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Heterozygous Microspore-Derived Plants in Rye

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Summary. In a particular experimental series involving anthers from F_1 hybrid plants of *Secale cereale* L., it was possible to induce the formation of 68 microspore-derived plantlets of which 61 were albinos and 7 green. 6 of the albino plants were haploid, whereas most of the others were diploid. All green rye plants were directly diploid and, after extensive screening, proved to be heterozygous. Evidence is presented suggesting that these latter plants arose from unreduced microspores. The significance of this finding is discussed.

Introduction

In previous publications we reported upon the production of haploid albino plantlets and diploid green plants from microspores of *Secale cereale* L. (Thomas and Wenzel 1975a; Thomas et al. 1975). It was assumed that the majority of non-haploid plants were derived from reduced microspores which had undergone some process of chromosome doubling during their development to embryos or calluses. Our experiments with rape, for instance, showed that diploid androgenic plants were derived from reduced microspores (Thomas and Wenzel 1975b; Hoffmann et al. 1977; Wenzel et al. 1977). Such a mechanism of spontaneous chromosomal doubling is well known (Murashige and Nakano 1966) and would be advantageous to breeders since it avoids artificial doubling, for example using colchicine. In rye, however, we now report that our first green plants derived from cultured F_1 hybrid anthers are heterozygous, a result which indicates not only the necessity to perform extensive homozygosity tests on non-haploid anther-derived plants but also the danger of using homozygous starting material for the development of techniques of anther culture.

Materials and Methods

Diploid F_1 hybrids (2n = 14) from crosses involving the following parents were used as experimental material: *S. cereale* var. Moscow dwarf (predominantly self-sterile, short awns and hairs below the ear); *S. cereale* var. Pekuro (high quality commercial line; self-sterile, normal awns and no hairs); *S. cereale* strain 408/67 (F_{10} derived from the cross *S. cereale* var. Heines Hellkorn × *S. vavilovii* Grossh.; cleistogamous-self-fertile, normal awns and no hairs); 4 mutants of strain 408/67 which had been selected for self-fertility, normal awns, no hairs and short stalk. The characters, self-fertility, normal awns and hairs below the ear, are dominant. All seed material was kindly provided by H. Kuckuck, Hannover (for further details see Kuckuck 1974). The F_1 hybrids, which are listed in Table 1, were vegetatively propagated for a period of six month before being flowered and subjected to anther culture.

The details of our greenhouse conditions, pretreatment of the spikes and of anther culture techniques have been described previously (Wenzel and Thomas 1974; Thomas et al. 1975).

Results and Discussion

From 84,000 anthers plated, 68 plants have been obtained. Of these, 61 were albinos (Table 1). The 7 green plants regenerated in this series of experiments were spontaneous diploids and could be cloned, vernalized, grown to maturity and flowered. They are referred to as the A_1 generation. All seven plants were self-fertile, although theoretically one would expect a mixture of self-sterile and self-fertile plants. If the population were bigger, one should expect a 1 : 1 segregation (Melchers and Labib 1970). The A_2 generation was analysed for awn length and hairy spikes and, as shown in Table 2, there was a clear segregation similar to that observed in the F_2. It could be demonstrated statistically that the green androgenic plants were as heterozygous as the corresponding F_2 plants. Neither the F_2 nor the A_2 showed the normal 9 : 3 : 3 : 1 segregation for awn length and hairs, as checked by Chi-square test. Nevertheless, the fact that they showed segregation indicates clearly that they are heterozygous.

This surprising result could be explained by assuming that the plants did not in fact arise from microspores but arose from somatic tissue. However, as anatomical studies showed, the culture conditions

Table 1. List of F_1 hybrids and results from the anther culture experiments

No.	number of anthers plated	A_1 plants green	A_1 plants albinos
1 Pekuro × Moscow dwarf	25 488	1	21
2 408/67 × Moscow dwarf	15 216	-	8
3 Moscow dwarf × 408/67	1 968	-	3
4 Moscow dwarf × Mut. 511	5 784	2	4
5 Moscow dwarf × Mut. 512	12 936	-	3
6 Moscow dwarf × Mut. 530	16 116	4	19
7 Moscow dwarf × Mut. 608	6 552	-	3
	84 060	7	61

Table 2. Comparison of the segregation patterns of the F_2 and A_2 generation

offspring F_1 No.	F_2/A_2	normal awns hairs	normal awns no hairs	short awns hairs	short awns no hairs
1	F_2	26 (31%)	23 (28%)	21 (25%)	13 (16%)
1	A_2	77 (49%)	41 (26%)	25 (16%)	15 (9%)
4	F_2	19 (31%)	16 (26%)	14 (23%)	13 (21%)
4	A_2	28 (46%)	11 (18%)	10 (16%)	12 (20%)
4	A_2	5 (26%)	3 (16%)	5 (26%)	6 (31%)
6	F_2	25 (41%)	19 (31%)	10 (16%)	7 (11%)
6	A_2	12 (60%)	5 (25%)	3 (15%)	0 (0%)
6	A_2	73 (45%)	54 (34%)	25 (16%)	9 (6%)
6	A_2	14 (42%)	9 (27%)	6 (18%)	4 (12%)
6	A_2	78 (43%)	56 (31%)	22 (12%)	26 (14%)

under which plantlets arise never allow growth of somatic tissue; furthermore the developmental stage of anthers used does not favour the formation of somatic callus. The conditions required for somatic tissue growth are either to completely omit phytohormones from the Nitsch and Nitsch (1969) basal culture medium (in which case 90 % of the anther walls proliferate), or to increase the level of auxins and at the same time use anthers containing very young microspores (in which case 90 % of filaments proliferate). Under the latter two conditions, dividing microspores were never observed. On the other hand, using anthers containing spores at the stage of the first mitosis, 0.1 % of the anthers contained large numbers of multicellular structures either still enclosed within the exine or just rupturing it. Direct proof that rye microspores can give rise to plants is the fact that 10 % of the albinos were haploid. In a later experiment with similar F_1 hybrids, one haploid green plantlet was observed but this was so weak that it could not successfully be transferred to potting compost.

A second explanation for the appearance of diploid heterozygous plants is more feasible: the heterozygous plants have arisen from unreduced microspores, that is, microspores which have not undergone, or not completed, meiosis. We have previously indicated (Thomas et al. 1975) that our pollen populations contained numerous abnormalities, including diploid chromosome numbers, giant microspores and incompletely separated ones (Fig.1a). We have now extended these observations to determine the percentage of abnormal sized microspores in pollen populations from plants grown under our greenhouse conditions. Using pollen circumference as a parameter (Fig.2), it has been shown that ripe pollen grains from the tetraploid *S. cereale* variety Tero (4x = 28) are significantly bigger than pollen grains from the diploid F_1 hybrids examined (t-values around 10.0). This indicates a correlation between ploidy level and pollen size. In Fig.2 it can be seen that pollen size in anthers from tetraploid plants predominantly follows a normal curve, but that in anthers from diploid F_1 hybrids there is a slight peak towards the diploid

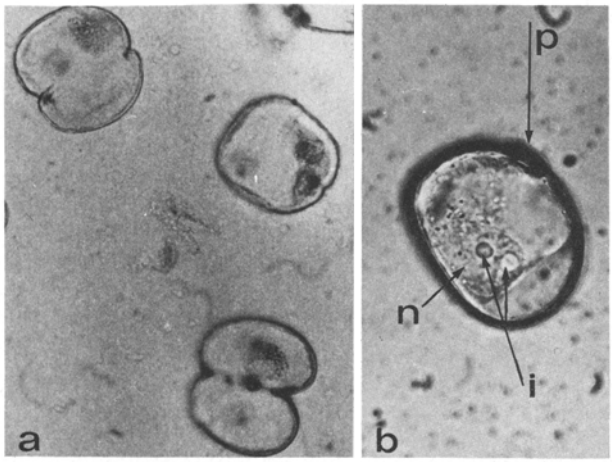

Fig.1. Abnormal development in microspores from diploid rye. a) Incompletely separated microspores indicative of aberrant development during or after meiosis (acetocarmine stained); b) isolated microspore containing one nucleus (n) with two nucleoli (i), p = pollen pore

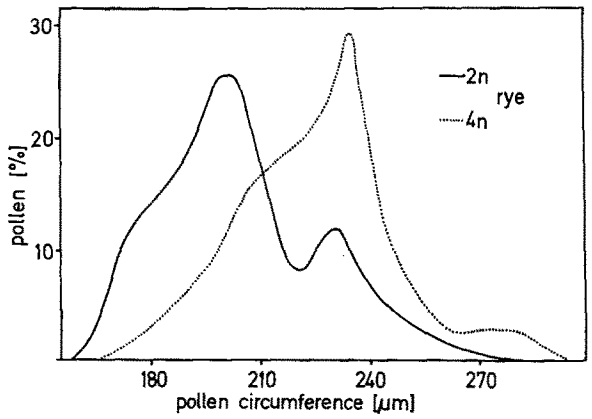

Fig.2. Comparison of the pollen size from diploid and tetraploid rye

pollen size, indicating that in our haploid pollen populations up to 10 % were abnormal.

This was confirmed using another parameter, the number of nucleoli. Reitberger (personal communication) has shown that interphase nuclei of haploid rye never possess 2 nucleoli, whereas up to 30 % of diploid rye nuclei contain 2 nucleoli. As shown in Fig. 1b, it was possible to find in suspensions of isolated microspores (prepared from spikes, pretreated in a similar manner to those used as anther donors) up to 3 % nuclei containing 2 nucleoli. This corresponds to an estimated percentage of about 10 % diploid microspores, many of which will probably be unreduced and consequently heterozygous.

Conclusions

The observations here reported strongly suggest that plants can arise from unreduced microspores as postulated by Engvild (1974) and Sunderland (1974). The critical proof that such abnormal microspores give rise to heterozygous plants can only be obtained when methods are available to induce the growth of rye microspore plantlets in complete isolation from somatic tissue. The development of a technique for inducing the initial growth of isolated rye microspores (Wenzel et al. 1975) is a step in this direction.

The reasons why our experimental conditions select either for heterozygous green or albino plants are not known. F_1 hybrid pollen probably contains many unfavourable gene combinations and the problems reported here may be overcome by inducing the growth of very large numbers of different microspores. Recent results with similar F_1 hybrids have shown it possible to increase 10-fold the percentage of responsive anthers, that is, those producing embryoids or macroscopic calluses. Nevertheless, a percentage of non-haploid microspore plantlets will be heterozygous and consequently all plants should be subjected to very careful homozygosity screening, before being used in crossing experiments. There would also appear to be a danger in using homozygous material as the source for developing anther culture techniques, since it would be impossible to decide with certainty whether the developed technique selects for the growth of unreduced microspores at the expense of reduced ones. Consequently methods developed on homozygous material may not be applicable to heterozygotes. It is now a feature of our work to alter the environmental conditions under which donor plants and anthers are grown, in an attempt to reduce the number of abnormal microspores.

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