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Induction of Haploid and Diploid Plants Though in Vitro Anther Culture of Haploid Wheat (n=3x=21)

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Summary. Five haploid plants of wheat were used for anther culture. Embryos were formed and six plants were regenerated. Of these, two were haploid (n=3x=21) and two diploid (2n=6x=42). The two diploids derived from the anthers of the same haploid wheat plant gave seeds, but the fertility was reduced in one of them showing, abnormalities at meiosis.

Key words: Triticum aestivum – Anther culture – Haploid

Introduction

In the last ten years, techniques for in vitro androgenesis have been developed in many laboratories. Among agricultural species, many cereals will give androgenetic plants: rice (Niizeki and Oono 1968), barley (Clapham 1973); wheat (Ouyang and al. 1973; Picard and De Buyser 1973), Triticale (Sun and al. 1973), rye (Thomas et al. 1975); and maize (401 Research Group, 1975). Since 1973, several hundred wheat plants of pollen origin have been obtained in our laboratory by culturing anthers in vitro (De Buyser and Henry 1979). The plants regenerated were haploid (n=3x=21) or diploid (2n=6x=42), but rarely tetraploid (4n=12x=84).

Haploid plants (n=3x=21) produced in this way were grown in the greenhouse and used for a second cycle of in vitro anther culture, as previously reported only by Chandy and Narayanaswamy (1971) who obtained haploid and diploid plants by anther culture of haploid *Datura metel* L. We have tried to obtain embryo formation and plant regeneration from anther culture of haploid wheat.

Meiotic behaviour of haploid wheat (*Triticum aestivum* L.) is abnormal and gives microspores with different chromosome numbers. In the case of wheat (*Triticum aestivum* L.) 2n=6x=42, with the AABBDD genomic formula, anther culture of haploid plants (n=3x=21) could be used to produce new chromosomal combinations and to obtain plants with one or two genomes.

Materials and Methods

Five haploid plants (n=3x=21) of wheat (*Triticum aestivum* L.), produced by in vitro anther culture, were used as a source of material. Two came from the spring cultivar 'Atys', and the three others, R_8, R_{97}, R_{114}, from a F_6 pedigree line. The haploidy of these plants was confirmed by chromosome counting. Under greenhouse conditions, the haploid plants gave spikes which were collected at the uninucleate stage and treated as those from normal diploid wheat plants (De Buyser and Henry 1979). Two anther culture media, B and B_4, were used. Regeneration of plants was achieved by transferring the developing embryos to a second medium R (Picard and De Buyser 1973).

Results

1 Embryo Formation

The haploid plants developed normal spikes in that anthers were produced. Meiosis was abnormal but gave some viable pollen. The plants were self-sterile although crosses could be achieved using pollen from diploid plants.

In culture, the pollen developed in the same way as in the normal diploid plants. After a few days of incubation, the first divisions occurred in some of the viable pollen and after fifteen days multinucleate embryos could be observed. Visible embryos emerged from the anthers after about four weeks. The results are described in Table 1.

All the haploid plants yielded embryos. More than 1% of the anthers from one haploid of the cultivar 'Atys' gave embryos, and on B_4 medium an embryo frequency of 2,3% was reached by the R_{97} haploid.

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Table 1. Results

Haploid Material		Atys 1	Atys 2	F_6R_8	F_6R_{97}	F_6R_{114}
Medium B	NA	1296	864	798	1329	2171
	NE	8	9	1	2	13
Medium B_4	NA			366	522	390
	NE			1	12	4

NA = number of anthers; NE = number of embryos

2 *Regeneration of Plants*

All the embryos were transferred to a second medium for differentiation. Six chlorophyllous plants were regenerated; none were albino. A few embryos gave only roots.

Four of the regenerants came from the 'Atys' haploid. Chromosome determination showed two of these to be haploid (n=3x=21). Unfortunately the other two died before counting. The two plants obtained from haploid R_{97} were both diploid.

The four surviving plants were grown in the greenhouse. A study of meiosis confirmed the chromosome determination made on the roots. However, one diploid showed some meiotic abnormalities in that univalents and trivalents were observed at the first metaphase.

At maturity, the haploid plants remained sterile and the diploids set seed, although the plant showing meiotic abnormalities had a reduced fertility.

Discussion

The results demonstrate that as in *Datura metel* L., pollen embryos and plantlets can be obtained in *Triticum aestivum* L. by culturing anthers from haploid plants. Moreover the low proportion of viable pollen in the anthers does not seem to affect in vitro androgenesis. Embryogenesis occurs in haploid anthers in the same manner as in those from a diploid plant. In haploid plants viable pollen is found but selfing is not possible.

Both haploid and diploid plants were regenerated from the embryos formed in culture. In the case of haploid *Datura*, of 80 plantlets produced, only three were haploid (n=12), all the others being diploid (2n=24). Chandy and Narayanaswamy (1971) proposed three explanations for the development of diploid plants: the formation of restitution nuclei; endoreduplication, and the functioning of multinucleate giant pollen grains. All these hypotheses are compatible with our results, except for the one plant whose chromosomes were not perfectly homozygous owing to some abnormalities in meiosis. We could postulate that here a translocation is preventing pairing between chromosomes which are thus no longer homologous. Such new chromosomal combinations are an invaluable tool in plant breeding as well as in genetic investigations.

Anther culture of haploid wheat has so far only produced haploid and diploid plants and we have not obtained plants with one or two genomes of wheat. We intend to continue studying the response of haploid anthers in culture, and to compare the progenies of the two diploid plants derived from the same haploid wheat.

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