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Pollen selection for selection of genotypes against different stress environments

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Abstract

In nature, pollen selection plays major role in evolution than ovule selection. Pollen selection can be defined as "application of selection pressure or stress to pollen grains (Gametophytic phase), so that, only those pollens carrying the desirable allele for the respective trait will be selected for fertilization, there by increased transmission of desirable alleles for targeted traits from gametophytic to succeeding sporophytic generation is achieved. The principle behind pollen selection is pollen competition. Whenever there is competition among the pollen grains lying on same stigma, only those pollens carrying the desirable alleles will be allowed for fertilization. Pollen selection is more effective than sporophytic selection.

Keywords: Pollen, Gametophyte, Haploid, Sporophyte

Introduction

A generally plants have two phases, sporophytic or diploid and gametophytic or haploid, which alternate in the life cycle. Research in plant breeding has been always directed towards the sporophytic phase, mainly because of ease of visualizing the traits under selection. But, selection at sporophytic phase has many constraints like:

1. Small population size decreases the efficiency of sporophytic selection.
2. Handling large set of population by sporophytic selection is difficult.
3. Requires several seasons or cycles of selection to improve any trait.
4. Developing critical selection environment is difficult at field level.
5. Can evaluate a plant for one stress at a time or per generation.
6. Selection is based on phenotype, which may involve environmental effect.
7. Selection for recessive allele is difficult.
8. Involves high cost, space & time.

The influence of the gametophytic phase is often forgotten, usually playing a "passive" role in most plant breeding methods. The concept of gamete selection for the first time was given by Stadler in 1944. It has only been in the 1970s that the possibility of gametophytic selection in plant breeding was put forward. The key moment was in 1979 when Mulcahy suggested the importance of gametophytic selection in the evolutionary progress of angiosperms. Since then, the use of gametophytic selection in plant breeding has developed, first by studying the overlap in the genetic expression between the sporophyte and the gametophyte and, later, by assessing the similarity in behaviour between the two phases in relation to different external stresses.

The basis of the gametophytic selection theory is that selection among haploid and heterogeneous male gametophytes could be positively correlated with changes in the following sporophytic generation (Ottaviano *et al.*, 1990). Therefore, pollen would not only act as a transmission vector for the genome, but also be an independent organism expressing its own genetic information.

Gametophytic selection is of two types

- A. Male gametophytic selection/ pollen selection
- B. Female gametophytic selection/ovule selection.

In nature, pollen selection plays major role in evolution than ovule selection. Pollen selection can be defined as "application of selection pressure or stress to pollen grains (Gametophytic phase), so that, only those pollens carrying the desirable allele for the respective trait will be selected for fertilization, there by increased transmission of desirable alleles for targeted traits from gametophytic to succeeding sporophytic generation is achieved.

Pollen selection has many benefits over sporophytic selection, like:

1. Large population size of the pollens allows screening of single plant for many stresses at a time.
2. Ease of handling large population.
3. Large population can be evaluated in very short period.
4. Haploid nature of the pollen cells allows selection in favour of recessive alleles as they are not masked by dominant alleles at haploid stage.
5. Pollen expression is independence from the maternal effect.
6. Drastically reduces the number of selection cycles in crop improvement programmes: alleles can be fixed in few generations, increases frequency of advantageous alleles in the progeny.
7. Allows the choice of rare favourable allelic combination that would be hardly detected using sporophytic selection.

The principle behind pollen selection is pollen competition. Whenever there is competition among the pollen grains lying on same stigma, only those pollens carrying the desirable alleles will be allowed for fertilization. If there is no pollen completion, then all the pollen grains have equal chance to enter in to fertilization, thus there would be no pollen selection operating. Pollen competition can be measured by four different components as:

- a) Fertility
- b) Viability
- c) Germination time & per cent
- d) Pollen tube growth rate

Among these four components, pollen tube growth rate is the best measure of pollen competitive ability as all pollen grains lying on same stigma may germinate, but may differ in tube growth rate.

Competition among pollen grains: pollen selection does occur in nature?

Pollen selection is not a man invented concept. Nature itself has developed and adopted this concept for its own evolution. Competition among pollen grains could lead to pollen selection in vivo (Mulcahy and Mulcahy 1987). The evidence we have so far is indirect, and is based on both unexpected segregation according to Mendelian ratios and correlation between pollen competitive ability and sporophytic vigour.

- a) Under intense pollen competition unexpected segregation for different genetic traits is obtained (Robert *et al.* 1989). While this fact has been repeatedly observed, there is no clear explanation of how it is produced. Since there is no male genotype that performs better in all crosses, it may be due to a selective action induced by the pistils, which lead to different kinds of pollen-pistil interactions within the crosses.
- b) Likewise, under intense pollen competition, a decrease in genetic variability of the offspring occurs, leading to a lower frequency of the extreme genotypes (Schlichting *et al.* 1987). There is substantial information supporting this view in several species: *Gossypium*, *Vigna*, and *Triticum*, *Dianthus chinensis*, *Raphanus raphanistrum* and *Cucurbita pepo*.
- c) Furthermore, a number of reports have demonstrated a positive correlation between pollen competition ability, evaluated mainly through pollen tube growth rate, and several sporophytic traits such as: seed dry weight, seedling weight, number of seeds per fruit, and root growth (Winsor *et al.* 1987). All these reports have as a

common factor the fact that the higher the pollen tube growth rate, the more vigorous the offspring. Thus, genes that affect sporophytic vigour could also be affecting pollen tube growth or, in other words, pollen tube growth and several sporophytic traits would be related genetically.

Levels of Pollen completion in nature:

Pollen competition could take place at two levels in nature: either through a direct competition among haploid gametophytes (Willson and Burley 1983) or through an interaction between the haploid and diploid genes (Mulcahy 1979).

A. Direct competition between gametophytes could be of two kinds:

1. Physical competition based on the rate of pollen tube growth
2. Chemical competition based on pollen inhibition. This allelopathic phenomenon has been recorded on an intergeneric basis in the genus *Parthenium*, where pollen grains contain germination and growth inhibitors for pollen of other genera (Kanchan and Jayachandra, 1980), it is unknown whether this phenomenon operates on an intraspecific basis or not.

B. Indirect competition: Indirect completion between pollen grains is through interaction between the male and the female tissue *i.e.* stigma-pollen interaction. Pollen tube kinetics are affected by the microenvironment generated in the pistil so that the growth of particular pollen tubes would be favoured giving them an advantage in achieving fertilization. This could explain the variations found in the progeny in relation to the female parent used. In natural conditions, plants could show a trend towards the enhancement of pollen competition by increasing the style length or by increasing the period of time between the arrival of pollen grains on the stigma and fertilization. However, the evidence we have so far is mainly indirect and circumstantial, although it may hint that gametophytic selection does actually operate in natural conditions.

Requirements of pollen selection:

Pollen selection has three important requirements to be satisfied for its success which are as follows;

- a) Expression of target gene in pollen.
- b) Overlap in the genetic expression between the sporophyte and gametophyte phases.
- c) Correlation between sporophytic and gametophytic responses to external factors.

Other are: Genetically diverse or heterogeneous pollen population (pollen fertility, germination, tube growth rate); Absence of epigenetic effects.

A. Expression of target gene in pollen:

Basically, we can find three kinds of genes, according to their expression in the two phases, gametophytic and sporophytic phase: (1) genes expressed only in the sporophyte (diploid expression); (2) genes expressed only in the gametophyte (haploid expression); (3) genes expressed in both gametophyte and sporophyte (haplodiploid expression). The third group contains the genes suitable for pollen selection. Actually, this could also be extended to the genes expressed only in the sporophyte, but which are closely linked to genes expressed in the gametophyte.

B. Overlap in the genetic expression between the sporophyte and gametophyte phases:

As pollen selection requires genes showing haplo-diploid expression, such genes can be identified by using different methods as follows;

a. Isozyme expression: Initial work in this field was carried out by comparing the genetic expression of single enzymes in the two phases of the plant life cycle, but without considering the possibility of a gametophytic selection. One of the first studies pointed out that the activity of alcohol-dehydrogenase in maize (*Zea mays*) is expressed both in the sporophytic and gametophytic phases (Schwartz 1971). Later, studies began to compare the genetic expression of different isozyme groups between the two phases. The initial research in this direction was limited to comparative studies of the expression of specific enzymes of the diploid and the haploid phases; overlap between both phases was found. There is no doubt that the key moment was in 1981 when Tanksley *et al.* published their work on tomato (*Lycopersicon esculentum*) isozymes. They looked at 9 genes and concluded that 62% of the isozymes studied were expressed in the gametophyte, 58% were expressed in both phases, and 3% only in the pollen grains. Further work, with different enzymatic groups and different plant species, supports these initial results. The overlap rates seem to be very similar. For example, 72% in *Zea mays*, 81% in *Prunus*, 60% in barley, and between 74 and 80% in *Populus*.

b. Nucleic acid overlap (cDNA-mRNA hybridization): Initial work in this field was restricted to RNA studies. It was shown that a part of the pollen mRNAs is presynthesized in the sporophytic phase; these mRNA are useful during the development of the pollen grain and the beginning of the pollen tube growth. Later, the hybridization between mRNAs synthesized by the gametophyte and cDNAs from the sporophyte was studied in *Tradescantia paludosa* (Willing and Mascarenhas 1984). These authors found about 30,000 different mRNAs in the sporophyte tissues and 20,000 in the gametophyte. Hybridization results confirm those of the isozymes: about a 60% of the sequences analyzed were expressed in both gametophytic and sporophytic tissues. However, these studies were done using only shoot tissue from the sporophyte; therefore, by studying additional diploid tissues, the percentage of overlap would presumably be increased.

c. Overlap in composition of particular biochemical substances: Some evidences indicate that there may also be an overlap in the composition of particular chemicals. This may open the possibility of using pollen selection for lipid quality in seeds based on significant correlation, for linoleic and linolenic acids, found in pollen and seeds of Brassica (Evans *et al.* 1988). In addition, glucosinolates have been also found in Brassica pollen (Dungey *et al.* 1988). These authors pointed out the possibility of its use for pollen selection, but further studies are necessary to have a clear picture of the correlation between the amounts of glucosinolates in pollen and seeds.

From the above methods it has been reported that about 60-80% of structural genes are expressed both in pollen and vegetative cells. (Tanksley *et al.* 1981)

Table 1: % of genes showing overlap in expression between gametophytic and sporophytic phase in different crops

Crop	% of genes	Reference
Tomato	60	Tanksley <i>et al.</i> , 1981
Maize	72	Sari-Gorla <i>et al.</i> , 1986
Prunus	81	Weeden, 1986
Populus	74-80	Rajora and Zsuffa, 1986
Barley	60	Pederson <i>et al.</i> , 1987

Applications of pollen selection

1. To screen genotypes at gametophytic generation to know their sporophytic performance for different stresses or growth characters.

2. For trait improvement: To transmit and increase the frequency of desirable alleles in the succeeding sporophytic generation via selected pollen.

A variety of selection pressures such as temperature, pathotoxin, herbicide, metal and water stress enforced during gametophytic generation were found effective in increasing the frequency of resistant individuals in the succeeding progeny. (Ravikumar *et al.* 2007)

Table 2: Pollen selection in different crops for different traits

Characters	Crops
Low temperature	Tomato, Maize, Potato
High temperature	Cotton, Pearl millet, Cucurbits, Lilli
Herbicide	Maize, Sunflower, Chickpea
Salinity	Barley, Tomato, Brassica
Metal toxicity	Silene alba, Mimulus guttatus, Tomato
Drought	Sorghum
Disease	Sunflower, Chickpea, Carnation, Maize, Brassica
Plant vigour	Maize, Alfalfa, Wheat, Oil flax, Dianthus, Petunia
Fatty acid composition	Brassica
Starch and protein	Maize and Brassica

Problems with pollen selection

1. It cannot be used for those genes which are expressed only in pollens.
2. Requires significant genetic variability in the gametic pool for effective selection.
3. Requires many cycles of selection to improve quantitative traits or sometimes it may fail to improve.
4. Transient Epigenetic effects like para-mutation.
5. Methods for separation of in vitro selected pollen from unselected and application of it to the styles need to be optimized.

Conclusion

Pollen selection is more effective than sporophytic selection. Though there so many literatures on pollen selection, it may take few more years for the breeders to understand its importance and practice it as a routine method in crop improvement.

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