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Factors affecting the induction of pollen plants of intergeneric hybrids of *Triticum aestivum* × *Triticum-Agropyron*

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Summary. Experimental results showed that the use of potato extract as a basic component of culture medium had a promoting effect on producing calli in anther culture of the intergeneric hybrids of *Triticum aestivum* × *Triticum-Agropyron* (intermediate type). The induction frequencies of pollen callus on the Potato-II medium containing potato extract as the main component was much higher than that found on N_6 and W_5 media. The induction frequencies of pollen callus and green plantlets in four intergeneric hybrid material inoculated at the late-uninucleate pollen stage were all higher than those inoculated at the mid-uninucleate stage. Appropriate increases in culture temperature significantly increased pollen callus induction frequencies of the intergeneric hybrids. The genotype and physiological state of anther donor plants also influenced pollen callus and green plantlet induction frequencies.

Key words: Anther culture – Pollen callus (plantlet) – Induction frequency – *Triticum aestivum* – *Agropyron glaucum*

Introduction

One of the most efficient ways for crop improvement and development of new varieties is to transfer desirable genes from wild species into cultivars. *Agropyron glaucum* is an important wild gene resource for improving wheat varieties. An intermediate type of *Triticum-Agropyron* (refered to as intermediate type hereafter) was selected from the progenies of crosses between common wheat (*Triticum aestivum* L., 2n = 42, *AABBDD*) and *Agropyron glaucum* (2n = 42, *BBEEFF*). The intermediate type was allo-octoploid containing alien chromosome group *EE* or *FF* as well as a complete set wheat chromosomes *AABBDD* (2n = 56). By crossing the intermediate type with common wheat, not only can the sterility in distant hybrids be overcome, but also disease-resistance, stress-resistance and other desirable characters of *A. glaucum* can be transferred into common wheat via chromosome addition, substitution and translocation (Li et al. 1980). However, the segregation of hybrids is random and the characters are not easily stabilized. It is time consuming to obtain a superior and stable strain having genes of disease resistance and stress-resistance from *A. glaucum*. The idea thus developed that by combining distant hybridization with anther culture techniques, various genotypes segregating from hybrids between wheat and intermediate type could be stabilized rapidly. Alien addition, substitution and translocation lines could also be obtained more quickly. Moreover, cytogenetic studies of these newly developed genotypes/lines may then be carried out. Thus, a combination of the two techniques could facilitate both fundamental genetic research and practical breeding. In recent years we have attempted to combine the techniques of distant hybridization and anther culture and have obtained a number of pollen plants from the anther culture of the intergeneric hybrid between the intermediate type and wheat. Several pure lines of wheat having some superior characters of *A. glaucum* have also been obtained after artificial chromosome doubling. The present paper concerns mainly the factors influencing the induction of pollen plants from the intergeneric hybrids between the intermediate type and wheat.

Materials and methods

Anther culture was conducted on F_1, F_2 and F_3 of intergeneric hybrids between wheat and intermediate type 1, 3 and 5

Fig. 1. Spikes of Intermediate Type 3×'Kechun 14' F_1 hybrid and its parents: *1* Intermediate Type 3; *2* F_1 hybrid; *3* 'Kechun 14'

(Fig. 1). Anthers containing microspores at the mid-uninucleate stage were used for culture except for the experiment in which the effect of anther stage on pollen plant induction was tested. Two to three of the uppermost and lowermost spikelets were discarded before the spikes were immersed in 0.1% mercuric chloride for 8 min, followed by three washes with sterilized distilled water. Anthers of the two basal flores of each spikelet were then taken for inoculation. Potato-II medium (Chuang et al. 1978) containing 2.4-D at 1 mg/l, kinetin at 0.5 mg/l and sucrose at 10% was used throughout for anther culture, except for in the medium test. For regeneration of pollen plants, calli of about 1–2 mm in size were transferred onto 190-2 medium (Zhuang et al. 1980a) supplemented with NAA at 0.5 mg/l, kinetin at 0.5 mg/l and sucrose at 3%. The preparation of the medium and microscopical observation were carried out as described previously (Ouyang et al. 1973; 301 Research group 1976).

Results

The following factors were found to influence the induction of pollen calli and plantlets in the anther culture of wheat-*Agropyron* hybrids.

1 Genotypes of the hybrids

Anthers from ten crosses of intermediate type×spring wheat and from nine crosses of intermediate type×winter wheat were cultured and the results are summarized in Table 1. In both groups of crosses the induction frequency of callus was higher in F_1 hybrids than in F_2 hybrids. For instance, the mean induction frequency of pollen callus was 20.0% for anthers of F_2 hybrids from six crosses between intermediate type and spring wheat, 1.8 times higher than that of F_1 hybrids from four crosses between intermediate type and spring wheat (7.1%). Although F_1 and F_2 hybrids vary in their origin, a similar tendency in different groups of crosses suggests that F_2 anthers respond better in culture than F_1 hybrids in different wheat-*Agropyron* hybrids. In some cases (e.g. in hybrids between intermediate type and spring wheat), few plantlets were regenerated from 100 calli derived from F_2 anthers (53.0%) than from those derived from F_1 anthers (63.2%). However, when calculated on the basis of 100 cultured anthers, F_2 anthers produced more green plantlets than F_1 anthers.

Anthers having spring wheat as one of the parents produced more pollen calli than those having winter wheat as one of the parents (Table 1).

Induction frequencies were significantly higher in anther cultures of complex crosses between intermediate type×wheat (winter or spring) and wheat (winter or spring) than in anther cultures of single crosses (intermediate type×wheat). The induction frequency of pollen callus is much lower in single crosses between intermediate type 2 and 'Xiaoyan 759' (3.9%) than in the complex crosses [(intermediate type 2×'Xiaoyan 759') F_1×Zhengfeng (41.0%) and (intermediate type 2 ×'Xiaoyan 759') F_1×'Huapei 2' (69.8%)].

Previous experiments in our laboratory (Ouyang et al. 1973; Chuang et al. 1978; Ouyang et al. 1983) and other laboratories (Heszky et al. 1976; Schaeffer et al. 1979; Shimada 1981) showed that the induction frequency of pollen callus and plantlets varied with the genotype of wheat. In the present experiment, F_2 anthers produced much more calli when using 'Kecun 14' as the wheat parent than when using 'Nongda 139' as the wheat parent, although F_1 anthers from both crosses produced very few calli.

In addition, growth conditions of the anther donor plants also markedly affected the induction of pollen callus. No callus was produced from over 200 anthers cultured from greenhouse-grown pollen plants derived from distant hybrids. However, when grown in the field, the same materials responded well in anther culture. Thus the developmental and physiological state of the anther donor plants may influence markedly the anther culture response.

2 Developmental stage of pollen at inoculation

For induction of pollen plants in common wheat, anthers are best inoculated at the mid-uninucleate

Table 1. Induction frequencies of pollen callus and plantlet in anther culture of F_1 and F_2 hybrids between the intermediate type and wheat

Cross	No. of crosses tested	Induction frequency of pollen callus (%)		% calli giving green plantlets		% calli giving albino plantlets		Induction frequency of green and albino plantlets (%)	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
(Intermediate type ×spring wheat) F_1	4	7.1	1.1 – 17.7	43.9	20.0 – 52.3	19.3	10.0 – 100	63.2	30.0 – 100
(Intermediate type ×spring wheat) F_2	6	20.0	10.6 – 38.1	35.6	15.6 – 42.1	17.4	7.3 – 62.5	53.0	29.6 – 78.1
(Intermediate type ×winter wheat) F_1	6	4.5	0.3 – 7.6	27.1	13.3 – 100	14.6	6.3 – 20.0	41.7	33.3 – 51.6
(Intermediate type ×winter wheat) F_2	3	13.4	6.7 – 17.2	18.2	9.9 – 33.3	29.8	18.5 – 32.1	48.1	41.6 – 58.5

Table 2. Induction frequencies of pollen callus and plantlet in anthers cultured at different developmental stages

Year	Materials	Stage of pollen development	No. of anthers plated	Induction frequency of pollen callus (%)	No. of calli transferred	% calli giving green plantlets	% calli giving albino plantlets	Induction frequency of green plantlets (%)
1981	79-156 (F_3)	Mid-uninucleate stage	400	5.3	14	28.6	35.7	1.5
		Late-uninucleate stage	502	4.4	25	4.0	64.0	0.17
	79-153 (F_3)	Mid-uninucleate stage	444	9.2	44	11.4	52.3	1.0
		Late-uninucleate stage	492	6.3	28	10.7	39.3	0.6
1982	Intermediate type 3×'Kechun 14'	Mid-uninucleate stage	419	130.3	108	36.1	19.4	46.9
		Late-uninucleate stage	418	19.4	36	30.6	16.2	5.9
	Intermediate type 3×'Zhengfeng'	Mid-uninucleate stage	420	58.6	40	30.0	10.0	17.5
		Late-uninucleate stage	420	38.1	96	41.7	11.5	15.8

microspore stage (Ouyang et al. 1973; 301 Research Group 1977). In oder to determine the optimum stage for inoculation in anther cultures of *Triticum-Agropyron* intergeneric hybrids, anthers containing microspores at mid- and late-uninucleate stage were inoculated. Results show that in all four tested hybrids, anthers inoculated at the late-uninucleate stage produced much more calli than those inoculated at the mid-uninucleate stage (Table 2). For instance, in (intermediate type 3× 'Kecun 14') F_2, 100 cultured anthers produced 130.0 calli when inoculated at the late-uninucleate stage, 5.7 times higher than those found upon inoculation at the mid-uninucleate stage, (19.4% calli from 100 cultured anthers). In most cases, the regeneration potential for green plantlets was also higher in calli derived from anthers inoculated at the late-uninucleate stage than in those from anthers inoculated at the mid-uninucleate stage (Fig. 2). Thus, anthers at late-uninucleate stages were proved to be more suitable for culture than those at mid-uninucleate stage in intergeneric hybrids between wheat and *A. glaucum*.

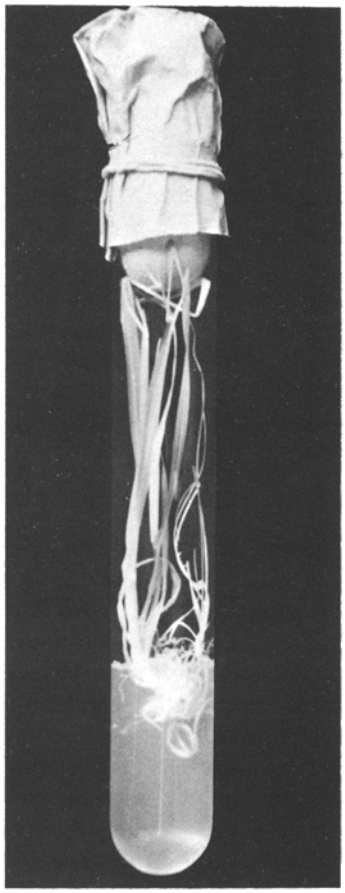

Fig. 2. A pollen plantlet induced from anther culture of Intermediate Type 3×'Kechun 14' F_2 hybrid

3 Medium composition

Anthers of four F_2 hybrids between the intermediate type and wheat were cultured on three basic media: W_5 (Zhuang et al. 1980b), N_6 (Zhu et al. 1975) nd Potato-II. Anthers from three of the four F_2 hybrids tested gave the highest yield of calli when inoculated on Potato-II medium, with a mean frequency of callus induction of four hybrids at 11.9%, about 1.5 times higher than that on W_5 and N_6 media (4.7 and 4.9%, respectively) (Table 3). The difference was statistically significant, suggesting that potato extract was beneficial to callus production in anther cultures of intergeneric hybrids between *T. aestivum* and *Triticum-Agropyron* (intermediate type).

The calli produced on the above three media were transferred onto 190-2 medium for plant regeneration. Data averaged from four F_2 hybrids show that calli produced on W_5 and Potato-II media were similar in their ability to regenerate plantlets (72.3% and 74.5% of the transferred calli regenerated plantlets). However, fewer calli produced on N_6 medium regenerated plantlets (61.4%) (Table 3). For regeneration of green plantlets calli produced on W_5 medium had the highest frequency of differentiation (47.9%) whereas only 34.7% and 33.9% of the calli produced on Potato-II and N_6 media regenerated green plantlets, respectively.

4 Cultural temperature

Cultural temperature plays important roles in pollen callus induction. In common wheat, a moderately

Table 3. Induction frequency and regeneration ability of pollen on W_5, N_6 and potato-II media

Materials	Media	No. of anthers plated	Induction frequency of pollen callus (%)	No. of calli transferred	% calli giving green plantlets	% calli giving albino plantlets	Induction frequency of green plantlets (%)
79-145 (F_2)	N_6	432	4.6	19	36.8	15.8	1.6
	W_5	432	8.1	35	31.4	34.3	2.5
	Potato-II	432	10.6	46	43.5	30.4	4.6
79-156 (F_2)	N_6	480	6.9	35	28.6	34.3	1.9
	W_5	432	4.2	15	46.7	20.0	1.9
	Potato-II	432	10.2	36	27.8	30.6	2.8
79-149 (F_2)	N_6	432	2.8	12	33.3	33.3	0.9
	W_5	432	2.8	9	33.3	33.3	0.9
	Potato-II	432	7.6	27	33.3	51.9	2.5
79-153 (F_2)	N_6	480	5.0	19	36.8	26.3	1.8
	W_5	432	3.7	10	80.0	10.0	2.9
	Potato-II	432	19.2	67	34.4	46.3	6.5

Table 4. Induction of pollen callus and plantlets under different cultural temperatures

Year	Materials	Temperature for inducing callus (°C)	No. of anthers plated	Induction frequency of pollen callus (%)	No. of calli transferred	% calli giving green plantlets	% calli giving albino plantlets	Induction frequency of green plantlets (%)
1981	79-149 (F_3)	26°C	450	3.1	12	8.3	50.0	0.25
		28°C	500	3.0	16	0	31.0	0
	79-153 (F_3)	26°C	489	5.9	30	26.7	46.7	1.57
		28°C	489	16.2	84	23.8	41.7	3.85
1982	(Intermediate type 5 ×'Orofen') F_1	26°C	504	1.4	7	42.9	0	0.60
		28°C	464	3.7	16	15.2	31.3	0.46
	(Intermediate type 5 ×'Nongda 139') F_1	26°C	294	0.3	1	100.0	0	0.30
		28°C	336	5.4	18	22.2	33.3	1.19

elevated temperature was found to be beneficial to pollen callus induction (Ouyang et al. 1983). In wheat-*Agropyron* hybrids, anthers from three of four tested materials produced more calli when cultured at 28 °C, instead at 26 °C, whereas anthers from the fourth material responded equally well at either 26 °C or 28 °C. However, the differentiation frequencies of both green and total plantlets were lower in calli derived from anthers cultured at 28 °C than at 26 °C. Nevertheless, when calculated on the basis of the number of anthers cultured, the yield of green plantlets was still higher in anthers cultured at 28 °C (Table 4).

In a number of varieties and intervarietal hybrids of common wheat, calli derived from anthers cultured at 28 °C had higher differentiation potentials than those from anthers cultured at 26 °C. The opposite situation in wheat-*Agropyron* hybrids in the present experiment may be accounted for by (1) genotypic differences and (2) the later transfer of calli onto differentiation medium.

Discussion

The results presented here show that for anther cultures of wheat-*Agropyron* hybrids, Potato-II medium is superior to the other two synthetic media in all the tested materials. Potato-II medium also proved to be superior for anther cultures of varieties and intervarietal hybrids in common wheat, suggesting that intervarietal hybrids of common wheat and intergeneric hybrids between common wheat and *Agropyron glaucum* are similar in their requirements for medium composition and hormones for induction of pollen callus. However, the overall induction frequencies of pollen callus were usually lower in wheat-*Agropyron* hybrids than in wheat. This may reflect the more strict or specific nutritional requirements by wheat-*Agropyron* hybrids for pollen callus induction. Further investigations need to be carried out to improve the medium for the anther culture of wheat-*Agropyron* hybrids.

The suboptimal culture medium and condition may not be the only reason for the lower induction frequency of pollen callus in wheat-*Agropyron* hybrids. It is known that in the F_1 hybrid, group E or F chromosomes form seven univalents during meiosis and that the abnormal distribution of these chromosomes may lead to the formation of anomalous microspores which may not be able to develop further during culture. It is difficult to overcome this problem. However, by using anthers of F_2 hybrids or complex hybrids (F_1 wheat-*Agropyron* hybrid backcrossed with wheat), the frequency of both viable microspores and callus induction can be greatly enhanced.

Segregation is complicated and numerous genotypes exist in F_2 intergeneric hybrids. Thus selection of good intergeneric hybrids and use of their F_2 or F_1 backcrossed with common wheat in anther culture may be recommended.

Although the induction frequency of pollen callus appears to be predetermined by the genotype and developmental and physiological state of the anther donor plants, modification of the culture procedure can improve markedly the callus yields. In anther cultures of (intermediate type×'Kecun 14') F_2, the induction frequency of pollen callus was as high as 130% when anthers were inoculated at late-uninucleate stage, 6.7 times higher than that in anthers cultured at mid-uninucleate stage (Table 2). This suggests that most (if not all) materials may have potential for pollen callus and plantlet regeneration in anther culture under appropriate manipulation.

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