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Rapid assessment of early embryo development in some wide crosses of cereals

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Abstract: The cleared-ovule technique was evaluated for rapid examination of early embryo development in selfed (barley, wheat) and crossed (barley × rye and reciprocal barley × wheat) material. The pistils were fixed in FAA and the isolated ovules were cleared in methyl salicylate. The development of embryos and endosperm were observed at 24, 48, 72 and 96 hours after pollination. The embryo development rate in barley × rye crosses was mostly similar but in some cases delayed or slightly faster than in control mother cultivars. In contrast to barley × rye crosses, in the reciprocal barley × wheat crosses the frequency of embryos was lower. The study shows that the technique of cleared-ovule in methyl salicylate may be applied for rapid assessment of early embryo and endosperm development in cereal plants. In comparison with traditional sectioning methods the clear ovule technique is simpler, more efficient and quicker.

Key words: barley, cleared-ovule technique, embryo, endosperm, interspecific crosses, rye, wheat.

Introduction

In some interspecific and especially in intergeneric cereal hybridization, disturbances in endosperm development and its degeneration often result in hybrid embryo collapse. In such cases, wide hybrids may be obtained by in vitro embryo culture. Very often, the number of hybrid embryos is limited and the optimum time of their isolation may vary in different cross combinations. Until now, hybrid plants have been mostly obtained through in vitro culture of immature embryos isolated 14-20 days after pollination (WOJCIECHOWSKA 1985, WOJCIECHOWSKA, PUDELSKA 1992). In crosses, when hybrid embryos die already at the globular stage, the culture of proembryos inside the ovule (1-4 days after polli-

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nation) can be applied. Successful ovule cultures in the Gramineae were described in intergeneric hybrids (NITZSCHE, HENNING 1976, AHMAD, COMEAU 1991, COMEAU et al. 1992, ŚLUSARKIEWICZ-JARZINA et al. 1994).

In wide hybridization studies it is very important to get quick information if fertilization takes place and about the stage of hybrid embryo development. Several techniques have been used to follow embryo sac and embryo development. The cleared-ovule technique (HERR 1971, MÓL 1988) has been applied mostly to study megasporogenesis and megagametogenesis, but embryo and endosperm development has been observed less often (BLACKBURN, CHRISTOPHEL 1976, BIDDLE, CHRISTOPHEL 1978, PRAKASH, HERR 1979, YANG 1986).

The aim of this study was to estimate the cleared-ovule technique for rapid examination of embryo frequency and early development stages in barley × rye, barley × wheat and wheat × barley crosses, and in control selfed barley and wheat material.

Material and methods

The pistils were removed from cross-pollinated flowers of *Hordeum vulgare* (cvs. Emir, Trumpf) × *Secale cereale* (cv. Strzekęcińskie), *H. vulgare* (cv. Trumpf) × *Triticum aestivum* (cv. Fukuhokomugi) and *T. aestivum* (cvs. Fukuhokomugi, Chinese Spring) × *H. vulgare* (cvs. Emir, Trumpf) and from self-pollinated mother plants of *Hordeum vulgare* (cvs. Emir, Trumpf) and *T. aestivum* (cv. Fukuhokomugi). The used cultivars of barley, rye and wheat showed a relatively good crossability in earlier research on production of barley × rye and reciprocal barley × wheat hybrids (WOJCIECHOWSKA 1985, WOJCIECHOWSKA, PUDELSKA 1992, 1993, 1995). The crosses were done under greenhouse conditions according to the procedure of WOJCIECHOWSKA (1985). The development of embryos and endosperm was observed at 24, 48, 72 and 96 hours after pollination. Pistils were fixed for 24 h in FAA (40% formalin : glacial acetic acid : 70% ethanol, 5 : 5 : 90) and stored in 70% ethanol. Then ovules were dissected from the pistils, dehydrated (for 1 h) in: 70%, 80%, 90% ethanol (one change) and 100% ethanol (three changes), and cleared (for 1-2 h) in one change of ethanol : methyl salicylate (1 : 1), one change of ethanol : methyl salicylate (1 : 3) and two changes of 100% methyl salicylate (YOUNG et al. 1979). Cleared ovules were stored in methyl salicylate in vials. The preparations were done according to HERR (1971). The slides were examined under the interference Nomarski contrast of a Nikon Diaphot-TMD microscope.

Results and discussion

Frequency and rate of embryo and endosperm development were analysed in three intergeneric combinations and in three selfed mother plants: 24, 48, 72 and 96 h after pollination. The results are presented in Table 1.

Table 1. Embryo and endosperm development in crosses of *H. vulgare* (cvs. Emir, Trumpf) with *S. cereale* (cv. Strzekęcińskie), *H. vulgare* (cv. Trumpf) with *T. aestivum* (cv. Fukuhokomugi), and *T. aestivum* (cvs. Fukohokomugi, Chinese Spring) with *H. vulgare* (cvs. Emir, Trumpf), and in self-pollinated cvs. Emir, Trumpf and cv. Fukuhokomugi

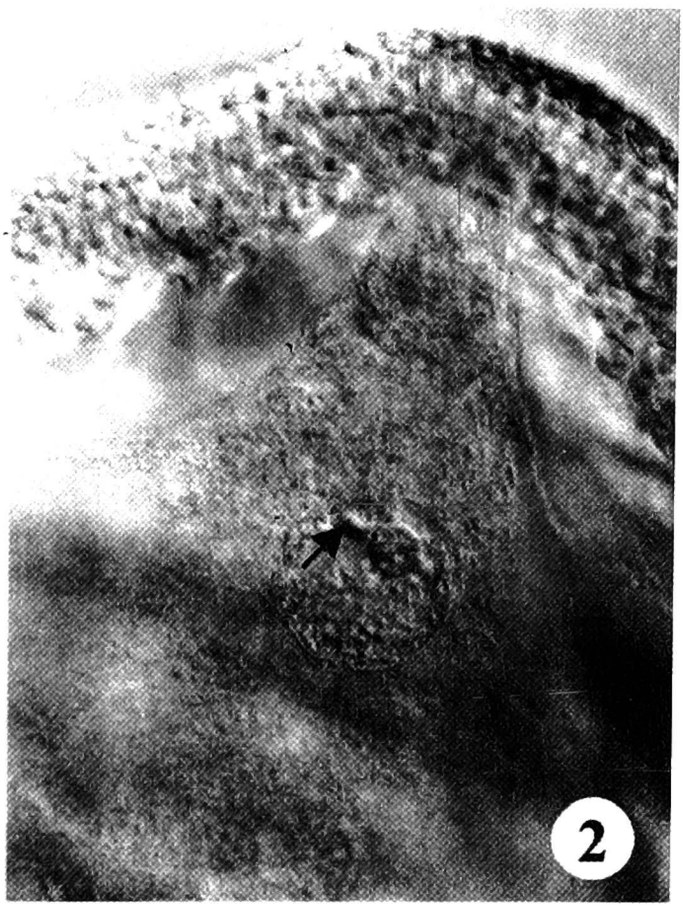
Combination	Numbers of ovules with embryos and endosperm / numbers of ovules observed after pollination							
	24 h		48 h		72 h		96h	
	Embryos	Endosperm	Embryos	Endosperm	Embryos	Endosperm	Embryos	Endosperm
Emir selfed	14*/15	14/15	12*/14	12/14	4^{+}/4	4/4	4°/4	4/4
Trumpf selfed	8*/9	8/9	8^{+}/8	8/8	7°/7	7/7	–	–
Fukuhokomugi selfed	13*/14	13/14	13*/15	13/15	7°/8	7/8	4°/4	4/4
Emir × Strzekęcińskie	9*/10	9/10	7^{+}/7	7/7	7^{+}°/8	7/8	12°/12	12/12
Trumpf × Strzekęcińskie	3*/3	3/3	–	–	–	–	–	–
Trumpf × Fukuhokomugi	1*/15	1/15	5*/17	4/17	5^{+}°/10	4/10	4^{+}°/6	4/6
Fukuhokomugi × Trumpf	0/11	0/11	0/29	0/29	0/5	0/5	2^{+}°/9	2/9
Fukuhokomugi × Emir	0/18	0/18	1*/11	1/11	0/26	0/26	3°/10	3/10
Chinese Spring × Trumpf	4*/9	4/9	5^{+}/16	5/16	–	–	–	–

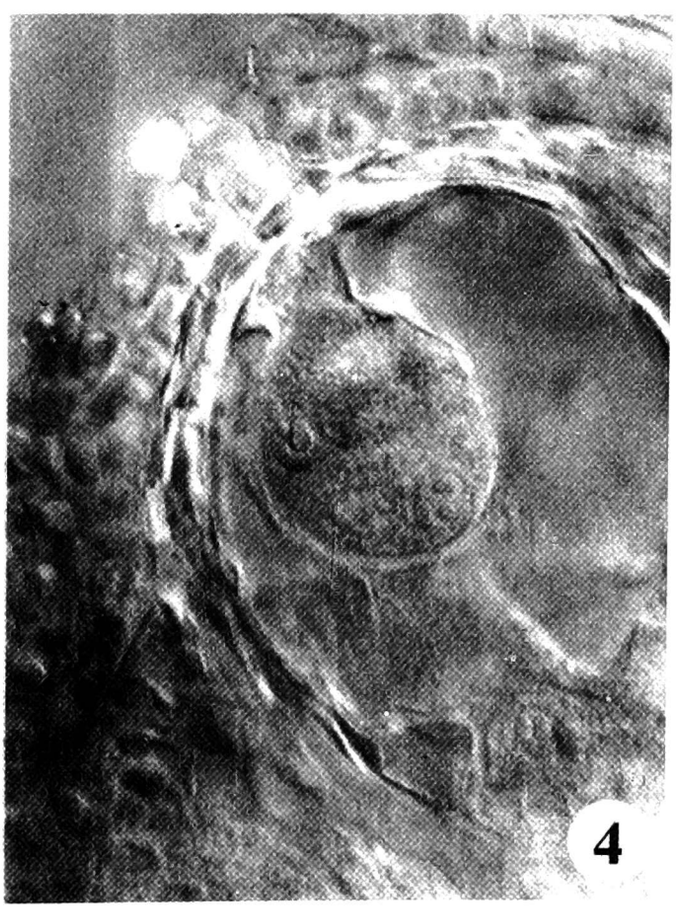
* 1-2-celled embryos
$^{+}$ 2-10-celled embryos
° > 10-celled embryos

Embryos and endosperm were observed in most ovules of *Hordeum vulgare* (cvs. Emir, Trumpf) pollinated with *Secale cereale* (cv. Strzekęcińskie) and in selfed barley plants. THOMPSON and JOHNSTON (1945) found that 90% of self-pollinated barley flowers and 80% of those pollinated by rye were fertilized. VISHNYAKOVA (1990) reported 45.5-80.0% of fertilized ovules depending on barley parents used in barley × rye crosses. FORSTER and DALE (1983a) studied early seed development in 17 genotypes of barley and 11 genotypes of rye. They found that the pattern of embryo and endosperm development was similar in all genotypes studied, but there were marked differences in developmental rates. FORSTER and DALE (1983b) concluded that (1) for successful barley × rye hybridization the embryo and endosperm developmental rates in parents must by similar, (2) development of hybrid embryos depends on early stages of endosperm development, and (3) mitotic rates in parental endosperms are more important than in embryos. In our study embryo development rate in barley × rye crosses was mostly similar but in some cases delayed (Figures 1, 2) or slightly faster (Table 1) than that in control mother cultivars. The results are similar to those obtained for barley × rye crosses by THOMPSON and JOHNSTON (1945), ODENBACH (1965) and BANNIKOVA and KHVEDYNICH (1974) who used traditional sectioning methods.

In contrast to barley × rye crosses in the reciprocal barley × wheat crosses the frequency of embryos was lower. This is in agreement with the frequency of embryos isolated 14-20 days after pollination, observed by WOJCIECHOWSKA (1985), WOJCIECHOWSKA and PUDELSKA (1992, 1993, 1995). The seed set and yield of embryos of barley × rye were higher than those of reciprocal barley × wheat crosses. However, the embryos of barley × rye were mostly undifferentiated while those of reciprocal barley × wheat were mostly differentiated and finally more hybrid plants of barley × wheat and wheat × barley were produced directly from cultured embryos than from barley × rye combinations in the our study. The rate of embryo development in Fukohokomugi selfed was more similar to that of Chinese Spring selfed reported by WOJCIECHOWSKA and LANGE (1977) than to that reported by BENNETT et al. (1973). Most probably the differences resulted from genotypes and environmental factors. The rate of embryo development in reciprocal barley × wheat crosses is generally similar to that in Fukohokomugi selfed.

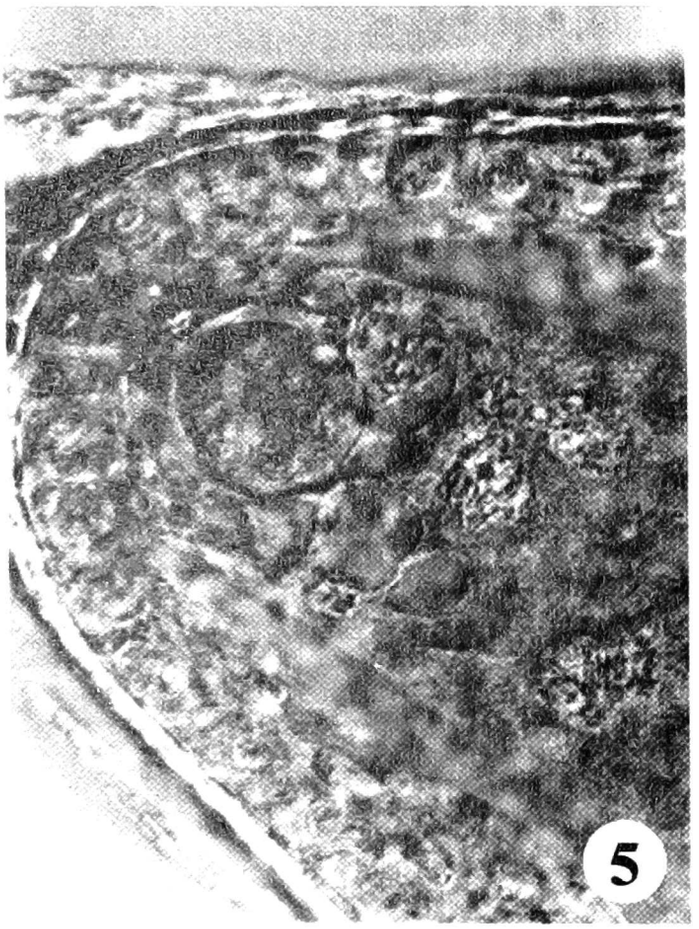
The present study shows that the technique of cleared-ovule in methyl salicylate may be applied for rapid assessment of early embryo and endosperm development in cereal plants. The stages before the fusion of sperm nuclei with egg cell nucleus and the polar nucleus (Figures 1, 2), through various stages of embryo development (Figures 3-5, 7 and 8), were observed on cleared ovule preparations. It was also possible to observe endosperm nuclei (Figures 5, 6) and their distribution in the embryo sac. The division of the polar nucleus occurred earlier than that of the zygote in all material studied. The subsequent endosperm divisions occurred in faster than the embryo cells divisions. However, the precise endosperm

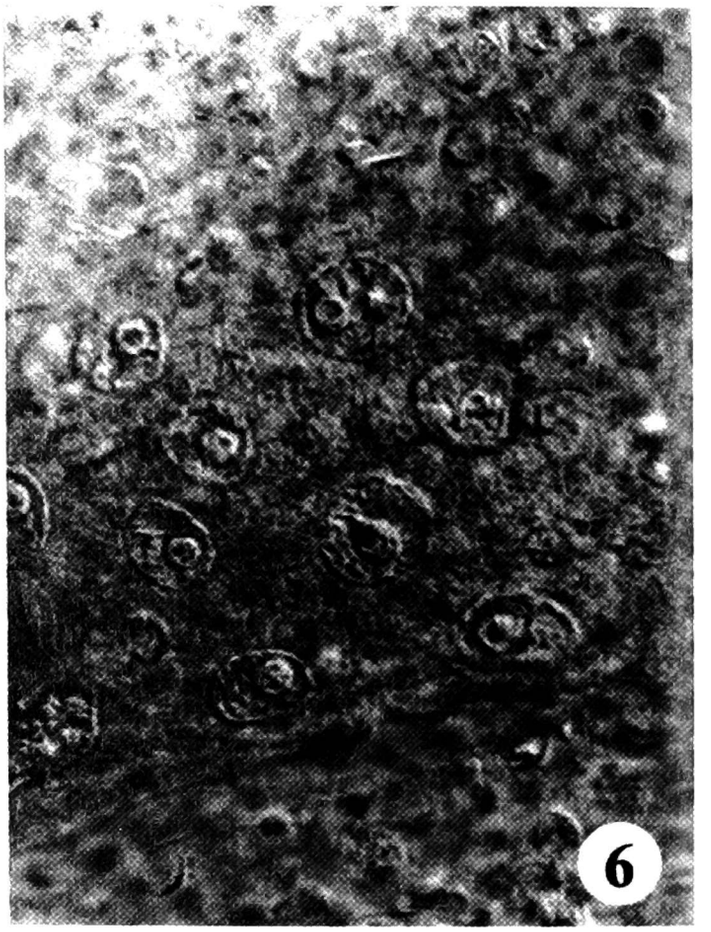




Figures 1-4. *Hordeum vulgare* cv. Emir. × *Secale cereale* cv. Strzekęcińskie

1. Male gamete (arrow) in an egg cell (24 h after pollination). 2. Male gamete (arrow) in a fused polar nucleus (24 h after pollination). 3. Two-celled embryo (arrow, 48 h after pollination). 4. Few-celled embryo (48 h after pollination).





Figures 5-7. *Hordeum vulgare* cv. Emir. × *Secale cereale* cv. Strzekęcińskie
5. Ten-celled embryo and endosperm nuclei (48h after pollination). 6. Ednosperm nuclei (48 h after pollination). 7. Multicelled embryo (72 h after pollination).

Figure 8. *Hordeum vulgare* cv. Trumpf× *Triticum aestivum* cv. Fukuhokomugi.
Multicelled embryo (72 h after pollination).

nuclei counting was difficult, especially from 48 h after pollination. It was only possible to estimate the general endosperm quantity. The cleared-ovule technique can complement the sectioning methods. In comparison to these is simpler, quicker and permits the observation of numerous ovules in a very short time, which is very important in wide hybridization studies. The advantage of this method is also possibility to examine the ovule in more than in one plane because it enables to change the position of the ovule under the cover glass.

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