in the environment ( $\sigma^2GE$ ):

$$\sigma_p^2 = \sigma_G^2 + \sigma_E^2 + \sigma_{GE}^2$$

There are different statistical designs used to capture the variances of yield trials carried out in a location (s) across year (s). The important ones are randomized complete block designs (RCBD) and the incomplete block designs (Figure 21).

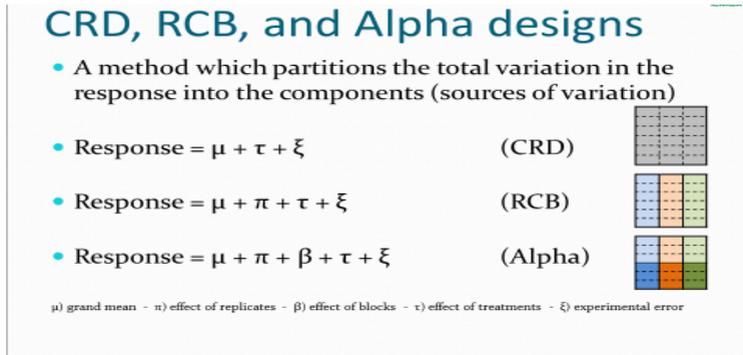


Figure 21: CRD, RCB and alpha designs

- Incomplete Block Designs are required:

When number of treatments in an experiment is large, implementing the experiment in RCB design would require large number of blocks and it is not practical to achieve homogeneity of experimental material within large blocks. As a consequence, intra block variance and CV increases and precision on treatment comparisons decreases.

- Resolvable Block Designs:

In this type of design, the general conditions are indicated below. The incomplete blocks could physically be arranged in such a way that the set of nearby incomplete blocks forms a complete replicate. Thus, in case the incomplete blocks (within a replicate) are not different in their effects, the replicate resembles a complete block of the RCB (except for randomization of the treatments).

One particular class of resolvable incomplete block designs recommended for variety trials is the Lattice Designs {Square Lattice; and Rectangular Lattice} where:

Number of varieties =  $s^2$  in square lattice; and number of varieties =  $s(s-1)$  in rectangular lattice. If the number of treatments to be assessed does not satisfy these conditions, then either some additional treatments have been added or some of the existing treatments have been deleted in practice.

- Alpha Designs

It is possible now to obtain a resolvable incomplete block designs, called an alpha design for any composite number of treatments and for a number of choices of block size. The Only restriction is that the block size must be a factor of number of treatments. In other words, it is possible to obtain an alpha design in  $v = s \times k$  treatments, where  $k$  denotes the block size and  $s$  is the number of blocks in each replication.

Example:

24 Genotype, 2 Rep, 4 Blocks (Block size is 6)

GenStat:

Stats → Design → Select Design → alpha designs → OK

| Rep | Plot  | 1  | 2  | 3  | 4  | 5  | 6  |
|-----|-------|----|----|----|----|----|----|
| 1   | Block |    |    |    |    |    |    |
|     | 1     | 2  | 21 | 24 | 14 | 12 | 17 |
|     | 2     | 7  | 10 | 11 | 16 | 4  | 18 |
|     | 3     | 1  | 20 | 6  | 22 | 5  | 13 |
| 2   | 4     | 19 | 3  | 23 | 15 | 8  | 9  |
|     | 1     | 3  | 4  | 17 | 24 | 20 | 13 |
|     | 2     | 10 | 8  | 11 | 19 | 6  | 2  |
|     | 3     | 16 | 18 | 5  | 23 | 14 | 12 |
|     | 4     | 9  | 22 | 21 | 1  | 15 | 7  |

## Analysis of Variance (ANOVA)

To undertake ANOVA using Genstat software, follow the following.

GenStat → Stats → Analysis of Variance → General; For CRD Design, use one-way ANOVA (no Blocking). The treatment in this design is only Entries/Genotypes. For RCBD we use one-way ANOVA (in Randomized Blocks). The factors are treatments and replications (blocks). In the case of Alpha design, the treatment structure is unbalanced with entries/treatment, and blocks (rep/block) (Table 7).

Table 7: ANOVA under CRD, RCBD and Alpha design

| <b>ANOVA (under CRD)</b>    |             |             |             |             |              |
|-----------------------------|-------------|-------------|-------------|-------------|--------------|
| <b>Source of variation</b>  | <b>d.f*</b> | <b>s.s*</b> | <b>m.s*</b> | <b>v.r*</b> | <b>F pr*</b> |
| <b>ENTRY</b>                | 24          | 474903      | 19788       | 2.8         | 0.001        |
| <b>Residual</b>             | 50          | 353236      | 7065        |             |              |
| <b>Total</b>                | 74          | 828140      |             |             |              |
| <b>ANOVA (under RCB)</b>    |             |             |             |             |              |
| <b>REP stratum</b>          | 2           | 78583       | 39292       | 6.87        |              |
| <b>REP. *Units* stratum</b> |             |             |             |             |              |
| <b>ENTRY</b>                | 24          | 474903      | 19788       | 3.46        | <.001        |
| <b>Residual</b>             | 48          | 274653      | 5722        |             |              |
| <b>Total</b>                | 74          | 828140      |             |             |              |
| <b>ANOVA (under Alpha)</b>  |             |             |             |             |              |
| <b>+ REP</b>                | 2           | 78583       | 39292       | 11.36       | <.001        |
| <b>+ REP. Block</b>         | 12          | 262974      | 21915       | 6.34        | <.001        |
| <b>+ ENTRY</b>              | 24          | 362117      | 15088       |             | <.001        |
| <b>Residual</b>             | 36          | 124466      | 3457        |             |              |
| <b>Total</b>                | 74          | 828140      | 11191       |             |              |

\*df= degrees of freedom; ss= sum of squares; ms = mean of squares; v.r= variance; F pr= Probability

GenStat software is a very handy software to analyse data from RCB, RCBD and alphatic designs. The software has many features for graphics and generation of randomizations and replications. However, it is unable to generate automatically randomizations and replications when the number of treatments is more than 100. In such instances, use cyclic design software. Another limitation is that if the number of blocks per replicate is less than number of plots per block, then design can have only 2 replicates. To overcome this, you can generate

2 randomizations of 2 replicates, then select 3 out of possible 4 replicates you have just generated. In Genstat, **You CAN**:

1. Use same land and same materials to get more precision
2. Use GenStat to generate and analysis Alpha designs
3. Analysis your data using simple ANOVA and ignore block information

But you **CANNOT**

1. Add blocks information in analysis step if you feel that your SED is high!
2. Generate design using simple randomization, say for an RCB case, and then add blocks!
3. Lose precision in Alpha design, worst case come when blocks have no effects and your replicates are homogeneous (RCB case)

## **6. RESEARCH HIGHLIGHTS FROM KEY LOCATIONS AND HOT SPOT SCREENINGS**

### **6.1. Resistance to diseases**

Developing and deploying genetically disease resistant varieties adapted to target growing environments is the most economical and environmentally friendly strategy for controlling rust diseases of wheat, particularly for resource-poor farmers. However, because of the co-evolution of the host and pathogen, the deployment of individual resistance genes often leads to the emergence of new virulent pathogen mutants, and hence the ‘boom and bust cycle’ of varieties performance continues. A new stem rust race Ug99 (TTKSK) was first detected in Uganda in 1999, and then spread to Kenya, Ethiopia, Yemen, Sudan and Iran. It became a global threat to the wheat industry of the world, because it soon became apparent that it over

comes many of the known and most commonly used stem rust resistance genes, such as Sr31, Sr24 and Sr36. Similarly, the breakdown of stripe rust resistance gene Yr9 in varieties derived from “Veery” in the 1980’s, and Yr27 in 2000’s in major mega-cultivars derived from the “Attila” cross such as Inquilab-91 (Pakistan), Kubsu (Ethiopia), PBW343 (India), and others such as Achtar in Morocco, Hidab in Algeria and many other cultivars in the CWANA region (Solh et al., 2012) has caused significant wheat production losses of up to 70%.

To combat these diseases, the ICARDA wheat breeding program carried out intensive screening and gene introgression activities. On annual basis, evaluation of elite genotypes for rusts and septoria is being carried out at Kulumsa research station in Ethiopia, and at Merchouch and Al-Altazi stations in Morocco (Figure 22). Furthermore, seedling and Adult plant screening for rusts is also carried out at the Izmir (Turkey). Highly resistant elite spring bread wheat genotypes have been developed as indicated in the figure below.

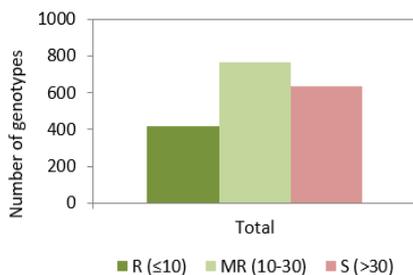
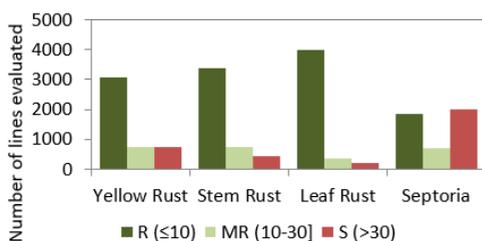


Figure 22: Diseases resistance; Response of 4600 ICARDA’s spring bread wheat genotypes to rusts and Septoria at Kulumsa (above) and at Merchouch (below)

### 6.2. Resistance to insects

Of all the insects attacking wheat crop, Hessian fly, Sunn pest, wheat stem sawfly and Russian wheat aphids cause significant economic losses. Hessian fly, *Mayetiola destructor*, damage can result in total loss of the crop if high infestations occur in the early stages of crop development. In Morocco, bread wheat and durum wheat yield losses due to Hessian fly have been estimated annually at 36% and 32%, respectively, amounting to about US\$200 million/annum (Lhaloui et al. 1992). On annual basis, more than 300 elite spring bread wheat genotypes from the spring bread wheat program of ICARDA are screened for Hessian fly resistance. As indicated in the figure 23 below, high yielding genotypes with Hessian fly resistance have been identified.

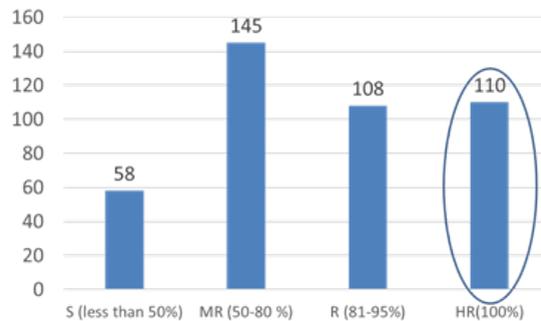


Figure 23: Response of high yielding, yellow rust resistant and drought tolerant elite spring bread wheat genotypes to HF, 2018

### 6.3. Evaluation for yield potential, drought and heat tolerance

Identification and development of wheat varieties combining high-yield potential with water use efficiency can help stabilize yield gains in the face of climate change. The development of wheat varieties with early vigour and cold tolerance has been a major target of wheat breeders in dry land areas, as early and complete canopy establishment shades the soil and

reduces evaporative loss from the soil surface, thereby significantly improving water productivity of wheat. High yielding and drought tolerant spring wheat genotypes have been identified from ICARDA's breeding program and their agronomic performance at the Sids Research Station in Egypt for yield potential, at the Wadmedani Station in Sudan for heat tolerance and at the Marchouch station in Morocco, as indicated in Table 8.

Table 8: Performance of elite bread wheat genotypes across key locations in Egypt, Sudan and Morocco, 2013/14

| Variety          | Marchouch (t/ha) | Sids (t/ha) | Wad Medani (t/ha) | Average (t/ha) | % of the check (Attila-7) |
|------------------|------------------|-------------|-------------------|----------------|---------------------------|
| IBW-IMAR         | 7.11             | 11.62       | 3.23              | 7.32           | 119%                      |
| IBW-AMAL         | 6.49             | 12.72       | 2.5               | 7.24           | 118%                      |
| IBW-HAMID        | 7.13             | 9.56        | 3.77              | 6.82           | 111%                      |
| IBW-WAHID        | 7.31             | 10.16       | 2.92              | 6.8            | 111%                      |
| IBW-BRIVAN       | 8.98             | 7.81        | 3.05              | 6.61           | 108%                      |
| IBW-FARID        | 6.39             | 10.23       | 3.03              | 6.55           | 106%                      |
| IBW-OMAR         | 6.96             | 9.72        | 2.65              | 6.45           | 105%                      |
| IBW-AKID         | 6.5              | 9.87        | 2.94              | 6.44           | 105%                      |
| IBW-WIDAD        | 6.51             | 8.46        | 3.87              | 6.28           | 102%                      |
| IBW-TARTUS       | 5.89             | 9.39        | 3.4               | 6.23           | 101%                      |
| Sids-1 (Check)   | 5.36             | 10.88       | 2.35              | 6.2            | 101%                      |
| Attila-7 (Check) | 6.19             | 8.4         | 3.86              | 6.15           | 100%                      |
| Pastor-2 (Check) | 6.24             | 7.49        | 3.42              | 5.72           | 93%                       |

As indicated below (Figure 24), those genotypes in the top right corner of the graph are the highest yielding in the contrasting environments indicating that those elite genotypes are high yielding at Sids station of Egypt (yield potential site), at Wad Medani station of Sudan

(Global heat platform) and at the rainfed (terminal drought) station of Merchouch in Morocco.

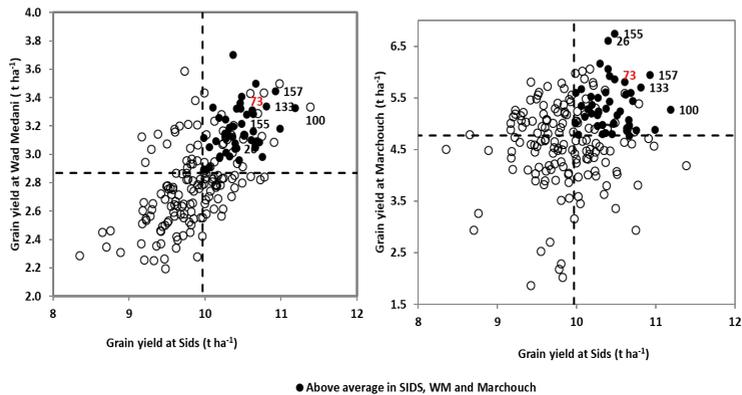


Figure 24: Yield of wheat genotypes across key locations

#### 6.4. Quality analysis

In most developing countries, apart from grain yield, disease resistance and drought/heat tolerance, grain quality has not been a strong criterion in variety selection. However, some developing NARS are critically looking for high-quality varieties suited for the preparation of a range of end products. The grain protein content was the first and more important quality trait focused in breeding programs. Breeding for grain protein content is difficult since yield gains have been generally associated with reductions in grain protein content due to a dilution of nitrogen compounds when carbohydrate deposition increases throughout photosynthesis (Jenner et al., 1991). Since most of the past wheat breeding progress worldwide can be attributable to the empiric selection for higher yields, grain protein genetic gain was negative during the 20th century in most countries like France, Italy or Spain (Brancourt-Hulmel et al., 2003; De Vita et al., 2007; Sanchez-Garcia, 2015) and in CIMMYT derived varieties (Ortiz-Monasterio et al., 1997). Most of these decreases are attributable to the replacement of the

low yielding landraces with high protein content by the first bred varieties, where the increase in grain yield was not matched with an increase in grain protein. However, recent advances in the agronomical management of nitrogen uptake and harvest index have allowed to reduce the loss of grain protein content in the newest released varieties or even to maintain it (Brancourt-Hulmel et al., 2003; Ortiz-Monasterio et al., 1997; Sanchez-Garcia, 2015).

In the CWANA region, varieties such as Achtar, Bezostaya, HD1220 and Pavon-76 are known for their excellent bread-making quality. These varieties are still dominantly grown in some countries not only because of their wide adaptation, high yield potential and stability but also because of their high protein content and bread-making quality. With this understanding, the wheat breeding program at ICARDA routinely undertakes evaluation of germplasm for quality traits following international standard grain quality procedures. Some of the most important small-scale tests used to determine physical, chemical and functional grain /flour qualities are indicated below.

**GrainScan:** Developed by CSIRO (Whan et al., 2014) Grainscan is a software that allows low cost and fast measurements of morphological characteristics of grains (length, width, area and perimeter) and color from images captured with flatbed scanners. More than 200 samples per technician and scanner can be analysed a day. If the grain samples are weighed after the scanning, TKW can also be calculated. The accuracy and precision of the method have been tested in ICARDA Quality Laboratory and  $R^2 > 0.9$  have been found for most of the parameters for bread wheat and durum wheat.

**Protein content:** Several methods can be used to estimate protein content, including the Kjeldahl methods, colorimetric/spectrophotometric assessments, and near infrared reflectance (NIR). Of these, the NIR analysis using either milled flour or whole grain is the most versatile and provides the fastest estimate. Its main advantages over other methods like

Kjeldahl are the fact that is a non-destructive method, it needs no chemicals and its use is very simple and fast.

**Grain hardness:** Rapid small-scale tests (based on grinding time, grinding volume, or particle size distribution) used to determine grain hardness make it relatively easy to screen for hardness as early as the F3 generation. NIR analysis of the particle size distribution of whole grain flour or analysis of intact grain samples are also available and the benefits are similar a for the protein. Rough estimates of hardness can also be made visually or by simply biting the grain.

**Flour/semolina yellow pigment:** Easy-to-use reflectance and pigment extraction methods are available to breeders. Semolina color is determined by xanthophylls, especially lutein, measured as pigment concentration or by colorimetric instruments. Semolina color heritability is high and largely additive. Color can be determined visually by comparison with standard samples or directly with a reflectance colorimeter.

**Enzymatic activity:** Rainfall at or prior to harvest can trigger alpha-amylase activity transforming starch in simpler sugars significantly reducing grain quality. From a breeding point of view, tolerance to preharvest sprouting is linked to grain dormancy. Dormancy inhibits the production of germinative enzymes, particularly alpha-amylase. Breeders can indirectly measure alpha-amylase levels using the Falling Number test (AACC, 1983) to measure the viscosity of a flour suspension, or dye-labeled starch substrates that measure alpha-amylase activity by change in the dye color over time to indicate tolerance to preharvest sprouting.

**Sedimentation test (Zeleny and SDS):** These tests can be used to obtain a semi-quantitative estimation of the amount of glutenins (or indirectly, of general gluten strength). The tests are widely used to screen early generation wheat lines for gluten strength (strong to weak).

Heritability of SDS-sedimentation is intermediate to high. Correlation between protein concentration and predictors of gluten strength differs among methods while SDS-sedimentation, used widely in early generation selection, and industrial standards, such as Alveograph and Mixograph parameters, are high. Selection in early generations using SDS sedimentation is frequently practiced from F2 onwards.

**Mixograph and Farinograph:** In the industries, in order to achieve high end-products quality, the dough must have an optimum rheology, dough kneading is one of the important test to characterize the quality of wheat flour, These tests can be used to determine dough rheology, Represent the behaviour of the dough under specific kneading conditions after it was brought to standard consistency of 500 BU (Brabender Unit) for the farinograph and 80 rpm for the mixograph (Figure 25).

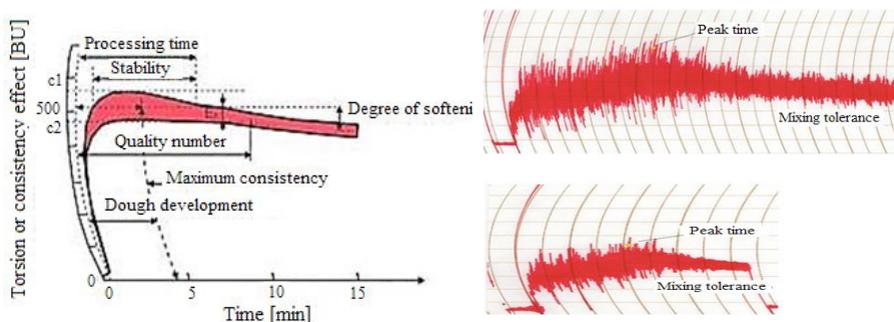


Figure 25: Interpretation of Mixograph curve

**Glutenins (HMW and LMW) and gliadins:** Sodium dodecyl sulfate-polyacrylamide gelelectrophoresis (SDS-PAGE) of whole protein extracts can be used in early generations to select lines with desirable HMW subunit composition and in advanced stages to define desirable HMW combinations in the progeny of new crosses.

Most of the currently available elite genotypes at ICARDA for both irrigated and rain-fed environments are acceptable to excellent in quality, with protein levels of 12 to 16%. Most of

these genotypes have the 5+10 (Glu-D1), 7+8 (Glu-B1) and 2\* (Glu-A1) alleles. These alleles, especially the 5+10 Glu-D1 allele, are known to be highly correlated with desired protein quality and are being used intensively as a selection criterion in our wheat breeding when making crosses and for identifying high end-use quality fixed genotypes.

There are several ways for assessing grain protein although nowadays near infra-red spectrophotometry (NIR) is the most used. A second and compatible way for increasing wheat end-use quality is breeding for gluten quality.

The gluten matrix properties of bread wheat are unique in terms of strength, tenacity and extensibility (Figure 26). These unique viscoelastic and rheological properties explain the wide range of end-uses of wheat flour and especially allow bread wheat dough to bear the fermentation process, producing high loaf volume without collapsing.

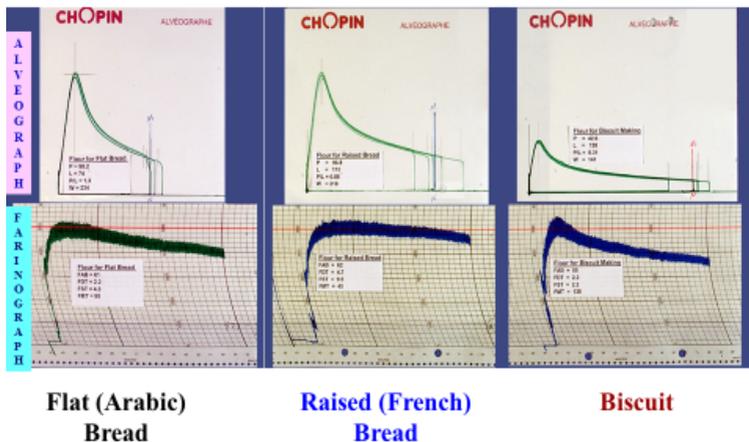


Figure 26: Dough characteristics for different bread wheat products

The gluten matrix properties are basically determined by a series of proteins called glutenins and gliadins. The strong influence of these proteins on the rheological performance of the dough explains the high heritability of these traits. However, some genotype by environment

interaction, mostly associated to variations in grain protein content, has been reported for dough extensibility (Cornish et al., 2001) and, to a lesser extent, dough strength. In fact, the traits associated with protein content are usually more affected by the environment and the variety x environment interaction than those related with protein quality and dough rheology (Williams et al., 2008).

High molecular weight (HMW) glutenins, encoded at the homeologous loci complex Glu-1, are the proteins with the highest effect over dough strength in bread wheat. In fact, their impact on final dough strength has been studied and associated to gluten strength indices that have shown to be highly correlated with final dough strength (Payne et al., 1987; Branlard et al., 1992; Table 9):

Table 9: Gluten strength scores for the HMW glutenin subunits. The allele nomenclature is expressed within parenthesis.

| Gluten strength index | High molecular weight (HMW) glutenin subunits |                          |                   |
|-----------------------|---|--------------------------|-------------------|
|                       | Glu-A1  | Glu-B1                   | Glu-D1            |
| 5                     |   | 7+8 (al)                 |                   |
| 4                     |   |                          | 5+10 (d)          |
| 3                     | 1 & 2* (a,b)                                  | 7*+8 & 17+18 (u,i)       |                   |
| 2                     |   | 7+9 (c)                  | 2+12 & 3+12 (a,b) |
| 1                     | null (c)                                      | 6+8; 7 & 20x+20y (d;a,e) | 4+12 (c)          |
| 0                     |   |                          |                   |

Adapted from, Payne et al., 1987 and Wrigley et al., 2009.

From a breeding point of view, this is of great importance since the high heritability of this trait implies that designing the crosses appropriately according to the parental lines alleles at the Glu-1 loci will lead to varieties with the suitable intended degree of gluten strength. However, the usual techniques used to ascertain most of wheat's rheological performance (Mixograph, Alveograph, etc.) important amounts of flour are usually necessary indicating that no screening for this aim can be done before the segregating populations are fixed

enough for having homogeneous grain to perform the tests. Therefore, the molecular screening of the segregating lines at early and advanced generations may allow the breeders to discard unsuitable combinations and select for the appropriate ones. For this aim, SDS PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis; Figure 27) of whole protein extracts can be used in early generations to select lines with desirable HMW subunit composition and in advanced stages to define desirable HMW combinations in the progeny of new crosses. Functional markers, especially for the alleles at Glu-1 loci, are also being developed and some of them are already available, especially at loci Glu-A1 and Glu-D1 and the Bx7 subunit at Glu-B1 (Ahmad 2000; De Bustos et al., 2000; Figure 27).

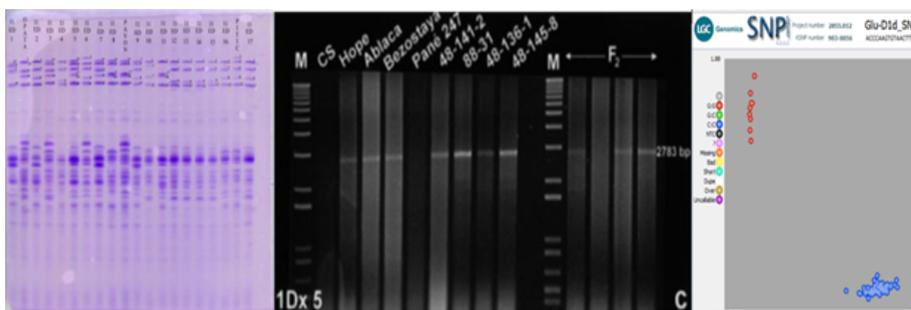


Figure 27: Example of SDS PAGE (left), agarose gel (middle) and KASP marker (right) for HMW glutenin subunit characterization.

Due to their, generally, lower impact on bread quality, low molecular weight (LMW) glutenin subunits and gliadins have been less studied than the HMW and, in the case of LMW the studies were performed mostly on durum wheat, where these proteins have an important impact on pasta-make quality. Also, the study of these proteins has often been neglected due to the increased difficulty of their identification in SDS PAGE. However, LMW glutenin subunits have been found to play an important role on dough extensibility (Cornish et al., 2001), making them of great interest for some wheat end-products quality. Thus, pasta end-quality relies on doughs showing low extensibility while for bread making quality

equilibrated doughs in terms of the tenacity/extensibility ratio are needed (Wrigley et al., 2009).

**Bread making and sensorial analysis:** Bread Making is one of the most ancient methods of cereals processing, the processing of bread can be divided into three basic operations: mixing or dough formation, fermentation and baking. The simplest bread making procedure is to mix all the ingredients (Water, Salt, Sugar, oil, Yeast and Flour) into a developed dough that is then allowed to ferment, during the fermentation the dough is punched one or more times. Thus, the transformation of dough to bread is a continuous process, it starts at 65 °C as the starch gelatinizes, the dough/bread becomes gas-continuous at 72°C and more elastic at 95°C to finally the bread. For this aim ICARDA scientist developed a low cost and fast method to determine the end-product quality characteristic (Loaf volume, alveoles sizes and number) (Figure 28). And then start the sensorial analysis to determine the crust texture and color, the crumb color, the pungent flavor and the chewiness.



Figure 28: Breads and Bread Maker

## 7. INTERNATIONAL NURSERY AND GERMPLASM DISTRIBUTION

Based on request, different forms of germplasm such as genetic stocks for crossing blocks, segregating generations and essentially finished (“fixed”) genotypes are distributed to the

CWANA NARS and other collaborators. The genetic stocks and the segregating generations are sent with the objective of decentralizing the breeding program and creating genetic variability to be selected under alternative conditions by the partners, while the fixed materials are sent with the objective of being considered for release as adapted varieties or used in new crosses by the collaborators. Experience obtained so far is that most NARS have released more varieties from directly introduced, essentially finished material than from early segregating populations. Research infrastructure, budget availability, and overall strength of NARS are the main factors accounting for these differences (Byerlee and Moya, 1993). At present, unless specifically requested, most of the germplasm distribution to NARS consists of essentially finished materials. Since 2005, the spring bread wheat program has distributed germplasm for heat tolerance, Hessian fly resistance, Ug99 resistance, drought tolerance and high yield potential for the CWANA region and beyond upon request. On an annual basis, the spring bread wheat program composes and distributes the following nurseries and yield trials.

- Spring Bread Wheat Observation Nursery for CWANA (CWANA SBWON)
- Spring Bread Wheat Observation Nursery for Heat Tolerance (SBWON HT)
- Spring Bread Wheat Yield Trial for Irrigated environments (CWANA ISBWYT)
- Spring Bread Wheat Yield Trial for Dryland rain-fed environments (CWANA DSBWYT) and
- Elite Spring Bread Wheat Yield Trial (ESBWYT)

Each of the observation nurseries are composed of 2000 genotypes targeted for the respective environments, while the respective yield trials consist of 50 genotypes in two replications with the 50th slot reserved for the local check commercial varieties. For winter bread wheat, selected genotypes from ICARDA are included in the Facultative and Winter Wheat

Observation Nursery (FAWWON) and the International Winter Wheat Yield Trial (IWWYT), which are distributed to more than 50 countries on an annual basis by IWWIP from Konya, Turkey.

In general, the observation nurseries are targeted to identify adapted varieties for release or use as parents in crossing by the respective NARS, while the yield trials are primarily distributed to identify widely adapted genotypes to be used in future crosses at ICARDA. All nurseries are distributed on an annual basis, based on requests from the NARS across CWANA and other regions of the world (Figure 29).

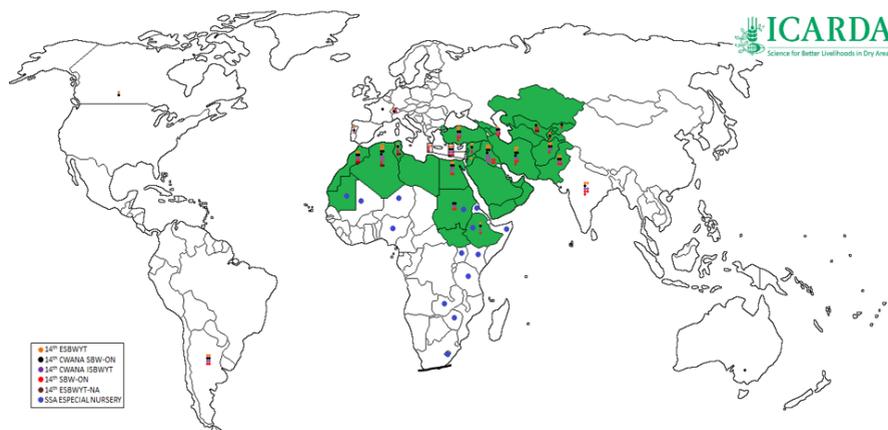


Figure 29: Distribution of ICARDA wheat international nurseries in 2013/2014

## 8. VARIETY RELEASE, ADOPTION AND IMPACTS

The national wheat research coordinating center of each country requests international wheat nurseries from CIMMYT and ICARDA and grows the nurseries for further evaluation and selection either for direct release or parental purposes. The germplasm acquisition, development, evaluation and release follow the general procedure outlined in figure 30 with slight modification at country levels.

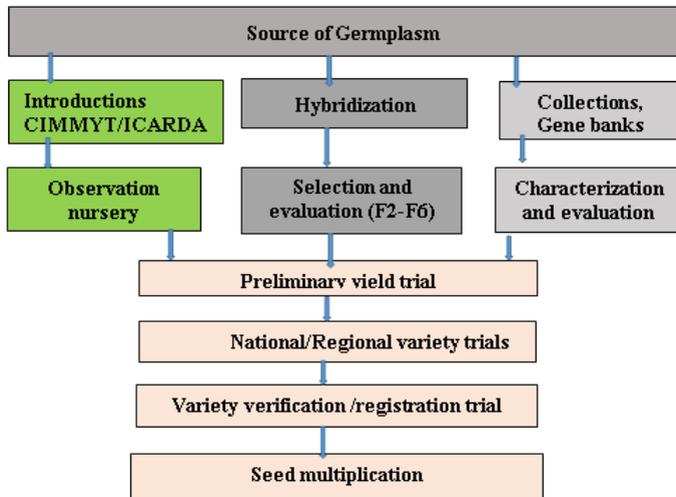


Figure 30: Germplasm evaluation scheme in the national breeding programs

The success of wheat improvement within the CGIAR has been remarkable, and today more than 70% of all spring wheat cultivars grown in developing countries are CGIAR-derived, reaching more than 90% in South Asia, parts of West Asia and North Africa (Bliyerlee and Moya, 1993). The adoption of improved varieties has increased from 93% in 2002 to 97% in 2014 and about 106 million hectares of the wheat area was covered by the CGIAR-related wheat varieties (Lantican et al., 2015). From the CGIAR wheat breeding program, it has been reported that more than 1500 wheat varieties have been released during the periods of 1966-1990 with average of 65 varieties released annually. More than 80% of the varieties released are spring bread wheats, which account for 77% of the wheat area in the developing world outside of China (Bayerlee and Moya, 1993). Among the many crosses developed to-date at CIMMYT/ICARDA, the VEERY cross (KVZ/BUHO//KAL/BB) and its derivatives have been by far the most successful cross enabling in the release of at least 65 varieties in more than 30 countries. Among the VEERYs, Veery 5 (CM33027-F-15M-500Y-0M) was the most

popular and released in many countries with different local names such as SERI 82 in Lebanon, Turkey, Mexico; Dashen in Ethiopia; Tilila in Morocco; Aziz in Yemen; Pirsabak 85 in Pakistan; Loerie in Zambia; MACS2496 in India; SCW101 in Zimbabwe; Rassol in Iran; GIZA 164 in Egypt; SASARAIB in Sudan and TAUSI in Tanzania.

After the VEERYs, Cham-6 (W3918A/JUP) also called Nesar was released and grown in Syria, Lebanon, Jordan, Iraq and Algeria. Similarly, Kauz (JUP/BJY//URES) has been released in many countries with different local names such as Cham-8 in Syria, Tanur in Lebanon, Mehdia in Morocco, Atrak in Iran, Bacanora in Mexico, WH 542 in India and with other different names in many other countries. After Kauz, many successful varieties have been originated from the Attila cross (ND/VG9144//KAL/BB/3/YACO/4/VEE#5) and grown in many countries as mega-cultivars such as Kubsa in Ethiopia; Imam in Sudan; Utique 96 in Tunisia; PBW343 in India; Chamran, Gaher and Shiroodi in Iran; Ziyabey 98 in Jordan; MH-97 in Pakistan; and with different names many other countries (Tadesse et al., 2010).

From 1994-2014, 4604 wheat varieties have been released globally by public (63%) and private (37%) breeding programs with the CGIAR-related varieties accounting 63% of the global releases. More than a quarter of such releases have been registered from 2010-2014 probably due to the introduction of Ug99 rust resistant varieties. At regional level, CGIAR-related varieties account 97% in South Asia of which more than 50% are direct releases. Similar levels have been reported in Central and West Asia and North Africa (CWANA) and Sub Saharan Africa (SSA) regions (Lantican et al 2015; Figure 31). International breeding efforts focusing only on winter wheat started since 1986 through the establishment of IWWIP. To-date more than 55 winter/facultative wheat varieties of IWWIP origin have been released in 10 countries of Central and West Asia including Afghanistan, Iran and Turkey (Tadesse et al., 2013). These new varieties have broad genetic diversity as they are developed from parents of diverse sources originated from ICARDA, CIMMYT, and from a wide range

of genetically un-related winter wheats from Turkey, Iran, Russia, Ukraine, Romania, Bulgaria, Hungary and US. Some lines such as OK82282//BOW/NKT and YMH//TOB//MCD/3/LIRA have been identified and released under different names in different countries indicating their broad adaptation. The former is released in Afghanistan and Kyrgyzstan while the later (Kinaci 97) has been released in Turkey, Afghanistan and Uzbekistan. However, MVs of winter wheat still cover a small area. Old cultivars such as Bezostaya and Gereek are dominantly cultivated in Turkey.

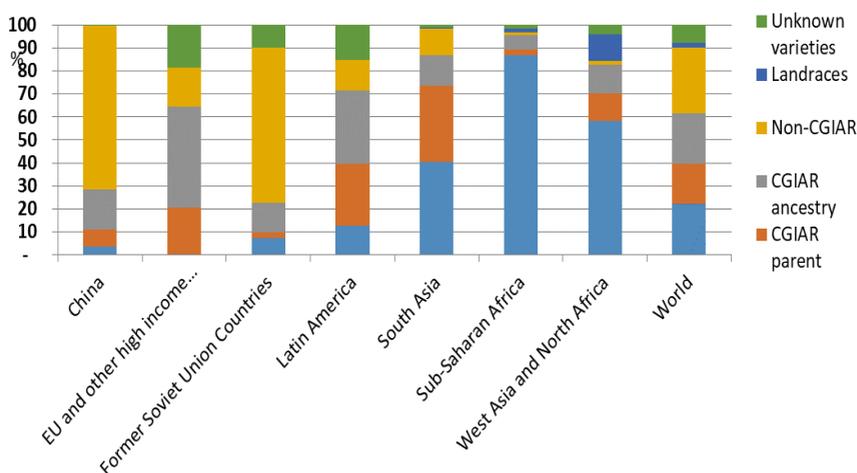


Figure 31: Percentage of wheat varieties grown in the world by region and origin (Lantican et al. 2015)

The impact of WHEAT has been witnessed not only by farmers, governments, policy makers and professionals but also by donors such as the World Bank. According to World Bank (2008), for no other major crop is the percentage of improved cultivars in farmers' fields in developing countries higher than for wheat. Annual benefit from the global wheat research ranges from 6.7-9.4 billion dollars. The benefit from CGIAR wheat research alone ranges from 2.3-3.1 billion dollar on annual basis (Lantican et al., 2015). The impact of international

wheat research, however, is beyond financial estimates. Its contribution in terms of capacity development, food security, job creation, and conservation of natural resources is huge.

## **9. BREEDING PROGRESS**

Development and identification of high yielding genotypes with wide adaptation and resistance to biotic and abiotic stresses remain the top priorities of the wheat breeding programs. There are different approaches of determining the breeding progress or the rate of genetic gain for grain yield and other traits. Some studies use yield of historical genotypes grown in the same environment while others have used mean yield to examine progress over time in highly productive environments. Trethowan et al. (2002) have used regression analysis using the mean of the five highest yielding entries expressed as per cent of the trial mean across years to determine the rate of breeding progress in elite spring wheat yield trial (ESWYT) and semi-arid wheat yield trial (SAWYT). Tadesse et al. (2010) have used success rate analysis of best lines for the high rain-fall wheat yield trials (HRWYT) of CIMMYT to demonstrate yield gains over years or trials. Genetic gain studies for the CIMMYT/ ICARDA wheat breeding program have shown continuous progress in yield and other traits (Trethowan et al., 2002; Sharma et al., 2012; Tadesse et al., 2010). Recently, Tadesse et al. (2013) have determined the breeding progress for the international winter wheat breeding program (IWWIP), and reported that the grain yield of the best line (BL) increased at a rate of 110 kg/ha/year ( $R^2 = 0.66$ ;  $P = 0.001$ ), while the trial mean (TM) increased at a rate of 91.9 kg/ha/year ( $R^2 = 0.53$ ;  $P = 0.007$ ) indicating a continuous yield improvement at IWWIP.

In addition to grain yield, significant progress has been made by the IWIN in developing resistant wheat germplasm to diseases and pests ensuring that developing and deploying genetically resistant varieties adapted to target growing environments is the best economical and environmentally friendly strategy for controlling rust diseases of wheat particularly for

resource poor farmers. However, because of the co-evolution of the host and pathogen, the deployment of individual resistance genes leads to the emergence of new virulent pathogen mutants, and hence the ‘boom and bust cycle’ of cultivars performance continues. Recently, a new stem rust race Ug99 (TTKS) has been first detected in Uganda in 1999 and then spread to Kenya, Ethiopia, Yemen, Sudan and Iran, and became a global threat to the wheat industry of the world for the very fact that it over comes many of the known and most common stem rust resistance genes such as Sr31, Sr24 and Sr36 (Singh et al., 2007). Similarly, the breakdown of yellow rust resistance genes Yr9 in cultivars derived from “Veery” in the 1980’s and Yr27 in 2000’s in major mega cultivars derived from “Attila” cross such as PBW343 (India), Inquilab-91 (Pakistan), Kubsu (Ethiopia) and others such as Achtar in Morocco, Hidab in Algeria and many other cultivars in the CWANA region (Solh et al., 2012) has caused significant wheat production loss. Through a coordinated international effort, many wheat varieties resistant to Ug99 and yellow rust have been released and replaced the susceptible cultivars.

In most developing countries, apart from grain yield and disease resistance, grain quality was not a strong criterion of variety selection. However, things have changed through time and some developing NARS are critically looking for better quality varieties suited for the preparation of different end products. Varieties such as Bezostaya, Achtar, Veery, HD1220, and Pavon-76 are known for their excellent grain quality. These varieties are still dominantly grown in some countries not only because of their wide adaptation, high yield potential and stability but also because of their high protein content and quality. With this understanding the wheat breeding programs at CIMMYT and ICARDA undertake evaluation of germplasm for quality traits following standard grain quality procedures. Most of the currently available elite genotypes for both irrigated and rain fed environments are excellent in quality with protein levels of 12 to 16%. Most of these genotypes have the 5+10 (Glu-D1), 7+8 (Glu-B1)

and 2\* (Glu-A1) alleles. These alleles, especially the 5+10 Glu-D1 allele, have been reported to be highly correlated with protein quality and are being used intensively as a selection criterion in wheat breeding for improving end-use quality.

## **10. ASSOCIATION MAPPING**

Association mapping (AM) is an important cost-effective approach for identification of marker-trait associations as it can utilize diverse sets of germplasm (landraces, cultivars, elite breeding lines, etc), and exploits the high marker coverage and higher resolution of the new genotyping technologies without the time and effort needed to develop bi-parental mapping populations (Crossa et al., 2007). Marker-trait associations using AM have been identified in different crops using different types of molecular markers. Breseghello and Sorrells (2006) and Tadesse et al (2015), among many others, have identified significantly associated markers with kernel size and milling quality in winter wheat. Similarly, association mapping studies have been carried out for resistance to foliar diseases (Crossa et al., 2007; Tadesse et al., 2014; Sukumaran et al., 2015; Jighly et al., 2015), soil-borne pathogens (Mulki et al., 2012) and resistance to major insect pests in (Joukhadar et al., 2013). Recently, Tadesse et al (2019) have identified QTLs related to yield and other agronomic traits under heat stress at Wadmedani (Sudan) and Sids (Egypt) stations. The *w SNP\_Ex\_c12812\_20324622* marker on chromosome 4A is significantly correlated with yield at both locations (Figure 32). At Wad Medani, *w SNP\_Ex\_c2526\_4715978* on chromosome 5A is significantly correlated with grain yield. Wheat genotypes carrying the cytosine base at the *w SNP\_Ex\_c12812\_20324622* and *w SNP\_Ex\_c2526\_4715978* markers out-yielded the ones carrying the alternative bases by 15% while genotypes carrying the cytosine base at only one of the two markers increased their yield by 7.9-10% suggesting the importance of using these markers for MAS in breeding programs to increase yield under heat stress. It is not difficult to identify QTLs linked to a particular trait on a given chromosome. However, the challenge is the stability of

the QTL across environments, seasons and locations. It is important to validate the QTL before using it for marker assisted selection in a breeding program.

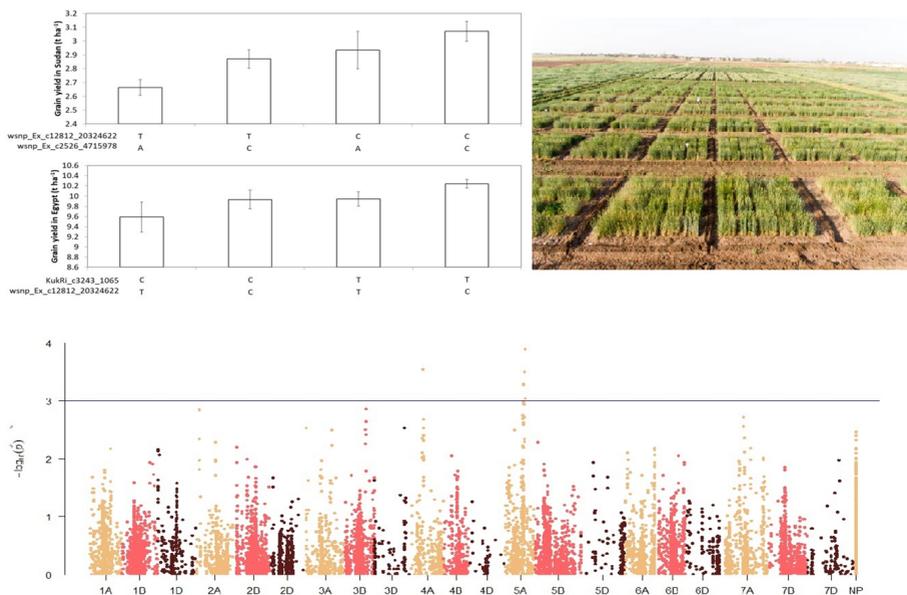


Figure 32: QTLs associated to yield under heat stress ( Tadesse et al 2019)

## 11. CAPACITY DEVELOPMENT

Germplasm distribution to the NARS on its own will not bring about the hoped-for result of releasing and adoption improved varieties, unless it is handled and managed by trained and qualified national breeders. Therefore the wheat breeding program at ICARDA organizes both short- and long-term training courses in wheat breeding and has trained hundreds of wheat breeders from all over the wheat growing NARS. This has helped in the promotion of the fundamental concepts of international wheat breeding, and the development and release of many new and superior wheat varieties. In the last decade, many new technologies such as molecular markers have been developed and utilized in the public and private crop breeding programs of the developed world. Such rapid advances in biotechnology and molecular

genetics not only provide unprecedented opportunities to enhance breeding efficiency, but also create new challenges in training breeders with skills integrating both conventional and molecular breeding approaches and techniques. To this end, the wheat breeding program at ICARDA has revised its training program to accommodate classical and molecular approaches for wheat improvement. Junior and mid-career scientists experience a comprehensive hands-on course on breeding for durable disease resistance, high yield potential and stability, drought and heat tolerance, end-use seed quality, and seed health issues using conventional and molecular tools (Figure 33). ICARDA believes that well-trained young scientists are the key to the future of NARS programs, in addition to improved wheat genetic materials.



Figure 33: Wheat breeding course participants at ICARDA, 2013-2019

## 12. FUTURE DIRECTIONS AND PROSPECTS

It is expected that by the year 2050 the world population will reach 9 billion and the demand for wheat reaches more than 900 million tons (Alexandratos, 2009). Increasing human population coupled with climate change and other production constraints such as increasing drought/water shortage, soil degradation, reduced supply and increasing cost of fertilizers, increasing demand for bio-fuel, and emergence of new virulent diseases and pests are becoming challenging issues to wheat production and genetic resources conservation. To

increase wheat production and feed the ever-increasing world population while conserving the natural resource base, the following points are important to consider.

1. Despite the existence of huge genetic resources in gene banks, the utilization level to-date is limited with some reports indicating it to be only around 10%. It is therefore important to design and apply efficient strategies and tools such as Focused Identification of Germplasm Strategy (FIGS), cytogenetic and genomic tools and bioinformatics in order to efficiently mine genetic resources for breeders sought traits. Wild relatives will be highly relevant to add novel diversity including for adaptation to climate change adverse effects, calling for strengthening pre-breeding efforts.

2. The availability of new molecular tools such as genotyping-by-sequencing (GBS) and advanced statistical analysis software would enable efficient characterization and mining of novel genes and alleles, gene introgression and pyramiding through marker assisted selection and undertake genomic selection in order to allow faster integration of desirable traits and improve breeding efficiency, especially for complex traits such as grain yield under optimum, drought, and heat conditions. However, it is noteworthy to keep the right balance in investment between marker technology and field level phenotyping. In the past, many QTLs have been identified with very little practical application in the breeding process.

3. Genomic Selection could be integrated with the doubled haploid breeding method at DH2 stage whereby the selected genotypes based on genomic estimated breeding value (GEBV) could be used to retrain the model, as parents and possible candidates for variety trials. Such approach enables to reduce yield trials across locations by 1 year and save resources which could be used for genotyping. The cost- benefit analysis of such practices varies across breeding programs in different countries and the correlation of the GEBV with the field-

based selections, and accordingly the decision to apply GS should be made by the respective programs.

4. Major efforts are needed to break yield barrier in wheat to increase wheat yield potential by 50% in order to cope the growing demand for wheat. Increasing the radiation use efficiency of wheat through modification of key enzymes (e.g. Rubisco) and biochemical pathways to increase photosynthesis, ear size and lodging resistance are key areas of wheat research through integration of physiological and molecular breeding methodologies to increase wheat yield potential.

5. Though there is no genetically modified (GM) wheat currently under production anywhere in the world, it is important to invest in this direction in the future as GM wheat would be particularly valuable for traits with limited or no genetic variation within the *Triticum* species such as herbicide resistance, fusarium resistance, novel quality traits, increasing yield potential through breaking the yield barrier and technologies for creating hybrid cultivars. In addition, GM technologies hold promise for enhancing drought and heat tolerance, as well as disease and pest resistance.

6. Future investment in hybrid wheat production is believed to increase wheat production as hybrid wheats provide higher grain yield, higher thousand grain weight, more tillers, higher biomass, deeper roots and better resistance to both biotic and abiotic stresses as compared to their parents. It is anticipated that the application of biotechnological methods will enable to capture increased heterosis by direct selection of favorable alleles, and development of new genetically based systems to control male sterility. It is important to collaborate with private sectors and leverage their technologies for the benefit of partners and stakeholders in the developing world.

7. The International Wheat Improvement Network (IWIN) coordinated by CIMMYT and ICARDA has been the most successful and efficient network for making available and widespread distribution of new wheat genotypes globally. Such a network needs to be strengthened through the establishment of other networks and collaborations in order to develop, disseminate, and market more productive, stress tolerant, and nutritive wheat varieties, and to perfect and promote production practices based on the principles of conservation agriculture that boost yields while conserving or enhancing critical resources like soil and water.

### **13. ACKNOWLEDGMENTS**

We sincerely acknowledge the wheat breeding team at ICARDA and M.Sc and PhD students working in the program for their valuable contribution to generate some of the information used in the preparation of this manual. The national programs in the CWANA and SSA regions in general and the NARSs at INRA, Morocco; ARC- EGYPT, ARC-Sudan, EIAR-Ethiopia and AUB and LARI in Lebanon are highly acknowledged for their partnership and support of our wheat breeding activities.

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This wheat breeding handbook summarizes wheat origin and evolution, challenges and opportunities of wheat production in CWANA and SSA regions, and current strategies of ICARDA's wheat breeding program in developing high yielding wheat varieties with resistance to the major abiotic and biotic stresses using both conventional and molecular approaches (gene introgression & pyramiding, shuttle breeding, doubled haploids, speed breeding, genomic selection, marker assisted selection, key location testing etc) in partnership with CIMMYT, NARS and other Advanced Research Institutes (ARIs). As this handbook will be freely available in printed and on-line versions, it will serve as an important reference for wheat breeders, geneticists, pathologists and others who are working in wheat breeding programs in the CWANA and SSA regions and beyond.

**Prof. Jacques Wery, Deputy Director General for Research, ICARDA**



ISBN 978-92-9127-5366

