

Use of Heterosis in Maize Breeding: History, Methods and Perspectives – A Review

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ABSTRACT

The hybrid vigor is one of the greatest practical contribution of genetics to the agricultural world and had its most significant expression in maize crops, being intensively explored by breeders and seed production companies. This review presents aspects of the genetic basis for heterosis, biometrical assessment, and improvement methods to obtain heterotic hybrids and reciprocal recurrent selection in maize. Contributions from Biotechnology and Molecular Genetics as tools for hybrid programs to diminish hand pollination work to obtain inbred lines and to form heterotic groups through molecular markers are also reported. Finally, the identification of QTLs to help select superior lines and confirm genetic hypotheses is presented to completely elucidate the heterosis phenomena.

KEY WORDS: *Zea mays*, Hybrid vigor, Breeding.

THE HETEROSIS CONCEPT

Heterosis is the genetic expression of the developmental differences among hybrids and their respective parents. The hybrid vigor is undoubtedly one of the greatest practical contributions of genetics to the agricultural world.

The heterosis concept, defined over a century ago, continues to be applied in the production of several hybrids from different vegetable species. In maize crops, however, the use of heterosis developed in such a unique way that hybridization was recommended as a valuable breeding method. It is unlikely that any other crop species has so significantly benefited from scientific research and presented such a large response to selection.

Prominent scientists, including Sprague and Eberhart (1975); Paterniani (1976); Jenkis (1978); Miranda Filho and Viégas (1987), Hallauer and Miranda Filho (1981); Paterniani and Campos (1999), have studied various aspects of maize hybridization. The competence of these authors makes scientific innovation

difficult to be achieved in this field and, consequently, this review aimed at collecting and compiling information and at reporting on heterosis studies and results recently published.

HISTORY

Kolreuter (1761) apud Brewbaker (1969) reported heterosis on *Nicotiana* sp. hybrids and emphasized two aspects of the phenomenon: a) that the hybrid vigor was related with the parents degree of genetic dissimilarity and; b) that it was important in the evolution, because the plant reproduction system suggests that nature favored natural outcrossing. Darwin (1876) apud Brewbaker (1969) showed that, in general, cross fertilization was beneficial while auto-fecundation was disadvantageous.

Shull (1908, 1909), in his work “The composition of a maize field”, established and used the concept of heterosis in a concise and definitive way, based on repeated observations of hybrid vigor. The author recognized “the complex hybrid” nature of each plant, the increase of homozygosity as a factor of vigor

reduction and the need to search for lines with superior behavior in crosses rather than per se. He also designed a generic procedure to obtain maize single hybrids that is, in essence, still used today: auto-fecundation of a large number of plants to obtain inbred lines; crossing the lines to obtain the largest possible number of hybrids; and experimentally evaluating the hybrids to determine the pair of lines with better performance (Paterniani and Campos, 1999).

Jones (1918) recommended the use of double hybrids from two single hybrids to lower seed costs. Commercialization became possible and a huge increase in hybrid maize acceptance in the USA started.

In Brazil, the first research with hybrid maize began in 1933 at the Instituto Agronômico in Campinas, SP. This work resulted in the production of the first Brazilian maize double hybrid in 1939 by Krug and co-workers. The use of heterosis has been intensively explored since then by breeders and seed production companies.

Paterniani (1974) described the main advantages of hybrids: the association in the same individual of distinct characters that are separated in the parents, obtaining superior genotypes in a relatively short time, taking advantage of gene interactions difficult to obtain and explore through other methods, producing uniform genotypes; and stimulating and promoting the development of the seed industry. On the other hand, he also mentioned the following disadvantages: the best genotypes are very difficult to obtain; heterosis is random (a limit is reached and it is difficult to overcome); it can only be used in species where the process of obtaining hybrid seeds is practicable and has a low cost (or the commercial product is expensive); and the maize hybrid scheme requires a developed social structure for production, processing, transport and commercialization.

GENETIC BASE

Two theories were proposed to explain the causes of heterosis once the hybrid vigor

phenomenon is well established:

1) The theory of dominance proposed by Davenport (1908), Bruce (1910) and Keeble and Pellew (1910) considers that the concentration and the interaction of several dominant genes (non-allelic interaction) in the hybrid is responsible for the vigor. As a didactic example, according to Ramalho et al. (1989), lines with different alleles in various loci are considered. Then,

$$\begin{array}{c} \text{Line 1 X Line 2} \\ \text{Aabbcc X aaBBCC (AA=Aa), (BB=Bb),} \\ \text{(CC=Cc)} \\ \Downarrow \\ \text{Hybrid 1 X 2} \\ \text{AaBbCc} \end{array}$$

The hybrid 1 X 2 presents three loci with dominant alleles, differently from the parental lines. Considering the complexity of quantitative traits like yield, it may be necessary to extrapolate these considerations to hundreds of loci.

The main objection to this theory lies in the fact that there is no homozygous lines as robust as the F_1 hybrids, and, according to this dominance hypothesis, a completely homozygous inbred lined with all loci carrying favorable alleles would have a vigor similar to that of the hybrid. It is known that the expression of vigor and of quantitative characters related to yield is extremely complex and that the number of alleles must be close to a few hundreds. According to Paterniani and Campos (1999), it would be difficult to obtain one completely homozygous line carrying all the favorable alleles even if the allele number was only 10.

A second objection to the theory of dominance was the apparent symmetry in the distribution of the F_2 obtained from the F_1 hybrids (Brewbaker, 1969). The distribution of the observations on metric traits showing heterosis was symmetrical, that is, it resembled the distribution of traits in which dominance did not occur. The critics argued that

under the theory of dominance hypothesis the distribution of the segregating generations should not be normal.

2) The overdominance hypothesis (heterotic genes) proposed by Shull (1909) and East (1936) was based on the hypothesis that heterozygosis itself was necessary for the complete expression of heterosis; in other words, overdominance would bring an advantage to the physiological activities of the hybrid.

In the example, the hybrid would be superior due to the intrinsic heterozygous state of its three considered loci.

$$\begin{array}{c}
 \text{Line 1 X Line 2} \\
 \text{Aabbcc X aaBBCC} \\
 (\text{AA}>\text{Aa}), (\text{BB}>\text{Bb}), (\text{CC}>\text{Cc}) \\
 \text{Hybrid 1 X 2} \\
 \Downarrow \\
 \text{AaBbCc}
 \end{array}$$

The main objection to the theory of heterotic genes is the fact that there is no evidence of heterosis contribution to overdominance ($d/a > 1$) when plant polygenic traits are considered. Also, heterosis does not necessarily involve heterozygosis *per se*.

The presently available results indicate that partial or complete dominance is the main cause of heterosis, and that the results suggesting the presence of overdominance are actually biased by gene linkage (Hallauer and Miranda Filho, 1981). It is possible, however, that some loci display dominance effects and others display overdominance.

It is necessary to take into consideration that the genetic effects in crosses producing superior hybrids can include epistasis, which is often present between genes controlling quantitative traits. Statistically, however, there are no results suggesting that the epistasis is responsible for an expressive amount of the genetic variation. The hypotheses are simplifications of the real situation, which may include complex interactions of all types for the manifestation of heterosis.

Hallauer (1997) alerted that several interactions present within an organism and between the organism and the environment may not allow a full understanding of the phenomena of the hybrid vigor.

TYPE OF HYBRIDS

Several types of hybrids can be produced:

- a) Single hybrid (HS): obtained from a cross between two inbred lines (Line A x Line B). The main characteristics are high uniformity and yield; seed production costs are high because the female plant is from a low yield inbred line.
- b) Modified single hybrid: follows the HS scheme, but uses as female parent the hybrid between two progenies of the same genealogy (A x A') and as male parent a line (B) or a hybrid between similar lines (B x B') of another genealogy. This procedure minimizes the costs of seed production because the female progenitor presents a certain vigor that manifests in a larger production.
- c) Three-way hybrid: obtained by crossing a single hybrid (A x B) with a line of the third genealogy (C). The male line must be sufficiently vigorous to allow interpersing the planting with the female hybrid parent and to produce enough pollen to ensure adequate grain yield in the female.
- d) Modified three-way hybrid: similarly to the modified single hybrid, the modified three-way is obtained by crossing a single hybrid (A x B) with a hybrid from a cross between lines of the same genealogy [(A x B) X (C x C')]. Therefore, its production requires two planting cycles.
- e) Double hybrid: obtained from a cross between two single hybrids (A x B) X (C x D). Its production involves four inbred lines and, consequently, this hybrid shows larger genetic variability (population homeostasis), less vulnerability and plant uniformity and smaller production costs than the former types.
- f) Multiple hybrid: results from the intercrossing of 6, 8 or more lines and does not have any commercial value. It contains a large genetic variability that can result in a wide amplitude of variation and, in advanced generations, it can be used as a source of inbred lines (Miranda Filho and Viégas, 1987)

g) Topcross: obtained from the cross between an inbred line and a variety of wide genetic base and used in hybrid programs to evaluate the lines combining abilities.

h) Intervarietal hybrid: obtained from the cross between two varieties. Although less productive than hybrids from inbred lines, they present the advantage of using heterosis without the labor intensive task of inbred line production, show greater rusticity and are indicated for planting under adverse or low technology conditions.

Comparison among the several hybrid types analyzed under the aspects of yield and production uniformity and stability are found in Miranda Filho and Viegas (1987). Duarte and Paterniani (1998, 1999) have analyzed the commercial hybrids regional adaptation and stability in the main cropping regions in the state of São Paulo.

The use of hybrids contributed significantly to the maize yield increase in the developed agricultural areas of Brazil. Maize is one of the main components of the pig and poultry production chain, reaching, nowadays, international standards in volume and technology.

BIOMETRICAL ASSESSMENT AND MEAN PREDICTIONS

Heterosis (h) or vigor can be defined by the expression:

$$h = \bar{F}_1 - \frac{\bar{P}_1 + \bar{P}_2}{2}$$

where:

F_1 is the hybrid F_1 generation mean;

P_1 and P_2 are the means of parents 1 and 2, respectively.

For each cross, heterosis will be maximum in the F_1 generation at the highest level of heterozygosis. The proportion of heterozigotos in the F_2 generation is reduced to 50% of that of the F_1 because self-fertilization reduces the heterozygote

proportion in 50%. Therefore, the mean of the F_2 generation can be estimated by the expression:

$$\bar{F}_2 = \bar{F}_1 - \frac{h}{2}$$

As $h = F_1 - \bar{P}$ $\left(\bar{P} = \frac{P_1 + P_2}{2} \right)$, then:

$$F_2 = F_1 - \frac{1}{2}(F_1 - P)$$

This procedure was extended by Wright to estimate the average of a synthetic variety, or the F_2 formed by intercrossing n inbred lines:

$F_2 = F_1 - \frac{1}{n}(F_1 - P)$ where, F_1 is the average of all possible F_1 between the lines and P is the mean of all lines.

In the F_3, F_4, \dots generations (selfing series), heterozygosity is reduced to half the previous generation at each selfing and the same happens with the heterosis. For example, the F_3 mean will be:

$$F_3 = F_2 - \frac{h}{4}$$

Generalizing,

The average of generation F_n will be:

$F_n = F_{n-1} - \frac{h}{2^{n-1}}$, where n represents the number of generations.

One of the most important contributions of Quantitative Genetics to plant improvement is the possibility of mean prediction. By estimating the double hybrid means from the single hybrid means and composite means from the parental varieties and their hybrid means, the performance of double and composite hybrids can be predicted without synthesizing them, which is in many cases impossible.

The prediction principle was proposed by Vencovsky (1987) in a clear and didactic way. Assuming the estimation of the mean of the M material, which is the F_1 generation of the cross between X and Y, it can be symbolically written:

$$M = [X] [Y]$$

The M mean can be obtained replacing X and Y for their respective constitutive materials. M can be a population resulting from crossing lines or open-pollinating varieties.

Example 1 – The mean of a double hybrid from inbred lines.

If X is the single hybrid (HS) A X B and Y the (HS) C X D, then:



$$\text{or, } X = (1/2) A + (1/2) B \text{ e } Y = (1/2) C + (1/2) D$$

Replacing X and Y in M, results:

$$M = [(1/2) A + (1/2) B] [(1/2) C + (1/2) D]$$

$M = 1/4(AC+AD+BC+BD)$; which is the well known expression of the double hybrid mean corresponding to the mean of the non-parental single hybrids (Jenkis, 1934).

Example 2 – F_2 generation of a double hybrid. It is a composite or population obtained by randomly intercrossing the plants of a double hybrid. The female and male gametes have the same double hybrid origin. The M mean corresponds to that of a population in Hardy Weinberg equilibrium, and $X = Y$, resulting:

$$M = [X] [Y] = [X]^2$$

As X has the genes of the A, B, C and D lines in equal proportions, the following expressions hold: $X = (1/4) A + (1/4) B + (1/4) C + (1/4) D$ and therefore:

$$M = [(1/4) A + (1/4) B + (1/4) C + (1/4) D]^2$$

$$M = 1/16(A+B+C+D+2AB+2AC+2AD+2BC+2BD+2CD)$$

Now the line means also contribute to the trait mean causing a decrease in it. This explains why yield decreases when seeds from the F_2 generation are used.

Example 3 – Three-way hybrid mean estimation. If X is the three-way hybrid resulting from the cross between (HS) A X B with a third line C, then:

$$M = [X] [Y]$$

$$M = [(1/2)A + (1/2)B]C$$

$$M = 1/2(AB+AC)$$

Example 4 – Mean of a composite formed by intercrossing n different double hybrids.

It is similar to example 2, intercrossing n double hybrids of different lines, then:

$$M = \frac{1}{n}L + \left(1 - \frac{1}{n}\right)F_1$$

Therefore, each line contributes with $1/n$ to the synthetic or composite mean. With a large n , the average of the single hybrids (F_1) predominates, resulting in a potentially good but heterogeneous population. If the double hybrids have common inbred lines, their mean (L) will contribute with more than $1/n$, and M will be lower, especially when dealing with the grain yield (Vencovsky, 1987). The formula above is identical to Wright's. Still according to Vencovsky (1987), the following assumptions are necessary for applying the $M = [X] [Y]$ formula for hybrid mean estimation:

- The M material cannot be endogamic;
- If the parental means are used to estimate the M mean, the parents must be in Hardy Weinberg equilibrium. Parental types in equilibrium can be: inbred lines (in any stage of endogamy), populations and varieties.
- The epistatic effects of the genes must be negligible or nil.

BREEDING METHODS OF SELECTION FOR HETEROSIS

Obtaining and improving the lines

Several types of hybrids can be synthesized, but the most common are hybrids from inbred lines. Selfing is the most used technique to obtain inbred lines.

Borém (1999) described the procedures to obtain controlled hybridization in main Brazilian crops and discussed aspects of flower structure, emasculation or tassel removal techniques and hybridization.

In the alogamous species, selfing involves the selection of a plant and protection of its female inflorescence (before the liberation of the stigma) with a plastic bag. After the emission of the stigma, pollen is collected with a kraft paper bag that is then placed on the ear for pollination, remaining there until harvest. One must be careful to avoid contamination with unwanted pollen.

Selfing leads to line homozygosity, but it only results in genetic improvement if some process of selection is simultaneously applied. To increase the probability of obtaining a superior hybrid it is necessary to increase the frequency of superior genotypes in the population (Miranda Filho and Viégas, 1987). This can be accomplished using intra-population recurrent selection. In the case of hybrids from inbred lines stemming from two populations, the increase in the frequency of superior genotypes is a direct consequence of the increase in the frequency of favorable alleles in the two populations. This can be more efficiently done using reciprocal recurrent selection (Miranda Filho and Viégas, 1987).

The main methods used to obtain inbred lines are:

a) Standard Method

The standard method uses successive self-pollinations and selection is carried out between and within progenies as endogamy increases. Plants are initially selected for desirable agronomic

characteristics, vigor and resistance to diseases and pests. After harvesting, during preparation for the next sowing season, they are also selected for superior ear and grain quality.

The selected ears will be sowed according to an ear-to-row scheme. In the second generation, the best rows and the outstanding plants within these rows will be selected. The process is then repeated forming selection cycles. There is a quick loss of plant vigor in the first selfing generations, with a tendency of stabilization in the subsequent ones, and after seven generations of selfing, the lines are considered pure or homozygous. The reduction of vigor does not occur equally in the different progenies. Certain inbred lines maintain high vigor, while others lose so much vigor that they cannot be reproduced.

Selection of germplasm for selfing is, presently, the most important factor to consider prior to inbred line extraction. The pioneer breeders in the use of maize hybrids did not have any choice other than selfing the varieties cultivated at the time. In the USA, the best varieties were the Lancaster Sure Crop and the Red Yellow Dent. In Brazil, since the early days of maize breeding, large heterosis effects obtained in crosses between the dent (Armour, Amarelão) and flint (Cateto) varieties were noticed.

Based on the genetic diversity, the concept of heterotic group formed by populations was developed. Heterotic groups are formed by populations whose derived inbred lines produce highly heterotic hybrids when crossed. These groups that are presently determined by diallel crosses represent valuable assets for the hybrid breeding programs.

Due to the limited number of rows that can be worked in a program, a careful sampling of the material to be submitted to selfing is required. After polling the opinion of 130 breeders, Bauman (1981) concluded that 500 individuals adequately represent an elite population. This number, however, is subject to a wide variation.

The use of biotechnology can accelerate the process of achieving homozygosity. Petolino (1989) described the method of anther culture, in which the tissue that generates the pollen grain is cultivated *in vitro* to produce regenerated plants. The greatest challenge lies in the low regeneration frequency of duplicated plants.

Presently, the tendency is to reduce hand-pollination in the efforts to obtain heterotic groups through the use of molecular markers in order to accelerate the selfing process.

b) Single Hill Method

The single hill method proposed by Jones and Singleton (1934) is a derivation of the standard method. It recommends the substitution of a row of several plants by a single hill with only three plants from each progeny (from selfing). The method has the advantage of reducing the experimental area and allowing selection of a larger number of progenies. However, progeny evaluation is made more difficult, hampering the selection for important traits as, for example, lodging and disease resistance.

c) Cryptic hybrid

The cryptic hybrid method was proposed by Hallauer (1967) and Lonnquist and Williams (1967), aiming to simultaneously obtain inbred lines and single hybrids. The method requires, therefore, the use of prolific plants where the first ear is crossed and the second selfed. It is based on the assessment of full-sib families (obtained from individual S_0 prolific plants) that are considered as cryptic (hidden) double hybrids. The plants used for crossing are also simultaneously selfed. The full-sib progenies ($S_0 \times S_0$) are evaluated in replicated yield trials to identify the best crosses. The selfed progenies (S_1) corresponding to the best crosses are planted in pairs in the next generation to produce new full-sib families ($S_1 \times S_1$) through crosses between individual plants of each S_1 family pair. These plants are also selfed to obtain the S_2 generation. This process continues until the desirable level of endogamy is attained in the lines that will be crossed to produce the single hybrids

($S_n \times S_n$). Repetition of the scheme allows the development of lines with adequate level of homozygosity and the production of superior single hybrids. These lines can, obviously, be crossed with other lines from different breeding programs.

d) Zygotic selection

Developed by Hallauer (1970), this method diverted from the former (item c) and is used when the breeder has a superior commercial line and wants a new line from a heterogeneous population to obtain a superior hybrid.

According to the description of Miranda Filho and Viégas (1987), plants from this population are simultaneously crossed with the standard line (L) and then selfed. The full-sib progenies ($S_0 \times L$) are evaluated in the same way as in item c. The S_1 progenies corresponding to the best crosses are again crossed with the standard line and selfed. The process continues until the desired endogamy level for the new line used to obtain the hybrid ($S_n \times L$) is attained.

This method can also be applied to obtain a three-way hybrid using a single commercial hybrid instead of the line as the recurrent parent.

Line breeding

a) Backcross method

The lines can present problems (susceptibility to diseases and lodging, for example) that should be corrected by the breeder. As the practice of selection is not possible because they are homozygous (uniform) genetic material, introduction of new traits should be done through backcrossing. Once a line is crossed with another marker that carries the desired trait (not present in the line), a series of backcrosses are done aiming to recover the original line genotype. Backcrossing is continued for three or more generations under selection, with the original line as the recurrent parent. This method is convenient for breeding simply inherited traits into an already stable line. If the trait of interest is not completely dominant, selfing and crossing should be alternated to guarantee the maximum expression of the gene

introduced in the line. Experimental data showed an improvement in performance of the recovered line and of the derived single hybrid (Sprague and Eberhart, 1975).

Some problems are detected in the method; for instance, the time delay due to backcrossing and a possible unwanted alteration in the recovered line performance. The backcross method is, however, the best way to improve extensively used lines.

b) Gametic selection

This process was suggested by Stadler (1944) for line substitution in superior hybrids but can also be used to obtain better lines (Lonnquist and McGill, 1954). The unit of improvement is the gamete.

The method consists in crossing an elite-line with a random sample of pollen from an open-pollinating variety. Therefore the F_1 plants which differ from the gametic complement of the variety are selfed and crossed with a tester. Crossing the elite-line with the tester serves as control. Any testcross showing better performance than the control will then have received a superior gamete from the variety.

Gametic selection has a disadvantage: the superior gametes identified cannot be isolated like homozygous zygotes.

The technique can also be applied to substitute a line (A, for example), in the double hybrid (A x B) X (C x D) as follows:

- 1) cross plants of an open-pollination variety or hybrid with line A;
- 2) self the selected F_1 plants. Cross these same plants with the C X D hybrid. Cross the original line A with C X D to be use as a control;
- 3) evaluate the hybrids obtained in item 2. Select the progenies (A x variety) with better performance to continue endogamy increase. Self the selected plants of the S_2 progenies;
- 4) self the selected lines. Evaluate again the hybrids obtained in the second and third year;

5) continue selfing the selected lines until homozygosity is achieved;

6) use the new line (E) in the original hybrid, that then will become (E x B) X (C x D).

This method is practically an assessment of the line combining ability, where the variety is used to increase the genetic base of the available material.

Line assessment

There is a reasonable diversity of techniques used to evaluate inbred lines and select an ideal tester. The assessment of a line must reflect its performance in hybrid combinations.

The original hybrid breeding methods were used to assess the lines when they already had a high level of homozygosity (in S_6 or S_7). Jenkins (1935) showed that "the lines attain their individual characteristics as parents in topcrosses very early in the process of endogamy increasing and remain stable from then onwards". Therefore, a tendency of anticipating the assessments followed. Sprague (1946) concluded that breeders should apply the early test in S_1 generation lines.

The early test differs from the selfing process in two aspects: 1) the plants S_0 are crossed with a tester simultaneously with the first selfing to allow the assessment of the combining ability and the general performance of the topcrosses; 2) the first selection and discard of materials allows the concentration of a greater breeding effort in the most promising S_1 and S_2 generation families, when there is a better chance to practice within line selection. This test is based on the fact that there is a large variation in the combining ability of different plants within a single population, and that sample selection based on general combining ability (GCA) provide better progeny selection results than phenotypic selection.

Richey (1927) argued against the effectiveness of the early test based on the following arguments:

- a) The testcross allows the assessment of a line performance at any endogamy level;
- b) The performance of a selfed progeny is not a good indicator of the combining ability before elimination by selection of the recessive major

genes of small frequency. The elimination of the recessive genes and the increase in homozygosity will render more reliable selfed progeny tests.

The text above shows that the line assessment method depends largely not only on the development phase of the maize hybrid program but also on the individual preferences of the breeder. An intermediate approach would be to practice visual selection during one or two years of selfing, and to perform combining ability tests in the S₃ generation. The best progenies would be reevaluated in S₄ or S₅.

The tester choice is another interesting subject that generates controversy between breeders. In general, the theoretical studies and experimental results lead to the choice of either a homozygous recessive tester or a variety with low frequency of important genes (Miranda Filho and Viégas, 1987). In practice, the tester also depends on the stage of development of the hybrid program.

Reciprocal recurrent selection

This method of interpopulation improvement has the objective of improving the reciprocal (in relation to each other) combining ability between two populations, aiming at improving them and their crosses to obtain better lines and superior hybrids.

Comstock et al. (1949) proposed the scheme of reciprocal recurrent selection (SRR) in which two populations are selfed. The S₁ progenies of each population are crossed with the other population and the derived hybrids are evaluated in competition trials. The best S₁ progenies of each population are identified and intercrossed to produce the next cycle of the respective improved population. The following scheme is used:

1) Simultaneous selfing of S₀ plants - the A and B populations. The selfed plants are used as the male

parent in crosses with a 4 to 5 plant sample of the other population;

2) Progeny assessment in competition trials;

3) The best S₁ progenies of each population are selected based on the test results and recombined to obtain the two first cycle A₁ and B₁ populations;

4) Beginning of a new cycle repeating the indicated steps.

Although rarely used, the reciprocal recurrent selection was accepted as theoretically correct under the genetical point of view.

Paterniani and Vencosvsky (1977), however, detected some practical limitations of the method:

1) Intensive labor: simultaneous selfing and crossing requires a lot of work and prolific populations;

2) Poor tester sampling: a sample of 4 to 5 plants cannot adequately sample the tester, diminishing the precision of the method;

3) Large time intervals between the cycles: leading to a smaller gain per year and a larger genotype X year interaction. This item can be minimized considering the possibility of out-of-season maize planting;

Considering these limitations, the following changes in the method of SRR were suggested:

a) Half-sib progeny reciprocal recurrent selection method

The method proposed by Paterniani and Vencovsky (1977) follows the steps presented in Figure 1.

First generation: half-sib families (open-pollination ears) of the A population are sowed in a ear row scheme in isolated plots and are emasculated (male inflorescence removal). Plants from the B population are used as male parents. In another isolated plot, the reciprocal sowing is carried out; families of the B population are sowed as female parents (emasculated) and plants from the A population are used as male parents. Three to five-meter rows containing 15 to 20 plants are sufficient in the 3:1 (female/male) row scheme. Around 200 half-sib progenies are used to represent each population.

Second generation: trial for assessment of the A x B and B x A half-sib families.

Third generation: based on the trial results, the best half-sib progenies from the A population (around 10 to 20%) are recombined using remnant seeds to obtain the A_1 population. The B_1 population is obtained similarly. The recombination can be done through hand-pollination of the selected progenies or in isolated plots using plant emasculatation of the selected progenies to be used as female parents and a mixture of the pollen of these selected progenies as male parents. Selection within male rows can be done through elimination of inferior plants before flowering while females can be submitted to selection during harvesting.

Steps 1, 2 and 3 are repeated to produce a second cycle, using new half-sib samples (around 200) from the A_1 and B_1 populations obtained from the female lines of the recombination plots. This scheme is simpler and less demanding than the original one since it does not require hand-pollination. The small sample problem was also solved and the selected progeny recombination process is more efficient.

b) Reciprocal recurrent selection with half-sib progenies from prolific plants

A new modification in SRR was proposed by Paterniani and Vencovsky (1978) adding other advantages to the method and using half-sib progenies from prolific maize plants. Prolificacy allows two types of progenies to be obtained from the same plant. Also, a significant progress is expected since prolificacy and yield are positively correlated traits.

The scheme follows the steps presented in Figure 2.

First generation: two isolated emasculatation plots are simultaneously sowed; in the first the A and B populations are used as female and male parents, respectively, and, in the second, B as female and A as male (reciprocal). At flowering, plants from the female rows must have their second ear protected before the stigma protrudes. Ears are counted from the top; the first ear is the highest and the first to flower, followed by the second and third.

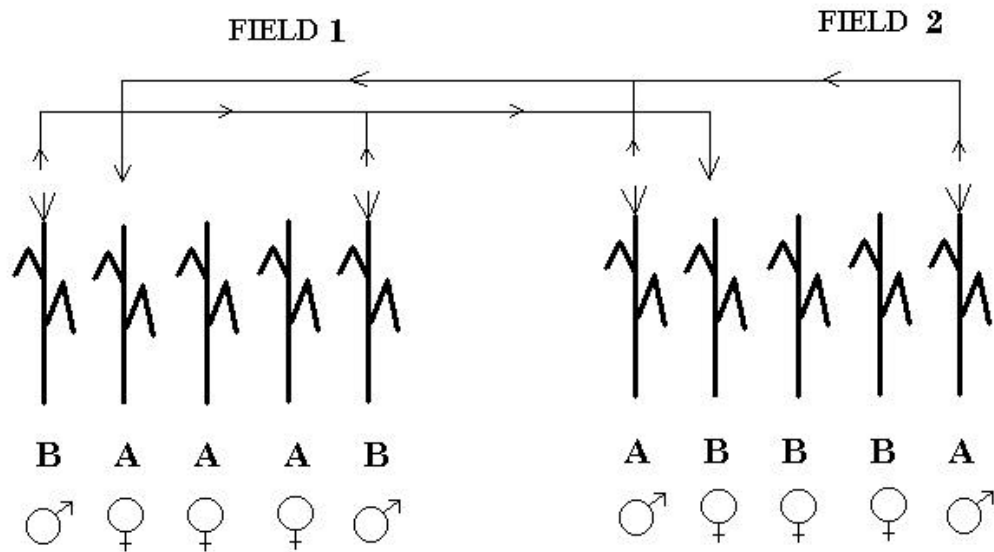
In each field, the first ears will be naturally pollinated with pollen from the contrasting or reciprocal population. The second ears will be pollinated with a pollen mixture from male rows of prolific plants from the other field. A mixture of pollen from around 50 plants of the male row (A, for example) is used to pollinate the protected (A) ears of the other field. Similarly, pollen from around 50 B plants is used to pollinate the protected (B) ears from the other isolated field. It is recommend to select the pollen source plants.

Two types of progenies are obtained: a) interpopulation half-sib progenies (first ear, open-pollinated) and b) intrapopulation half-sib progenies (second ear, hand-pollinated), which will be used in step 3, according to the combining ability result of the plant with the contrasting population.

Second generation: the intrapopulation progenies (A plants x B population and B plants x A population) are evaluated in yield tests. Based on these results, plants showing higher combining ability with the contrasting population are identified.

Third generation: seeds of the intrapopulation progenies (second ears, hand-pollination) corresponding to the plants showing superior combining ability are planted in new isolated plots to begin the next cycle. In the first plot, the female rows are A_1 and the male rows are B_1 , while in the second, the female rows are B_1 and the males A_1 . Following the procedures described for the first generation, selected progenies are recombined and new crosses are done to allow combining ability assessment.

1st Generation:



♀ = ear (half-sib progenies) sowed in rows.

♂ = Mixture of seeds from naturally pollinate ears of the contrasting population.

2nd Generation: interpopulation (crossed with the contrasting population) half-sib progeny trials.



Third generation: similarly to the first generation, seeds from hand-pollinated ears (intrapopulation half-sibs) of the selected plants that produced the best progenies are used to begin the 2nd cycle.

Figure 2 - Graphical scheme of the reciprocal recurrent selection method with half-sib families from prolific plants.

The populations obtained after each cycle of selection can be immediately used as line sources, pairwise crossing or can be submitted to further reciprocal recurrent selection cycles.

The following advantages are observed in this scheme: plants are submitted to selection every year; prolificacy in one year and combining ability in the following. Therefore, the improved populations must be more productive per se (due to selection for prolificacy) and also show high combining ability.

The scheme is manageable and allows testing a large number of genotypes. Paterniani and Vencosvsky (1978) reported results of three cycles of SRR, with yield progress ranging from 3.1 to 6.2%.

Some recent SRR works show the efficacy of the method: Landi and Frascaroli (1995) used two synthetic varieties submitted to two cycles of SRR based in a selection index and obtained a 4,9%/cycle progress in the interpopulation due to the 12,2%/cycle in grain yield. Sawazaki (1996) applied half-sib progeny SRR on the SAM (South American Mushroom) X IAC 64) popcorn interpopulation. The mean interpopulation heterosis for yield (PG) was of 47.65%, suggesting a high between population genetic diversity.

Betrán and Hallauer (1996) applied nine cycles of SSR on the Iowa Stiff Stalk Synthetic (BSSS) and Iowa Corn Borer Synthetic $n^{\circ}1$ populations and observed that interpopulation single hybrids yielded 54.5% more than the single hybrids obtained from the original populations. Menz and Hallauer (1997) submitted the Tuxpeño and Suwan 1 maize varieties to SSR aiming an increase in genetic diversity through the introduction and adaptation of tropical material. The predicted gains in the first cycle of SRR was of 1.42 t.ha⁻¹ (24,3%) in yield and - 5,3% (18,8%) in grain moisture reduction.

GENERAL AND SPECIFIC COMBINING ABILITY

Commercial production of hybrid maize requires extensive assessment of inbred lines and the diallel

cross method is widely-used by breeders with such a purpose.

Griffing (1956a) defined the diallel cross as a complex of n^2 possible genotypes obtained from n pure lines. These n^2 genotypes are: a) the inbred lines; b) the $n(n-1)/2$ F_1 hybrids and; c) the complex of $n(n-1)/2$ reciprocal F_1 hybrids.

The assessment of the lines in the diallel cross proposed by Griffing (1956a, 1956b) is based in the general and specific combining ability concepts developed by Sprague and Tatum (1942). These latter authors defined general combining ability of an inbred line as the average performance of such a line when in hybrid combination and the specific combining ability as the quality that makes certain hybrid combinations superior or inferior to the average performance of the other tested lines.

The combining ability is not a fixed property of a line, depending on the genetic constitution of the tester population used.

Griffing (1956a, b) considered four types of diallel tables. The complete diallel includes the parents and their hybrids and reciprocal hybrids, while the other three are derived from the suppression of some of the components, such as the parents or the reciprocal hybrids. A methodology of analysis was developed for each situation aiming to obtain information on the combining ability of the involved parents.

The growing interest for open-pollinating varieties and intervarietal hybrids is encouraging the development of new genetic models for the analysis of the diallel table means, involving a fixed group of random mating parental varieties and their crosses (Gardner and Eberhart, 1966; Eberhart and Gardner, 1966). These methods allow for the assessment of the combining ability of pure line, lines with any level of endogamy or open-pollinating varieties.

One of the biggest problems faced by maize breeders working with hybrids from lines has been

the evaluation of the n parental lines. For large n values, the assessment of all the hybrids become impracticable because the number $[(1/8).(n-1).(n-2).(n-3)]$ of possible double hybrids is enormous. The topcross method (Davis, 1927), which consists in the assessment of a large number of lines with a common tester, can be used to avoid the problem.

The use of single hybrids as testers is a largely used process because it allows the assessment of a large number of lines and also provides information of more immediate use (Miranda Filho and Viégas, 1987). The greatest limitation of the topcross method is that it only provides information on the combining ability of the lines with the tester, and not about the combining ability of a line with the others. In practice, the complete diallel limits the number of materials to be analyzed because of the number of hand-pollination required to obtain all the necessary crosses.

The partial diallel method (Kemphorne and Curnow, 1961) proposed the analysis of a sample of the possible crosses between the lines of a population. The new partial diallel scheme substituted the intrapopulation diallel crosses using different populations to obtain the hybrids.

Vencovsky (1970) suggested the assessment of the general and specific combining abilities of two sets of varieties, according to a method similar to Griffing's Method 4 (Griffing, 1956b) where only intravarietal hybrids are evaluated.

Miranda Filho and Geraldi (1984) proposed a model to analyze the partial diallels between distinct groups of varieties as an adaptation of the complete diallel analysis of Gardner and Eberhart (1966). An adaptation of the Griffing's Method 2 (Griffing, 1956b), where the variety means and the inter-group hybrid means are evaluated was presented by Geraldi and Miranda Filho (1988).

Still trying to solve the problem of evaluating a large number of genotypes, studies on circular partial diallel analysis were carried out for assessment, where one group of n lines is crossed with another

group of s lines resulting in ns hybrids (Gonçalves, 1987; Dantas, 1992, 1988; Andrade, 1995). The results indicate that the methodology is highly efficient in practice to identify promising single, double, three-way and intermediate hybrids.

PERSPECTIVES OF HETEROSIS AND THE BIOTECHNOLOGY CONTRIBUTION

The diallel cross allows estimation of the general and specific combining ability of the lines, but the labor-intensive characteristic of the method, which requires hand-pollination and hybrid testing, renders it impracticable as the number of lines increases.

Molecular biology methods have been presented as a quick and efficient assessment alternative of the between lines genetic diversity in order to provide information on heterotic groups for synthesis of new hybrids (Lanza et al., 1997).

The molecular marker technique allows the assessment of the between- lines genetic divergence in the laboratory. This data can be used to identify those lines that could generate superior hybrids. The molecular markers analyze the genetic diversity directly at the DNA level and, therefore, are not subjected to environmental effects.

Various classes of molecular markers were described and those most used in plants for genetic map construction, mapping of genes of interest and study genetic diversity are: RFLP (Restriction Length Fragment Polymorphism); RAPD (Random Amplified Fragment Polymorphic DNA); micro-satellites and AFLP (Amplified Fragment Length Polymorphism).

ESTIMATION OF THE GENETIC DIVERSITY BETWEEN MAIZE LINES USING MOLECULAR MARKERS

Since the pioneering work of Shull (1909), several studies have showed consistent evidence that the cross between genetic divergent lines frequently produces superior progenies. Positive correlation between the genetic diversity of the parents with the

grain yield of the F₁ generation and with the level of heterosis in maize was detected by Moll et al. (1962, 1965) and Paterniani and Lonquist (1963).

Estimates of genetic diversity and selection of highly divergent lines in maize hybrids suggest that the RFLP and RAPD data can detect "pedigree" relationships between the lines, correlation with yield data and can also be used to group the lines in different heterotic groups (Lee et al., 1989; Godshalk et al., 1990; Melchinger et al., 1990a; Melchinger et al., 1990b; Smith et al., 1991; Messmer et al., 1991; Dudley et al., 1991; Livini et al., 1992; Lanza et al., 1997).

Around 500 markers well-distributed in the genome RFLPs are presently mapped in maize (Helentjaris et al., 1988; Burr et al., 1988; Coe et al., 1988) and they reveal a high level of polymorphism in the elite germplasm (Smith et al., 1990; Melchinger et al., 1991; Boppenmaier et al., 1992).

However, most of the work was conducted with a narrow genetic base material (line of tempered climate) and it is necessary to expand the study to include materials with wide genetic base (lines from tropical climate regions).

The micro-satellites, also known as SSR (Single Sequence Repeats), consist in a subclass of repetitive DNA formed by short sequences that were successfully used in maize in genome mapping (Chin et al., 1996) and diversity (Ramash et al., 1995) studies.

The AFLP markers are also obtained and detected after a PCR reaction (Polymerase Chain Reaction) involving primers marked with radioactivity (Zabeau and Vos, 1993; Lin and Kuo, 1995). In general terms, the AFLP technique can be considered a greatly improved RAPD, where up to 100 loci (around 10 times more than with RAPD) can be sampled in one single, high resolution and reproducible gel. Smith et al. (1994) detected a high correlation between AFLP data and F₁ yield, heterosis and pedigree relationships and RFLP data of maize lines.

In general, markers have been presented as a quick and efficient alternative to assess line diversity. They are used to predict line performance in single hybrid combinations in the laboratory, before the field assessment, thus helping to reduce the time, quantity of resources and efforts allocated for hybrid evaluation.

QTL MAPPING

QTL's (Quantitative trait loci) can be defined as loci where the various genes responsible for the expression of a quantitative trait are located. These loci are characterized by a continuous distribution of the phenotypic variation and by the large influence of the environment on the trait expression.

In maize, the analyses of the QTL's with molecular markers based on saturated linkage maps allowed the identification of chromosome regions related to morphologic traits of agronomic interest, such as: drought tolerance (Ribaut et al., 1997); plant height and time of flowering (Khairallah et al., 1998); heterosis and genotype X environment interactions (Stuber et al., 1992).

The identification of the QTL's involved with the expression of maize yield components was reported by Stuber et al. (1992), Ajmone-Marsan et al. (1995, 1996), Austin and Lee, 1998, Frova et al., 1999, using germoplasma from temperate regions. The estimated number of QTL's ranged from three to eight and the expected magnitude of any QTL genetic effect varied from 5% to 35% of the phenotypic variability. In these studies, the distribution of the genetic effects associated with the QTL's controlling grain yield suggest the presence of a single loco or a few loci with large effects associated to other additional QTLs of relatively small contribution to the phenotypic variation.

In the tropical germoplasma, the yield QTLs identified by Ribaut et al. (1997) were unstable in relation to the genome location and the percentage of the explained phenotypic variance. Recent studies with tropical germplasm have been conducted in

Brazil with the objective of improving the precision of detection and location of QTLs related to the maize grain yield.

Besides providing important contribution to the breeding programs and improving the selection process of superior lines, the QTL mapping can help elucidate the phenomena of heterosis and confirm the hypotheses proposed to explain the genetic causes of hybrid vigor.

RESUMO

Utilização da Heterose no Melhoramento de Milho: Histórico, Métodos e Perspectivas – Uma Revisão

O vigor de híbrido é uma das maiores contribuições da Genética à agricultura mundial e foi na cultura do milho que a heterose teve sua maior expressão, sendo intensivamente explorada por melhoristas e empresas produtoras de sementes. Na presente revisão, apresentam-se aspectos da base genética da heterose, avaliação biométrica, métodos de melhoramento visando à obtenção de híbridos heteróticos e de seleção recorrente recíproca em milho. Relata-se ainda sobre a contribuição da Biotecnologia e da Genética Molecular como ferramentas aos programas de híbridos, visando à redução de trabalho de polinizações manuais para obtenção de linhagens e à formação de grupos heteróticos através de marcadores moleculares. Finalmente, aborda-se a identificação de QTLs, que poderá ser útil na seleção de linhagens superiores e na confirmação das hipóteses genéticas para elucidar completamente o fenômeno da heterose.

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