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Breeding durum wheat for biotic stresses in the Mediterranean region

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SUMMARY – Durum wheat has evolved into a cereal speciality crop with numerous end uses characteristic to the Mediterranean region. In this region however, numerous biotic and abiotic stresses limit the profitability from this crop. Different fungal and viral diseases as well as insects and aphid pests are causing losses in yield and quality of durum wheat crops in the Mediterranean countries. Breeding for resistance or tolerance to biotic stresses has helped in the past and will permit to improve and stabilise yield without heavy use of chemicals. This paper is a review of breeding efforts of southern Mediterranean region NARs in concert with the CIMMYT/ICARDA program as supported by the West Asia and North Africa Dryland Durum Improvement Network (WANADDIN) IFAD project. Results of research on breeding for resistance to leaf rust, yellow rust, septoria, root rot, powdery mildew, hessian fly, smuts and bunts, saw fly and barley yellow dwarf virus are evaluated. Good or moderate resistance to most of the prevailing diseases is available on a separate basis in adapted material, interspecific hybrids progenies and/or in old collections or populations. The exchange of material and the introgression of multiple resistance in adapted and good grain quality material is being made by national and international breeding programs.

Key words: Durum wheat, biotic, stress, resistance.

RESUME – *"Amélioration du blé dur pour des stress biotiques dans la région méditerranéenne". Le blé dur est une culture typiquement méditerranéenne vu les utilisations spéciales qui en sont faites. Cependant, dans la région méditerranéenne, plusieurs stress biotiques et abiotiques limitent le profit de l'agriculteur de cette culture. Plusieurs maladies cryptogamiques, virales et insectes ravageurs causent des pertes de rendement et de qualité du blé dur. L'amélioration de la résistance génétique du blé dur a aidé dans le passé et permettra de limiter l'utilisation des produits chimiques polluants. Cet article est une revue des efforts de l'amélioration de la résistance génétique aux maladies conduits dans plusieurs pays de la Méditerranée du Sud et du programme international CIMMYT/ICARDA aidés par le projet IFAD : 'West Asia and North Africa Dryland Durum Improvement, WANADDIN'. Les résultats de recherches sur la résistance génétique aux rouilles brune et jaune, à la septoriose, aux pourritures racinaires, au blanc du blé, à la cécidomyie, au cèphe et au virus de la jaunisse nanisante sont évalués. Une résistance génétique bonne à modérée existe pour presque toutes les maladies sus-mentionnées dans du matériel parental, ségrégant ou avancé. L'échange des ressources et l'introgression de résistance à plusieurs maladies dans du matériel ayant une bonne adaptation et qualité du grain est en cours dans des programmes nationaux et internationaux de la région.*

Mots-clés : *Blé dur, biotique, stress, résistance.*

Introduction

Durum wheat is important in the Mediterranean basin and in several large cereal producing countries in the world. In the Mediterranean countries the importance of durum wheat is due to the particular utilisation characteristic to the region and also because durum wheat constitutes the largest part of the staple food in the southern Mediterranean countries. Drought and heat are common in most of the regions and are unpredictable stresses. Cold is common in fewer countries and its occurrence is more predictable. Biotic stresses occur in all the countries and their type depend heavily on the climatic conditions and some diseases depend on the occurrence of abiotic stresses.

The development of genetic resistance to a given disease can be evaluated by the advance in activities leading to the resistant variety. This process can be divided in several phases: (i) diagnosis of the disease and its importance; (ii) study of the pathogen; (iii) methodology for culturing or rearing the pathogen and inoculation of the host plant; (iv) studying the genetics of virulence as well as resistance and/or tolerance; (v) screening for resistance in the host; and (vi) introgression and combination of resistance into adapted material possessing other researched plant traits.

The objective of this paper is to report advance in research and breeding for genetic resistance to the major diseases in some southern Mediterranean countries. Most of the disease resistance in the southern Mediterranean countries can be easily translated to all Mediterranean countries.

During the growing seasons of 1995-1996 to 1997-1998, national and international durum wheat nurseries were evaluated for their reaction to the different diseases occurring in the developing Mediterranean countries. These nurseries were constituted from world collection accessions and populations, advanced and adapted lines as well as segregating populations. These segregating populations were coming from conventional crosses as well as crosses with related wild species coming from the breeding programs crossing blocks. A nursery comprising entries with multiple resistance was compiled and evaluated. The nurseries were evaluated for leaf rust, root rot, septoria diseases, tan spot, powdery mildew, yellow rust, barley yellow dwarf viruses, bunts and smuts and saw fly. Material for Hessian fly resistance was evaluated in separate nurseries.

Leaf rust research in Morocco

Leaf rust (caused by *Puccinia recondita* f. sp. *tritici*) is the most widely distributed of the three rusts of wheat in North Africa. It occurs on both bread and durum wheat. However, an increased susceptibility is found among the newly developed cultivars of durum wheat. Due to the presence of a functional alternate host, *Anchusa italica*, the pathogen responds rapidly to changes in cultivar resistance. Therefore, an effective use of resistance to leaf rust cannot be achieved without the characterisation of the pathogen species and the assessment of temporal and spatial changes in the virulence of the pathogen.

The objectives of this collaborative work were:

(i) The identification of the prevailing pathotypes of the pathogen causing leaf rust and of the effective resistance genes and sources of resistance.

(ii) The establishment of appropriate screening methodology to test the performance of durum breeding material.

(iii) Identification of lines with resistance to leaf rust (seedlings and adult plants).

Surveys were conducted in the main wheat growing areas of Morocco, Algeria and Tunisia. Notes were taken on the incidence and severity of foliar diseases of both bread and durum wheat. Leaf rust samples were collected to be analysed for their virulence. Variable numbers of leaf rust samples were collected or sent by co-operating countries and were analysed for their virulence. The virulence analysis was conducted on 18 single gene differentials. The tested genes were *Lr1, 2a, 2c, 3, 3ka, 9, 10, 11, 16, 17, 19, 24, 26, 29, 30, 32, 33, 34.*

For the selection of virulent pathotypes, seedlings of the commercially grown cultivars of bread and durum wheat in Morocco were tested against cultures of *P. recondita* f. sp. *tritici* in order to select virulent pathotypes on durum. The selected pathotypes are or will be utilised to screen for resistance in the seedling stage and to conduct inheritance studies. A hot spot, where the alternate host is naturally infected was identified in the Safi region (Morocco) since 1992. A number of nurseries were tested in this location in the years 1993-1999. The plant material was also put in the experimental stations, especially at Douyet, where heavy leaf rust infection were frequently observed.

A large number of lines from the CIMMYT/ICARDA program, ICARDA and national programs were found to be resistant or moderately resistant. However, a proportion of these were not resistant in all test locations and growing seasons. The ICARDA nursery Durum Leaf Rust Gene Pool (DLRGP) accessions were found to be resistant in most tested locations and years. The lines constituting this gene pool did not possess good adaptation traits such as plant height, earliness and yield potential. The number of durum breeding lines (from national yield trials) possessing good to acceptable resistance were showing slightly lower yield and narrower adaptation to the different test sites and in the different growing seasons. The utilisation of the new sources of resistance is current in some research institutions of the region as well as in the CIMMYT/ICARDA durum breeding programme. A large and long term database on virulence of observed pathotypes is available and being sustained by new field research (ICARDA yearly reports 1995 to 1998).

Hessian fly research in Morocco

Hessian fly, *Mayetiola destructor* (Say) is the most destructive insect pest of wheat (*Triticum* spp.) in the major cereal growing areas of the world. In North Africa, and especially in Morocco, grain yield losses have been estimated to 42 and 36% in bread wheat using an insecticide control and near isogenic lines respectively. In durum wheat, losses have been estimated to 32%, which is equivalent to losses estimated on bread wheat. Ten genes of resistance (*H5*, *H7H8*, *H11*, *H13*, *H14H15*, *H21*, *H22*, *H23*, *H25* and *H26*) were selected as conferring resistance to Hessian fly in the field in Morocco. Most of these genes are located on the A or the D genomes. For a long period, no resistance was found in a durum wheat background. Breeders and entomologists in Morocco and ICARDA have decided to transfer the genes that are located on the A genome into durum wheat. Up to date, the *H5* gene has been successfully transferred from bread into durum wheat .

Durum wheat varieties adapted to Morocco and the Mediterranean region were used as recurrent recipient parents in a backcrossing program with the breadwheat variety Saada, carrying the *H5* resistance gene. Some advanced lines possessing wide adaptation in the West Asia and North Africa region were used in the crossing program. The crosses and backcrosses were made at Settat and Rabat research stations (INRA), Morocco, and at ICARDA, Aleppo Research Centre, Syria, starting from the 1988-1989 growing seasons. The F_2 segregating populations with a durum wheat phenotype were tested for Hessian fly resistance under artificial infestation in the greenhouse in Morocco and were either used for further backcrossing cycles to durum wheat or advanced in the field in Morocco and Syria. The same operation was repeated for the subsequent cycles. In Morocco, F_3 and later generations were grown in the fields of the Jemaa Shaim, Sidi El Aydi and Marchouch experiment stations under natural Hessian fly infestation. The objectives of the field selection stage were to advance, increase and screen large numbers of entries for Hessian fly resistance, other biotic stress resistances and adaptation criteria. In the field selection process, natural Hessian fly infestation was enhanced with the use of delayed planting. In ICARDA, Syria, crosses were made in the same procedure: families were advanced and selected for better drought and heat tolerance and sent back to Morocco for final testing for Hessian fly resistance. The emphasis was mostly given to increasing the chance of obtaining drought and heat tolerant material by increasing the tested family size. Yield trials were made in order to discard the low yielding and un-adapted lines in multiple sites in both Morocco and ICARDA, Syria.

Numerous promising lines (F_{10} to F_{12}) derived from single crosses are available and four are currently proposed for registration after being subjected to advanced yield trials on experiment stations and on farm yield trials. More than 300 advanced lines derived from single and multiple backcrosses with field and greenhouse tested resistance are now available in the breeding programs and are in the multilocation testing for adaptation and yield stability (ICARDA yearly reports 1995 to 1998).

Root rot research in Morocco and in Tunisia

Common root rot appellation has been widely used to point out a group of diseases that are characterised by necrosis of stems, roots and crowns. Common root rot fungi are ubiquitous, and troublesome wherever cereals, especially wheat and barley, are grown and of economic importance. Under Moroccan conditions, the major causal agents are *Fusarium roseum* f. sp. *culmorum* or *cerealis* and *Helminthosporium sativum* or *Bipolaris sorokiniana.* Root rot disease is one of the great challenges that face wheat production and particularly durum wheat and the estimated losses varied from 4 to 11%. The durum wheat varieties were documented to be very susceptible to root rot and foot rot. In the field, the disease is recognised by the development of white heads in addition to discoloration of the roots and crown necrosis. Under field conditions, white heads could be an excellent character in screening for disease resistance. Unfortunately, few sources of resistance are known for durum wheat improvement. Therefore this study was involved in screening for common root rot disease of durum wheat.

A mixture of four different isolates of *Bipolaris sorokiniana* and another four of *Fusarium culmorum* f. sp. *cerealis* was inoculated to a natural soil brought from Sidi El Aidi experimental station. The origin of the inoculum is Chaouia (from a monoculture of wheat), Abda, and Doukkala regions (Morocco). The concentration was adjusted to 16 10^4 conidia/ml and the seed of each accession was sown in a row of 10 cm long. Ten to fifteen grains made one row of the wooden box containing a total of 30 accessions. At the end of each week, the entries were all watered with a nutritive solution adapted for the type of soil and its quantity used in each box. Two evaluations were made during the growth stages of the durum

wheat. One at the tillering stage and the other at the heading stage. The evaluation scale is composed of five categories: highly susceptible, susceptible, moderately susceptible, moderately tolerant, and tolerant. The procedure conceived in this research aims to screen for both the seedling blight and root rot disease at the same time.

Among the 932 accessions screened in Morocco, only 78 were tolerant to moderately tolerant. All the highly susceptible lines exhibited seedling blight and most of them died before heading (ICARDA yearly reports 1995 to 1998). The method discriminated very well between the susceptible and the tolerant entries. Therefore, good evaluation of the root rot disease resistance even under the greenhouse conditions could be obtained. White heads were also obtained on the very susceptible entries and are characteristic of susceptibility. It appeared that a number of the tolerant entries were moderately late in heading times when compared to the susceptible ones.

In Tunisia, a survey has been conducted in the northern regions in order to determine the relative importance of *Fusarium* species in these areas. Virulence characterisation of several isolates of *Fusarium culmorum* has been also undertaken to determine the relative importance of *Fusarium graminearum* in the durum wheat growing areas.

Over 350 durum wheat lines were screened for resistance to *Fusarium culmorum* in an artificially contaminated plot (ICARDA yearly reports 1995 to 1998). The lines were screened for resistance to three to four isolates of *Fusarium graminearum.* Resistance to *Fusarium culmorum* was assessed on emergence of cultivars. The effect of *Fusarium* on germination indicated that this disease could reduce significantly the stand establishment of durum wheat. Only twenty five accessions were found to be resistant to all isolates. Four of these cultivars were highly resistant, five were moderately resistant and sixteen were less resistant. These latter accessions had lower final grain yield and generally limited kernel number and weight. The most resistant cultivars did withstand the effect of the disease and had grain yield and yield components comparable to the non inoculated check. Resistance to *Fusarium graminearum* was assessed using four isolates. Artificial inoculation was carried out at heading time onto the spikelets of the middle of each head. The disease effects were then evaluated using information on the spread of the disease into neighbouring spikelets. Only six lines were found to be resistant to *Fusarium graminearum*. Three of those cultivars are listed in the national durum wheat collection of INAT (Tunisia), two are in the durum wheat collection of Kef (Tunisia) and one from ICARDA durum wheat lines. Most of these lines are landraces collected over the past five years from several wheat growing areas. Twenty five percent of a total of 231 accessions showed acceptable level of resistance to *Fusarium graminearum*. However, the level of resistance is partitioned as following: 4.8% highly resistant, 9.6% resistant and 10.5% moderately resistant. Although resistance to *Fusarium* is available in distant genetic pools, this resistance appears to be unstable and depends upon the prevailing growing conditions. The screening in a large durum wheat collection may provide new sources of resistance. Based on our results, it is apparent that the use of landrace in a breeding program is crucial to improve combined resistance to both *Fusarium culmorum* and *Fusarium gramenearum*.

Powdery mildew research in Morocco and Tunisia

During the last decades, the importance and the economic impact of powdery mildew on wheat was only considered in regions with high rainfall and maritime or semi-continental climate. However with the improvement of cultural practices such as nitrogen fertilisation, irrigation and utilisation of genetically uniform semi-dwarf varieties, the impact and the severity of this disease in the more arid regions increased. During the same period, research moved toward the survey of the physiological specialisation of the pathogen and the identification of sources of resistance. However, the different studies made in Europe and in the United States showed that known genes for resistance that are extensively efficient and potentially useful are few, because of the germplasm exchange and the utilisation of the same related material in different improvement programs. Currently, there is little or no information on the resistance to powdery mildew of durum varieties adapted to North Africa nor on the virulence spectrum of the pathogen. The knowledge of the host's resistance can also be combined to the virulence studies in order to foresee and to anticipate the virulence change in the pathogen populations.

The objective of preliminary studies in Morocco and Tunisia (ICARDA yearly reports 1995 to 1998) were therefore: (i) to characterise the virulence spectrum of the pathogen agent *E. graminis* f. sp. *tritici*; (ii) to postulate for the identity of the sources of specific resistance to the powdery mildew present in the

durum and bread wheat varieties with the utilisation of pathogen pure cultures of known virulence genes and the application of the gene-for-gene hypothesis; and (iii) evaluation of resistance in shared germplasm and local varieties.

Numerous isolates were collected, purified and characterised against international differential variety sets. Physiological specialisation has been found in most collections of pathogen isolates. The reactions of most of the local varieties and shared germplasm have been characterised against the purified pathogen isolates. The determination of virulence genes and resistance genes present in these two countries is ongoing. In the last two seasons, screening of international nurseries has been made using purified or mixed virulent Isolates (ICARDA yearly reports 1995 to 1998).

Barley yellow dwarf virus research in Morocco

Barley yellow dwarf virus has become the most widespread and economically important virus of cereals. The virus is persistently transmitted by several aphid species. Since the chemical control is not effective, the disease is only effectively managed by growing resistant cultivars. Screening for BYDV resistance has been carried out in several Mediterranean countries since 1986. During the 1995-1996 to 1997-1998 growing season, a large amount of durum wheat germplasm from ICARDA/CIMMYT, Canada and Moroccan national program was screened in different experimental stations and in the greenhouse.

All entries were grown in greenhouse flats 5 plants per row. Each entry was planted in a single row. The sensitive cultivar 'Clintland 64' oat was included in each 20 entries of the experiment. Seedlings were then infested for 5 days with viruliferous oat-bird cherry aphids *Rhopalosiphum padi* (L.) which were reared in growth chamber and were carrying PAV-type. After the inoculation feeding period, the aphids were eliminated by spraying with the insecticide (deltametrine 2.5 a.i. g/litre). The BYDV visual score was based on a 0 to 9 rating scale, where 0 was assigned to plants with no symptoms and 9 to plants with severe symptoms.

Compiling the reaction scores observed in all nurseries and in all growing seasons, 1% of the entries had a score between 1 and 3, 33% of the entries had a score between 4 and 6 and 66% were scored between 7 and 9 (ICARDA yearly reports 1995 to 1998). The search for sources of genetic resistance to a pathogen is usually based on a systematic screening of the available germplasm. The reaction of some moderately resistant genotypes could be persistently obtained in more than one growing season.

Tan spot research in Morocco

Tan spot of wheat is caused by *Pyrenophora tritici-repentis.* Tan spot has become important in many countries in recent years. Reports from numerous wheat growing areas and surveys conducted during the last decade in Morocco and Algeria showed this disease was often the first in importance for durum wheat while bread wheat was less affected. Yield losses were shown to range from 12 to 18% under moderate field infestation using the current moderately susceptible cultivars. Even though tan spot is favoured by wet climate, it is important in dryland areas as it develops steadily throughout the season.

The objectives of this study were to systematically screen new germplasm from ICARDAs' key location disease nursery (DKL) and part of the world durum collection. Durum breeding lines and accessions were tested under straw inoculation in the Sidi-El-Aydi experiment station. The DKL nursery was also tested in Rabat Morocco under favourable disease conditions. Triticale checks were planted in every 20 entries. Disease evaluation was made using one of the two scales: (i) 0 to 9 scale for disease severity alone (0 being immune and 9, most susceptible); and (ii) disease severity and leaf coverage 1-9 and 0-100 respectively. Disease assessment was made during the mean post heading growth stages.

The tan spot scores of the triticale check reaction ranged from 1 to 4 depending on the year and site. Reaction of the durum lines and accession were predominantly in the moderately susceptible to highly susceptible classes. The tan spot scores of the DKL nursery showed that all the entries had a disease severity from 7 to 9, meaning that the disease has reached the uppermost leaf at evaluation time. The leaf coverage percent was variable from 25 to 100%. The leaf coverage percent was correlated to the growth stage: late maturing lines appeared to be resistant but became susceptible later (ICARDA yearly reports 1995 to 1998).

Septoria research in Tunisia

Septoria tritici is a prevailing disease causing an important yield reduction in most wheat growing areas in the Mediterranean. Recent surveys in Morocco and Tunisia demonstrated that Septoria glume blotch (*Septoria nodorum*) is becoming a serious concern and causing major grain yield loss in the humid cereal growing areas. Septoria disease affects all classes of wheat, and commercial cultivars are largely susceptible. Resistance is the most appropriate means of control in the southern Mediterranean side countries.

Durum wheat nurseries from the national programs and schools as well as CIMMYT ICARDA nurseries were tested for resistance. Assessment of *Septoria* resistance was based on natural infection at this site. Estimation of the disease severity was based on two scoring techniques:

(i) 0-9 scale (Saari Prescot scale): (0 = No disease,... 7 = Flag leaf diseased, 9 = Spike diseased).

(ii) 0-100%: Percentage of the diseased leaf area. Five scoring data were made with 10 days interval, from tillering stage to grain filling. Grain yield was estimated per m^2.

The results of the different screening (ICARDA yearly reports 1995 to 1998) have shown that there is wide difference in septoria scores. Furthermore tolerance to septoria diseases may be an alternative way of research; lines with same degree of septoria disease may present different levels of yield loss. The data from these evaluation showed that the breeding program in Tunisia possessed material with good level of resistance.

Yellow rust disease in Syria and Turkey

In Syria, yellow rust disease attacks durum and bread wheat and causes considerable losses in yield. Suitable temperature is 5-12ºC with high humidity. Dew is necessary for yellow rust infection. This study aims to identify the fungus races available in Syria, and to find out genotypes tolerant to yellow rust disease. Promising lines and differential varieties for yellow rust were planted in different sites and with multiple replications.

The data showed that there are several yellow rust races and that sometimes, these races do not exist in one area. The disease survey for yellow rust and leaf rust on farm trials showed that the promising lines Douma 20602, Bicre/Cham, Outrob 2, and Syrian 2 are more resistant to leaf rust than the planted cultivars in addition to their resistance to yellow rust.

In Turkey the breeding program is currently using selected pathotypes in artificial field inoculation. Several lines with good resistance to the disease were released (ICARDA yearly reports 1995 to 1998) and others are in the breeders' pipeline.

Common bunt research in Syria

Common bunt disease attacks durum and bread wheat, as well as barley. It causes considerable losses if the seed has not been subjected to treatment. Varieties have differing susceptibility to common bunt. The objective of the work was to identify the resistant material – or less susceptible – to common bunt.

Differential varieties for common bunt (29 entries) were planted in three replicates and grown under natural infection. Another set consisting of 35 durum new releases and promising lines was grown under similar conditions in addition to artificial inoculation. The disease was visually evaluated. The data showed (ICARDA yearly reports 1995 to 1998) that under artificial inoculation there are some promising lines more resistant to common bunt than the planted cultivars.

Wheat stem saw fly research in Syria

The wheat stem saw fly is a spreading insect pest in Syria and causes considerable losses. The objective of this work (ICARDA yearly reports 1995 to 1998) is to identify resistant material to wheat stem saw fly. Eighty-five lines were planted in 2 sites for two consecutive years and stem saw fly damage was

evaluated visually. Several lines with different genetic background have shown resistance to sawfly attack. This trial took place under natural infestation only.

Conclusions

Genetic resistance studies and breeding are more advanced in leaf rust, septoria and yellow rust. For root rot, tan spot and BYDV, there are promising results that should be improved jointly by both breeders and pathologists. Hessian fly resistance program has been ongoing for a relatively short time in comparison to the other diseases but genetic resistance within acceptable genetic background is available and should be combined with other desirable traits in the coming breeding cycles. Genetic resistance to disease is mostly available on separate basis. Work in the future should concentrate on integrating numerous aspects of genetic resistance, other desired traits and providing material with a wider genetic base.

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