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ANTHER CULTURE OF PERENNIAL RYEGRASS
(*Lolium perenne* L.)

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Introduction

Perennial ryegrass is a natural cross-pollinating species characterized by a fairly efficient self-incompatibility system, which seriously limits possibilities of inbreeding. Reliable methods for producing homozygous doubled haploids could lead to improved selection efficiency and more accurate determination of combining abilities in conventional ryegrass breeding. Thus the inclusion of doubled haploids in the selection work would potentially improve homozygous balance leading to recombinant inbred lines in the long term, and on short term provide homozygous plants of high genetic potentials for use in synthetic or hybrid varieties.

The anther culture methods have improved considerably during the last 15 years. Although the methods are most advanced for self-pollinating cereals such as rice, wheat and barley, results have recently been obtained also for cross-pollinated forage grasses including perennial ryegrass (Olesen, 1987, Olesen et al., 1988).

Characteristics of ryegrass anther culture

A large number of green plants have been regenerated from ryegrass anther culture and successfully established in soil (Olesen et al., 1988). Their origin from reduced microspores was confirmed by isozyme analysis, and chromosome count revealed 50-60 per cent of the plants obtained from diploid donor plants to have undergone spontaneous doubling to the diploid level (Olesen et al., 1988; Hayward et al., in press).

A consistent disturbed segregation of isozyme markers amongst androgenetic plants originating from two diploid donor plants has been demonstrated. Since different alleles predominated in different progeny sets, a hypothesis of linked loci affecting anther culture response or of linked lethal alleles leading to gametophytic selection have been put forward (Hayward et al., in press).

Donor genotypes is the major determinant for the results with ryegrass anther culture (Olesen et al., 1988; Halberg et al., in press). Estimates of variance components from replicated trials with 20 diploid and 10 tetraploid donor clones showed genotypes to account for about 73 per cent of the total variation in yield of calluses/embryos. In this study embryos were obtained from all clones, plants were obtained from all clones except one, and the total yield of plants (albino plus green plants) ranged from 0 to 61 plants per 100 cultured anthers. Green plants were obtained from 5 diploid and 7 tetraploid donor clones, however, with few exceptions in a very low number (Olesen et al., 1988).

By far the majority of regenerated plants in ryegrass anther culture is of the albino type (Olesen et al., 1988; Morris et al., 1988), which is a common problem in anther culture with graminaceous species. The ability to produce green plants depends on the genotype of the donor plant (Halberg et al., in press), as is also the case for wheat (Andersen et al., 1987) and barley anther culture (Knudsen et al., 1989). However, genotypes capable of giving green plants in anther culture may be found among ordinary ryegrass breeding material (Olesen et al., 1988; Morris et al., 1988).

The genotypic approach to improved anther culture response

The anther culture method for perennial ryegrass needs to be improved to be of more value in the breeding work, and two approaches can be followed. Either modification of media and culture conditions can be attempted, or emphasis can be placed on donor genotypes. For a widespread utilization of the haploid technique in the breeding process, the methods should be efficiently applied to a wide spectrum of genotypes. Ideally this should be accomplished by a general improvement of the anther culture methods. However, the very strong genotypic effect suggest that improvements for breeding purposes at present will be most efficiently obtained by adopting the genetic approach.

In a study of anther culture response in F_1 of four hybrid families obtained by the pairwise hybridization of three very responsive diploid parent plants capable of producing green plants, a very large genetic variation for anther culture response was found both within and between the four families and their parents (Halberg et al., in press). In this study a total of 41.432 anthers were cultivated, yielding 28.502 calluses/embryos, 9.830 albino plants and 1.390 green plants (average 3.4 green plants per 100 anthers). Substantially higher yielding genotypes were obtained through the hybridization and superior hybrid clones were found in all four cross populations. Out of the 55 hybrid clones, the 6 best produced from 11 to 59 green plants per 100 cultivated anthers, while their parents in the same experiment only produced one green plant or less per 100 anthers. This improvement was mainly obtained by a higher embryo formation and improved percentage of green plants, while increase in regeneration frequency was less pronounced.

Conclusion

The donor genotype is a major determinant for yield of embryos, albino and green plants in ryegrass anther culture. In general, the yield of green plants is far to low for practical breeding purposes. However, responsive genotypes can be found within ordinary breeding material, and it should be possible to identify agronomically acceptable ryegrass clones with good anther culture potential. Due to variation for anther culture response within varieties and even within genetically highly related material, it is important to work with single clones rather than with families/varieties. Clones with superior anther culture potential can be produced by the hybridization of responsive genotypes. Work in progress will show whether such clones can be used as general "haploid inducers" to transmit the anther culture capacity to ordinary breeding material by hybridization.

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