LESSON 18: GAMETOCLONAL VARIATION

Introduction

There is considerable evidence that haploid plants regenerated from callus cultures show genetic variation, that is gametoclonal variation. For example, gametoclonal variation for heading date, plant height etc. has been reported in rice. Such a variation may be subjected to selection at the haploid level, and the chromosome number of the selected plants may be doubled to obtain homozygous plants.

Gametoclonal variation appears to be more desirable than somaclonal variation because:

1. The mutant characteristic is expressed in the Ro plants, i.e., plants regenerated from cells in vitro, and
2. Cells having detrimental mutations may be expected to regenerate much less frequently in the case of haploid than in diploid cells. However, regeneration of haploid plants is feasible in fewer plant species than regeneration from somatic cells.

To distinguish somatic-derived somaclones and gametic-derived gametoclones, three parameters are considered:

1. Both dominant and recessive mutant genes induced by gametoclonal variation will express directly in haploid plants regenerated from microspores of diploid anthers (since they will have only one copy of each gene)
2. Recombinants recovered in gametoclones would be the result of meiotic crossing- over
3. The gametoclone can be used only after having stabilized by doubling its chromosome number.

The value of gametoclonal variation in crop improvement is evident from the development of double-haploid lines by anther culture of F1 hybrid plants of wheat and rice (Zeng 1983). Several double- haploid plants were recovered that expressed mixed characters of the two parents. Based on these observations, it is suggested that gametoclonal variation is the result of factors that cause meiotic recombination and mutation prior to initiating anther culture. Mutations may, however, occur even after or by the doubling process. Further, the genetic changes associated with the use of microspores for regeneration equally contribute to gametoclonal variation.

Variation in chromosome number of gametes or gametophytic tissue plays an important role in gametoclonal variation. This is evident from a range of aneuploids and mixoploids recovered from anther cultures of wheat, maize, and sexual hybrid of wheat and triticale (Hu 1983). Another aspect to be taken note of is that variants uncovered following androgenesis are never recovered from somatic protoplast cultures. Further several reports suggest that gene amplification contributes to reduction in the yield of gametoclonal variant plants. Thus, the mutation spectrum obtained by gametoclonal variation may differ from that obtained by somaclonal variation.

Applications in Plant Breeding

Somaclonal variation and gametoclonal variation represent useful sources of introducing genetic variations that could be of value to the plant breeders.

Analytical Breeding

Beeding of autotetraploid (4x) crops like potato, alfalfa etc. presents problems due to the complex segregation patterns at the autotetraploid level. In comparison, breeding at the haploid level of crops like potato is much easier due to much simpler segregation patterns and smaller breeding populations involved. Haploids of tetraploid (4x) species are called dihaploid since they have two similar (in case of autotetraploid species) or dissimilar (in case of allotetraploid species) genomes. Potato dihaploids (2x) are fully fertile, and have enough vigour to allow hybridization and selection at this level. The selected dihaploid clones are treated with colchicine to obtain tetraploid clones which are used as varieties. The scheme involving extraction of dihaploids from tetraploid species, breeding at the dihaploid level and then chromosome doubling of selected dihaploid lines to obtain tetraploid varieties is called analytical breeding. This approach is becoming increasingly popular in crops like potato.

Use of dihaploids in breeding of crops like potato offers the following advantages:

i. much simpler segregation ratios and, as a result, smaller segregating populations,

ii. a greater efficiency of selection, and

iii. much easier hybridization with diploid wild species which offer many genes of great value.
Several elegant and attractive schemes for analytical breeding, including a scheme for extraction of monoploids (haploids of dihaploids; monoploids have a single genome) and then protoplast fusion of 4 selected monoploids to obtain autotetraploid varieties, have been proposed for use in crops like potato.

Achievements

Haploids have been effectively coupled with breeding programmes of crops like rice, wheat, barley, tobacco etc. This has resulted in the development of several varieties (Table 1.). In China, over 100 rice varieties were developed using anther culture route. Many of them represent yield enhancement of 10% or more (upto 27%) and were cultivated in substantially large areas (upto 15 million ha in some cases). One rice variety, Hua-03 has a higher protein content in addition to a 10% yield advantage over the check variety. In Canada two barley varieties, Mingo and Gwylan, produced using the H. bulbosum route were superior in resistance to powdery mildew and barley yellow mosaic virus, and other yield attributes. F 211 is an excellent tobacco cultivar developed in Japan by anther culture route; it is resistant to bacterial wilt and has a mild smoking quality. However, this approach has not yielded any variety in India.

Advantages

1. Production of homozygous DH lines using haploids saves atleast 4 years in comparison to selfing/close inbreeding.
2. Selection among DH lines is atleast 6-8 times as efficient as that among segregating populations; this greatly reduces the size of breeding populations. In addition, the confusing effects of heterozygosity are absent in DH lines.
3. Haploids may be useful for the isolation of mutants, since even recessive mutant alleles will express in the M1 (mutagen-treated) generation itself. Desirable mutants may be selected at the haploid level, and their chromosome number may be doubled to obtain homozygous mutant lines in a single generation.
4. Pollen embryos are generally highly regenerative. Therefore they can be used for gene transfers mediated by Agrobacterium (this has been achieved in Datura) or by a technique like particle gun.

TABLE 1. Selected examples of varieties developed through haploid production by anther culture or hybridization with H. bulbosum.

Haploid produc-	Crop	Varieties	Country
tion route			
Anther culture	Rice	Tanfeng 1, Tan Fong 1, Hua Yu 1,	China
		Hua Yu 2, Ta Be 78, Xin Xiu,	
		Xhonghua 8, Xhonghua 9 etc.	
Anther culture	Wheat	Hua Pei 1, Lung Hua 1, Jinghua 1,	China
		Yunhua 1, Yunhua 2 etc.	
		Florin	Europe
Anther culture	Tobacco	Tan Yul, Tan Yu2, Tan Yu3etc.	China
	(N. tabacum)		
Anther culture	Tobacco	F211	Japan
	(N. tabacum)		
H. bulbosum	Barley	Mingo, Gwylan	Canada

Limitations

1. In many crops, the application of this technique is not yet possible since the technique for haploid production is not available.
2. In many other crops, its application is not feasible because large numbers of haploid plants are not easily obtained. Therefore, a wider application of pollen culture in crop improvement depends primarily upon the development of techniques for quick production of large numbers of haploid plants.
3. High cost of obtaining haploids and doubled haploids is still a major problem.
4. Sometimes deleterious mutations may be induced during the in vitro phase.
5. A non random recovery of haploids may reduce the spectrum of variability recovered.
6. A sophisticated tissue culture laboratory and a dependable greenhouse are essential for success.
7. Specialized skill for carrying out the various operations are required.
8. Plants other than haploids are often recovered from anther cultures/ interspecific hybrids; this necessitates cytological analyses.
9. Occurrence of grametoclonal variation may limit the usefulness of pollen embryos for genetic transformation/ gene transfer.
10. High frequency of albinos are produced in anther cultures of monocots, especially cereals. This problem needs to be resolved for a successful exploitation of haploids in breeding of such crops.

Conclusion

The technique of gametoclonal variation is also a useful source for introducing genetic variations like somaclonal variations. This has been extensively applied in analytical plant breeding. The techniques employed for induction of somaclonal and gametoclonal variation are relatively easier than recombinant DNA technology. In particular, the improvement of crops with polygenic traits by conventional and non- conventional breeding methods have proved very difficult. But, with these methods, genetic manipulation of such crops has beome easier.

Questions

1. How will you distinguish between somatic-derived somaclones and gametic-derived gametoclones?
2. Discuss the usefulness of gametoclones in plant breeding.
3. Write down the achievements, advantages and limitations of gametoclonal variation.

Note