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The breeding methodology for the future of durum wheat in Italy

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SUMMARY - Breeding of autogamous crop plants involves the creation of genetically variable populations for selection, population improvement and variety development using different selection strategies. Generally the genetic base is small, therefore its broadening is very important. A survey of several new breeding strategies are discussed.

Key words: Breeding, genetic variability, hybrid, recurrent selection, molecular marker.

***RESUME -** "Méthodologie d'amélioration pour l'avenir du blé dur en Italie". L'amélioration génétique des plantes autogamètes suppose la création de populations pour pouvoir effectuer la sélection, l'amélioration des plantes mêmes et le développement variétal. Généralement la base génétique est petite et pourtant son enrichissement est très important. On va présenter de nouvelles stratégies d'amélioration génétique.*

***Mots-clés :** Amélioration génétique, variabilité génétique, hybride, sélection récurrente, marqueur moléculaire.*

Introduction

The main aim of plant breeders is to obtain a variety with higher grain yield, or a superior biomass production per unit area. A resistance of plant to biotic and abiotic stresses and an increased harvest index are important aspects of the new genotypes. Genetic control of these traits was known since the first green revolution when Nazareno Strampelli, using the Japanese variety Agakomuki, developed highly successful cultivars having short-stature.

For durum wheat, the qualitative and nutritional aspects are particularly important. However, selection for these traits at early generations is very difficult in this species because of strong genotype-environment interactions.

Economical and biological aspects, complicate durum wheat breeding even more. A better understanding of the genetic basis of the characters would promote accelerating breeding processes.

Fig. 1 shows average yields of Italian durum wheat in five-year intervals during the period of 1928-1989. One could see that the period of stability in 1928-1962 was followed by a considerable increase of the average yield in the 70's years; in the last years, the increase slowed down. The period of quick yield increasing was coincident with the diffusion of some new genotypes like *Creso* and *Val series*, while, during the second period, varieties Adamello, Appio, Arcangelo, Duilio, Grazia, Latino, Ofanto, Simeto were introduced. The last varieties had improved agronomic and morpho-physiological characteristics and met new requirements for durum wheat.

Strategies in the breeding of durum wheat

At the present, breeding for new highly-productive genotypes is complicated, firstly, by over-production of durum wheat at the Community level; secondly, by increasing cost of the production, and, thirdly, by the fact that highly-productive varieties appear to be more susceptible to biotic and abiotic stresses and have low technological quality.

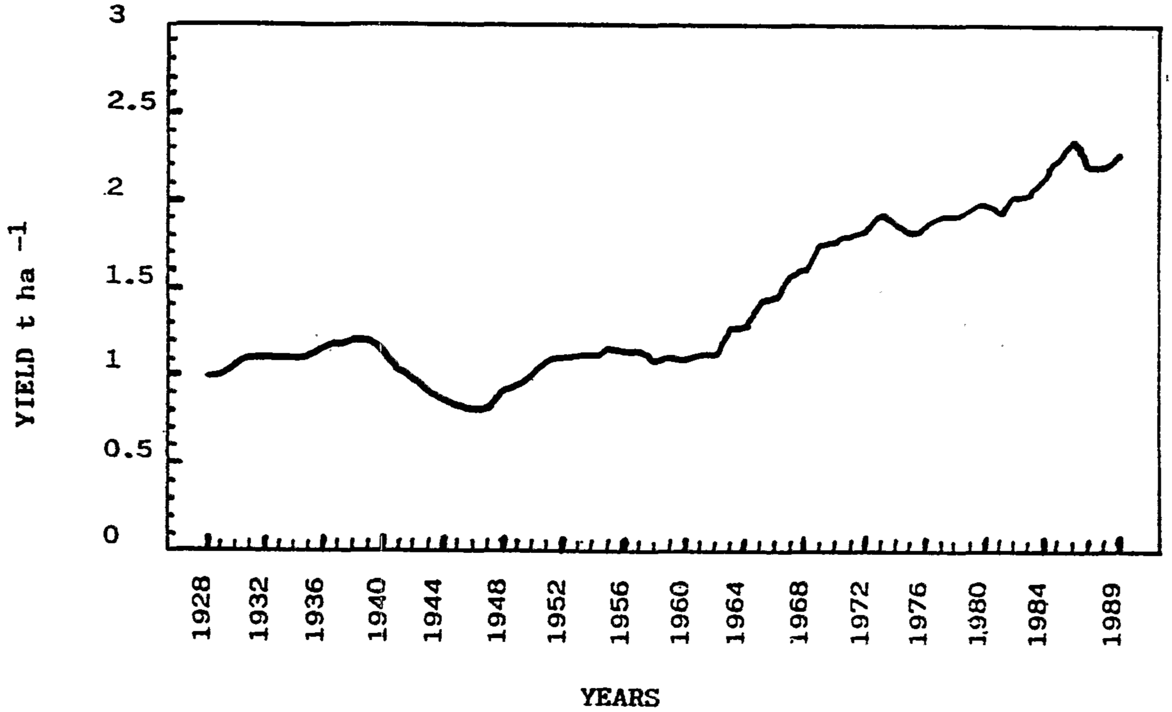

Fig. 1. Durum wheat yield (t ha^{-1}) in Italy in the period 1928-1989 expressed as five-year moving averages, Boggini *et al.* (1992).

The actual purpose is to overcome these problems and to maintain durum wheat cultivation, particularly in the typical Southern areas of Italy, where the drought stress seriously restricts the yield as compared with the Centre and North Italy. This aim can be reached by development of a new ideotype of durum plant. This ideotype should be able to respond to the demands of grain quantity and quality optimal for the Mediterranean region, of the low cost of production and of the resistance to the environment.

Conventional, and, mostly, non conventional breeding strategies would probably allow to attain the objective faster. In this way, distant hybridization, hybrid wheat production, recurrent selection, doubled haploids, plant breeding using genetic markers are proved to be useful tools.

Inter-species, inter-genus hybridization and transfer of useful genes

Species of the genus *Triticum*, wild or cultivated, are closely related and most of them can be crossed rather easily; hybrids between cultivated wheats and the Triticinae (*Agropyrum*, *Haynaldia*, *Secale* and *Eromopyrum*) and Hordeinae (*Hordeum*, *Elymus*, *Treniathernum*) species can be also obtained.

For many years, breeders use inter-species and inter-genus hybridization to transfer genes present only in wild species and genus in the cultivated species of *Triticum*.

A cross between *T. durum* and *T. aestivum* was mostly frequent. This type of hybridization was successfully used also in Italy; the aim was to transfer from common wheat into durum spike fertility, earliness (Maliani, 1964), cold resistance (Rusmini, 1961; Alessandroni *et al.*, 1966), dwarfness (Vallega and Zitelli, 1973). The hybridization with other species of *Triticum* was also used to transfer some main disease resistances (Zitelli and Vallega, 1968) and, recently, to increase the technological quality of wheat through the transfer in durum wheat of genes *Glu-D1* and *Gli-D1*/*Glu-D3* (Ceoloni *et al.*, 1993).

A considerable success was reached in the S. Angelo Section of the Experimental Institute for Cereal Research when cold resistance genes of *Agropyrum* where transfered into durum wheat by means of inter-genus hybridization, so that it became possible to cultivate durum wheat in the North of Italy (Bianchi, 1973).

The use of the inter-species and inter-genus hybridization could be considered as one of the main strategies in durum wheat breeding.

This methodology also implies chromosomal manipulations aiming at transferring to the cultivated species of chromosome regions of limited length. Involving genes ph1 and kr4, facilitating homoeological pairing, simplifies the task (Luo *et al.*, 1993). Many useful results, especially in *T. aestivum*, obtained by using this methodology, have been presented at the last Wheat Genetic Symposium (Bejing, China). Recently the cross *T. durum* x *T. dicoccoides* also gave a remarkable success. Wild wheat *T. dicoccoides* may contribute much into durum wheat because it carries valuable genes conditioning resistance to biotic (several diseases) and abiotic (drought and salt tolerance) stresses, and influencing positively on some agronomic and qualitative characters (for example, providing high protein content) (Pasquini *et al.*, 1992). Joppa and Cantrell (1990) have shown that the *major* gene(s) which increases protein content is located on the chromosome 6B of *T. dicoccoides*.

Recently, electrophoretic analysis allowed to reveal uncommon alleles at the glutenin loci *Glu-A1*, *Glu-B1* and *Glu-B3* in some accessions of *T. dicoccoides* (Ciaffi *et al.*, 1993).

The selection of good alleles for gliadin and glutenin through electrophoretic procedures hopefully can help in obtaining lines of durum wheat characterized by a superior pasta making quality derived from the *T. durum* parent, and a high protein content together with disease resistance derived from *T. dicoccoides*.

By using of inter-genus hybridization among different species of *Triticum* and species of other genus, *Hordeum bulbosum*, *Zea mays* and *Tripsacum dactyloides* poly-haploid lines were obtained (de V. Pienaar, 1993; Mujeeb-Kazi *et al.*, 1993); they were hardly obtainable in *T. durum* by means of *in vitro* culture of anthers.

Hybrids

The discovery of cytoplasms which induce male sterility in wheat and of nuclear genes which allow to overcome this male sterility has allowed a hybrid wheat approach (Maan, 1973).

Recently, chemicals have been discovered which can induce male sterility in wheat. Even with a satisfactory chemical, however, seed production will remain a problem because of low cross-pollination (Borghi *et al.*, 1988).

Most of the work on hybrid wheat concerned *T. aestivum* while very little attention was paid to *T. durum*. However, in Italy, De Ambrogio *et al.* (1989) showed the yield advantage of 19% of the F_1 hybrid durum wheats over the highest yielding inbred variety. These results are encouraging because the parents for these crosses were selected just for the F_1 seed set and not for their combining ability for yield.

More recently, new sources of male sterility were found. Genetic (Jingyang, 1993) and physiological (Jiannan *et al.*, 1993; Murai and Tsunewaki, 1993) male sterility are now available and an improved level of hybrid production may be searched.

Recurrent selection

Recurrent selection is a term which was applied to several breeding schemes. Sprague and Brimhall (1950) described the following steps in their approach called *truncation selection*: "evaluate a series of individual plants for a given character, truncate the frequency distribution at some desired level, and intercross the individuals. This recombination would then serve as a source material for new cycle of selection". Recurrent selection means any form of cyclical selection with the help of which the frequency of desired traits in a given gene pool is enhanced.

Lines selected by techniques which reduce chromosomal recombination to a single meiotic event may have limited genetic variation because they retain blocks of linked (unseparated) genes. These blocks can be eliminated through recurrent selection procedures which continually promotes genetic recombination.

Jensen (1970) has mounted three objections against recurrent selection: (i) the number of genotypes involved in the programme is low, (ii) the genetic variability is reduced because of the limited initial gene pool and limited recombination potential, and (iii) the tendency toward inbreeding, which favours the retention of linkage, is excessive.

After the final intermating cycle, the population may be considered as improved germplasm and used in further breeding programmes or for extraction of individual lines in order to evaluate them as new cultivars. Recently, Chinese researchers (Jiang and Wu, 1993; Leqing *et al.*, 1993; Zongying, 1993) showed excellent results by this technique of selection, especially for resistance to scab (*Gibberella zeae*). They concluded that recurrent selection for scab resistance, when using the dominant male-sterile gene Tal (Ms 2), was feasible and effective.

The authors of this article have initiated a recurrent selection, by using chemically induced male sterility, for: (i) increased genetic variability for yellow index and (ii) genotypes with higher tolerance to drought stress and increased yield stability.

Doubled-haploids

Haploid breeding procedure produces plants with the gametic chromosome number. The chromosome may subsequently double, resulting in homozygous diploids. A number of homozygous plants can be produced from a cross in a single generation. This is the most valuable in breeding, if doubled haploid plants represent a random set of gametes from the parental cross.

The successful culture of microspores derived from the anther wall tissue (not from anthers) was obtained and reported for cereals. Microspores are an attractive target for *in vitro* manipulation, for example, for genetic transformation. The limitations of using this approach for cereals are connected not with methodological aspects but rather with the low efficiency of embryo formation and regeneration of green plants from microspore cultures. In bread wheat, many breeding programmes were set up for obtaining doubled haploid plants from *in vitro* anther culture. However, only a few commercial cultivars have been developed in France and China (Hu *et al.*, 1983; De Buyser *et al.*, 1987). In durum wheat, moreover, the low level of pollen callus induction as well as the extremely low yield, if any, of green plantlets restrict an application of androgenesis in plant breeding (Foroughi-Wehr and Zeller, 1990).

Recently, about 30% of embryoids were obtained in cultured anthers of the durum wheat lines homozygous for the 1BL/1RS wheat-rye translocation (Cattaneo *et al.*, 1991). However, the regeneration frequency was low as compared with the bread cultivar Veery, and only two green plantlets were obtained from 124 androgenetic embryoids in this line. These results suggest, nevertheless, that the 1BL/1RS translocation has positive effect on the androgenetic embryoid induction in durum wheat.

Choo *et al.* (1985) compared haploid breeding with other breeding methods and reached the following conclusions: (i) the pedigree method may be more effective in selecting for characters with high or moderate heritability but is less effective for characters with low heritability, such as yield; (ii) haploid and single-seed descent systems are equally efficient in deriving homozygous lines from F_1 hybrids in a short time; and (iii) haploid methods have no advantage as compared with backcrossing in transfer of one or a few desirable characters to a cultivar which excels in many attributes.

Genetic and molecular markers

Another very interesting way to follow is the genetic transformation. Although the first gene was isolated from a plant cell only 14 years ago and the first exogenous DNA was transferred into tobacco cells 10 years ago, genetic transformation was already successfully applied to dicotyledon species such as tomato, potato, tobacco, cotton, carrot etc.).

Transgenic plants were obtained also in monocotyledon rice, maize and sugar cane. Vasil *et al.* (1992) obtained wheat transgenic plant while Weeks *et al.* (1993) set up a method to transform embryogenic calli of wheat through particle gun bombardment. More recently, Anderson *et al.* (1993) obtained transformed plants with transient expression for bialaphos herbicide in wheat callus subjected to particle gun bombardment.

The current opinion is that recombinant DNA biotechnology will provide a strong contribution to the next green revolution, mainly in terms of environmental protection, productivity and plant protection.

Restriction fragment length polymorphism (RFLP) was firstly proposed by Botstein *et al.* (1980) to be used as a genetic marker. Theoretically, this approach allows to construct a high-resolution genetic map for any crop.

Such a map, being used for analysis of quantitative trait loci (QTLs), can greatly increase the selection efficiency for these traits. DNA markers facilitate the transfer of qualitatively expressed genes, such as those conditioning pesticide and disease resistances, from wild species into a cultivated background.

Mapping and application of RFLPs in polyploids lagged behind diploids being complicated by a number of factors including (i) a large number of segregating genotypes, (ii) comigration of fragments, (iii) poorly characterized genome constitution and/or chromosome pairing behaviour, and (iv) difficulties in genotype characterization due to multiple fragments (Sorrells, 1992).

Construction of linkage maps for diploid relatives has several advantages, especially in the Triticeae where gene synteny is conserved among the three genomes of cultivated wheat and in diploids (Kam-Morgan *et al.*, 1989).

Wheat is amenable to cytogenetic manipulations which facilitate genetic mapping and introgression of alien germplasm. Moreover, nulli-tetrasomic and ditelosomic lines can be used to place a fragment onto a specific chromosome arm by their disappearance in the critical stock. Therefore, virtually all fragments can be located to chromosome arms, whether or not they are polymorphic for a particular mapping population.

In addition, RFLPs can be used to predict, at least in part, heterosis and to identify new genotypes for increasing genetic variation in breeding programmes. Analysis of genome organization and the implementation of map-based cloning of tightly linked genes are other potential applications.

Many factors affect the breeder's choice of gene target, how many are used, and the stage of selection or inbreeding at which they are applied. Lande and Thompson (1990) have elaborated theoretical aspects of marker-assisted selection for improvement of quantitative traits. They concluded that the efficiency of conventional methods of phenotypic selection could be substantially enhanced by combining them with marker-assisted selection. The relative cost of marker-assisted selection at various stages of inbreeding or testing depends on the number of individuals evaluated, and, to a lesser extent, the number of markers used.

Conclusion

Many selection strategies are at the disposal of a breeder who deals with autogamous plant species.

Generally, self-pollination leads to more or less isolated homozygous lines, and recombination must be forced by the breeder. The primary purpose of recurrent selection programmes is to increase the recombination frequency. In the future, routine gene transfer can be of great importance to overcome crossing barriers between species and to make use of genes from other species in a breeding programme. Optimization of the procedure needs an efficient estimate of population parameters such as components of variance and number of genes. Investigation in plant populations have demonstrated quite conclusively the effectiveness of molecular markers for identifying and locating QTLs. Homozygous lines can be developed easily by rapid continuous selfing (SSD). Thus, development of homozygous lines via doubled haploids is not the quickest method in every case.

Generally, only genotypes which prove their potential under field conditions will become good varieties. This test phase (an examination at many places over several years) is an essential and time-consuming part of all breeding programmes.

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