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Somatic embryogenesis in polyembryonic *Secale cereale* L.

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Summary. The progeny of polyembryonic *Secale cereale* L., was used to study the in vitro response of the immature embryos. The formation of embryogenic calli was very high, and this response and its distribution was statistically different to that shown by the normal regenerated plants and the original population. This behaviour seems to be related to a genetic condition which favours the presence of supernumerary embryos, in vivo as well as in vitro.

Key words: Gramineae - *Secale cereale* - Somatic embryogenesis - Polyembryony

Introduction

Morphogenetic response in vitro is influenced by a number of factors including the genotype of the explant (Beckert and Quing 1984, Tomes and Smith 1985). These observations suggest that the morphogenetic response in vitro could be manipulated and improved through conventional breeding (Bingham et al. 1975, Petolino et al. 1988).

In earlier studies we have observed a differential response between different cultivars and among plants within the same cultivar of rye, an allogamous species (Linacero and Vazquez 1986, 1990). We now describe in vitro embryogenic response in the progeny of in vitro regenerated plants and in a polyembryonic plant.

Material and methods

Immature embryos from an open-pollinated population of rye (*Secale cereale* L. cultivar Elbon),were cultured on MS medium (Murashige and Skoog 1962) supplemented with 2 mg/l 2,4-D, as previously described for *Secale vavilovii* (Vazquez et al. 1991). In all the cases, regenerated plants were obtained from 4-6 month old embryogenic calli induced in darkness from immature embryos.

The "embryogenic response", is defined as the frequency of embryogenic calli (embryogenic calli/total calli) obtained from the immature embryos belonging to one individual plant. The regenerated plants formed the R_0 generation. Selfing was not performed because too little seed is obtained as the cultivar used is highly self-incompatible. The next generation, R_1, was obtained by manual crosses between two R_0 regenerated plants, and all the R_1 plants from the same R_0 cross constituted a family. The R_2 generation was obtained through manual crosses between two R_1 plants from the same family.

We examined the embryogenic response of R_2 progenies from 10 different R_0 plants, by analyzing the immature embryos obtained from 4 to 11 R_1 crosses per regenerated plant. No less than 30 R_2 embryos, recovered on the plant used as female in each cross, were cultured. The embryogenic response was evaluated after 8 weeks.

The in vitro response of immature embryos obtained in the R_2 progeny of a immature embryo regenerated plant, from Elbon, which showed polyembryonic kernels in its progeny, was also examined.

Results and discussion

The embryogenic response per plant in the original Elbon population varied between 0 to 25%. This increased up to 50% in the progenies of normal regenerated plants. Figure 1a shows the plant distribution according to their embryogenic response. A one-way ANOVA was performed to find if the differences between the original Elbon population and the regenerated plants were significant. The result ($F_{1,58}$=7,602)showed that they were highly significant. Therefore, embryo culture per se could be used as a method to select genes which increase the level of response. Similar results have been previously reported in other crops (Bingham et al. 1975, Petolino et al. 1988).

One regenerated plant segregated polyembryonic kernels (Figure 2) in its R_2 and progenies. The frequency of polyembryonic seeds varied among the different R_1 crosses from 3.17% to 30%, while in the normal population this frequency was 0.17%. The genetic analysis of these progenies (to be published elsewhere) indicated that the aptitude to induce polyembryonic seed is a heritable trait transmitted through the pollen, and seems to be a dominant character with nuclear determination. However, for

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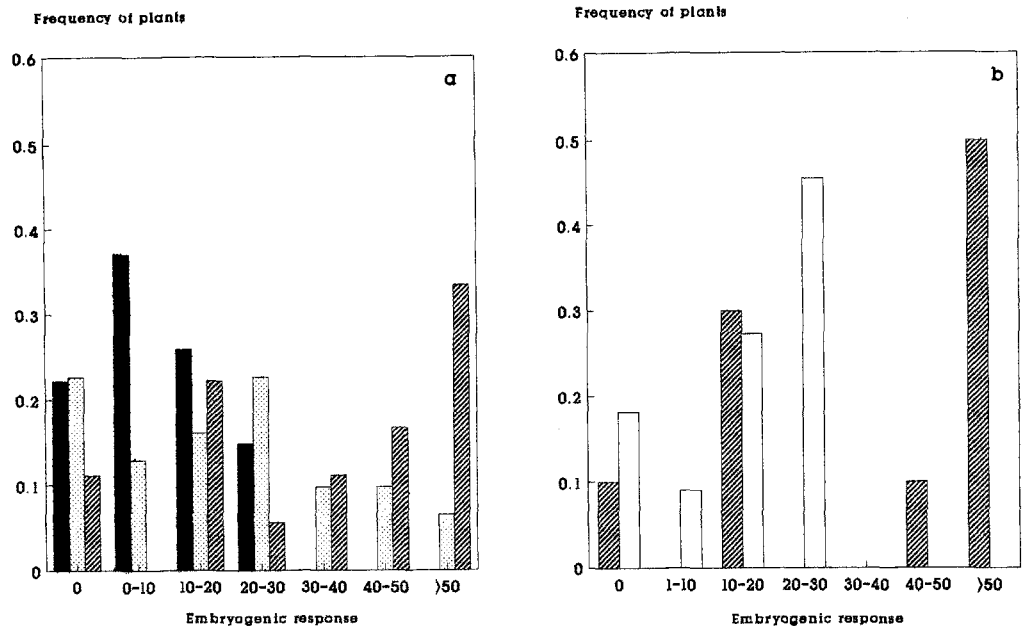

Fig.1. Distribution of plants according to their ability to produce embryogenic calli in vitro. **a**, ■ plants from Elbon original population, ⊡ R_1 plants, ▨ R_1 plants from the polyembryonic plant. **b**, ▨ R_1 plants obtained when the regenerated polyembryonic plant was used as female in the R_0 cross, □ R_1 plants obtained when the polyembryonic plant was used as male in the R_0 cross (reciprocal cross).

expression of the phenotypic trait the female parent should have a specific cytoplasm because when R_0 reciprocal crosses were performed, one segregated polyembryonic seeds and the other did not. On other hand, the level of phenotypic manifestation of the trait (frequency of polyembryonic seeds) varied between plants with the same genotype. Therefore the character has a low penetrance and expressivity. We will refer to this plant as a "polyembryonic plant". This type of transmission has also been proposed to explain the genetic control of the semigamy phenomenon in cotton (Turcotte and Feaster 1974), and maternal haploid induction in maize (Lashermes and Beckert 1988).

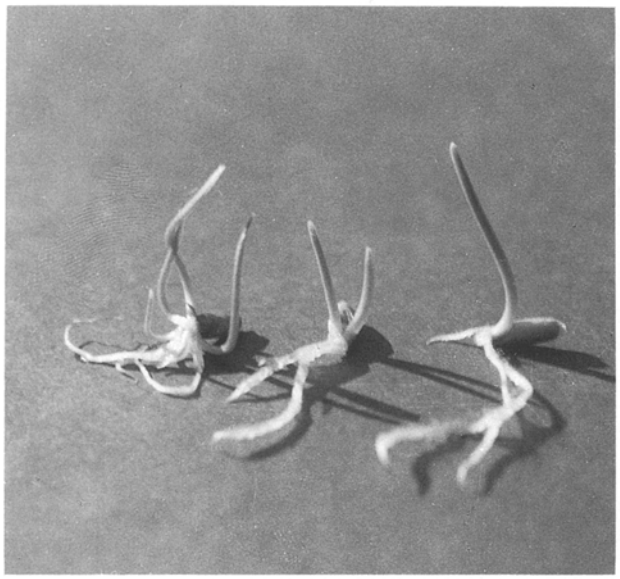

Fig.2. Polyembryonic seeds with 4 and 2 embryos respectively, and one normal seed (right).

The in vitro embryogenic response of the immature R_2 embryos formed on R_1 polyembryonic plants varied from 0% to 65%. This response and its distribution (Figure 1a) were different from those of the normal regenerated plants, and of course the original population. One-way ANOVA of the results between the polyembryonic plant and the other regenerated plants differed at the 5% level ($F_{1,49}$= 6.343). The differences between the polyembryonic plant and the original population were highly significant ($F_{1,44}$=29.8).

The increased response in the progeny of the polyembryonic plants could be due to the fact that it was a regenerated plant itself. However as stated earlier, the corresponding reciprocal families (same R_0 parents), which have the same genetic background, did not show polyembryonic seeds in any generation. Figure 1b shows that there were differences between both reciprocal families, which were statistically significant when a one-way ANOVA was performed ($F_{1,20}$=6.978). On the other hand, the non-polyembryonic families showed the same in vitro response as the other regenerated plants, and the differences between them were not statistically significant ($F_{1,30}$=0.072).

This characteristic of the polyembryonic plants could be also related to a genetic condition which favours

the presence of supernumerary embryos, in vivo as well as in vitro. If these is true, we would have expected crosses showing a high morphogenetic in vitro response, and also showing a high frequency of polyembryonic seeds.

To determine if both characters, polyembryony and a high morphogenetic in vitro response, are related we have analyzed the behaviour of different crosses from the polyembryonic families. Some of the embryos obtained, in each analyzed cross, were cultured in vitro as immature embryos, and the others completed their development on the spikes and were later germinated to verify if the kernels were polyembryonic. We selected only the crosses showing polyembryonic seeds, to study if the expression level of the polyembryonic phenotype was correlated with the level of induction of somatic embryogenesis induction. Each cross had two variables, the polyembryonic seed frequency, and the embryogenic calli frequency (Figure 3). We applied the transformation in the sine-arc scale to the data,

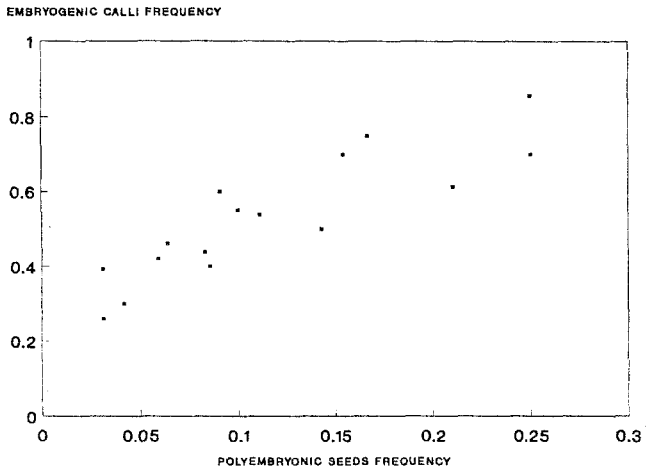

Fig.3. Relationships between embryogenic potential and polyembryonic seed formation in different crosses from the polyembryonic plant. The frequencies of embryogenic calli and polyembryonic seeds were estimated for each cross.

and a test was applied to ascertain if both data were correlated. The correlation coefficient ($r = 0.89$) was highly significant and indicated that both frequencies were positively correlated. The factor implicated in the development of supernumerary embryos in vivo also increased the in vitro somatic embryogenesis. The best crosses for embryogenesis in vitro also showed a high frequency of polyembryonic seeds. In conclusion, the capacity of the immature embryos of rye to form embryogenic calli is closely related with in vivo polyembryony.

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