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The response of anther culture to culture temperature in *Triticum aestivum*

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Summary. The response of anther culture to culture temperature was studied in detail using many varieties, F_1 hybrids and pollen-derived lines of wheat (*Triticum aestivum*) as materials. The suitable culture temperature for inducing pollen callus (or embryoids) in wheat anther culture ranged from 26 °C to 30 °C, varying with genotypes. But for the great majority of wheat genotypes the suitable culture temperatures lay between 28 °C and 30 °C. The most significant genotypic variation in the response to culture temperature was observed in the comparison between the culture at 33 °C for eight days followed by culture at 25 °C (or 26 °C) and the continuous culture at 25 °C (or 26 °C). This genotypic variation in the response to culture temperature is a heritable character which may be controlled by multiple genes. The effect of culture at 30 °C for eight days followed by culture at 26 °C was similar to, or in some cases, better than that of continuous culture at 28 °C, and the effect of culture at 32 °C for eight days followed by culture at 28 °C was similar to that of continuous culture at 30 °C. In the range from 26 °C to 32 °C, the overwhelming majority of pollen calli emerged before the 40th day after anther inoculation, and the higher the culture temperature, the earlier and more concentrated the emerging period of the pollen callus. The pollen callus obtained at high temperatures above 28 °C should be transferred in time onto the regeneration medium at 25°–27 °C to induce shoots.

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

Introduction

Culture temperature is a very important factor in the induction of pollen callus (or embryoids) from microspores in anther culture, yet reports of studies on this factor have so far been infrequent, and furthermore the majority of these studies were rather simple. The early studies relating to this factor are those carried out by Sunderland (1971) and Corduan (1973) in *Nicotiana tabacum;* Sopory and Maheshwari (1976) in *Datura innoxia;* and Pan et al. (1975); Picard et al. (1975 b) and Li (1978) in *Triticum aestivum.* In these early studies the temperatures tested were all below 28 °C except the work of Sopory where 30 °C was tried, so that the effects of high temperatures above 28 °C were almost unrecognized. Afterwards, workers began to pay attention to the role of high temperature. Keller et al. (1978, 1979) studied the effect of culture temperature on anther culture of *Brassica napus* and *B. campestris* and found that culture at temperature above 30 °C for several days prior to transfer to 25 °C had very good effects. Ho et al. (1978) reported the obtaining of relatively high yields of wheat pollen callus and plantlets at 28°–32 °C. But in our Institute, 301 Research Group (1977) and 302 Research Group (1974) observed in wheat and rice respectively that at high culture temperatures, although the pollen callus induction frequencies were increased, the plantlet regeneration abilities of the pollen calli tended to decrease, especially when the callus was transferred to regeneration medium rather late. This result suggested that it was necessary to make further studies on the effects of culture temperature. Hence in the last three years we continued studying in detail the response of wheat anther culture to culture temperature, especially the response to various temperature changes between 26 °C and 33 °C during the process of induction and growth of pollen callus.

Materials and methods

The materials used belong to *Triticum aestivum* including five varieties, three F_1 hybrids and two anther culture derived pollen lines of spring type, two varieties and four F_1 hybrids of winter type as well as two semi-winter type materials, 'Xiao-

yan 759' and the F_1 hybrid of 'Xiaoyan 795' × 'Xiaoyan blue grain'. 'Xiaoyan 759' and 'Xiaoyan blue grain' are alien addition line and alien substitute line respectively selected from the progenies of the cross *T. aestivum* × *Agropyron elongatum* backcrossed repeatedly with *T. aestivum* by the North-west Institute of Botany (China). The materials were grown in the field or in the greenhouse. The anther inoculation time of the field-grown materials was in summer, and that of the greenhouse-grown materials was in spring.

The anthers with microspores at mid-uninucleate stage were used for inoculation. Prior to inoculation, the upper and basal parts of the anther donor spikes which bore spikelets lagging in development were cut off, and the middle main parts were sterilized with 0.1% mercuric chloride for 8 min followed by washing with three to four changes of sterilized water. Then the anthers of the two basal florets of every spikelet were taken for inoculation. It was reported that there were differences in response to in vitro culture among anthers of different spikes and even among anthers at different places of the same spike, although their microspores were all at the same developmental stage (Picard et al. 1975 a; Wang and Chen 1980). In order to minimize the influence of these differences in response on the accuracy of experiment we distributed the anthers of every two neighbouring spikelets on a spike equally to all the test treatments (including the control) of an experiment.

The medium for inducing pollen callus was the Potato-4 medium which was substantially the same as Potato-2 medium reported previously (Chuang et al. 1978) with only a slight modification that the concentration of KNO_3 was increased from 1,000 mg/l to 1,150 mg/l. The medium for plantlet regeneration from pollen callus was MS medium (Murashige and Skoog 1962) but with the major inorganic salts reduced to half their original strengths, the thiamine-HCl increased to 0.4 mg/l and supplemented with 0.5 mg/l each of naphthalene-acetic acid and kinetin. The preparation procedures of the media were the same as described in previous papers (Ouyang et al. 1973; Chuang et al. 1978).

Various temperatures for inducing pollen calli were tested between 22.5 °C and 33 °C. All the tests were carried out in dark in the incubators. The relative humidity of the incubators were regulated at between 40% and 60%. The temperature was measured with accurate thermometers laid on each shelf inside the incubators, since the temperature readings of top-inserted thermometers were often 1–3 °C different.

The pollen calli visible to naked eyes (usually 0.2–1.5 mm in diameter) were counted and transferred to regeneration medium in three batches from the 26th day to the 50th day after inoculation. We agree with Picard et al. (1975 a) that the majority of the cell masses induced from wheat pollen grains were embryoids. But for convenience the embryoids as well as calli are all called calli in this paper. The pollen calli obtained from various temperature treatments, after transferred to regeneration medium, were all cultured at 25°–27 °C (but 21°–26 °C for the greenhouse grown materials in 1980) in a culture room illuminated 9–11 h daily with fluorescent tubes with an intensity of about 1,500 lux.

The main indexes used to show the experimental results are calculated according to the following formulae:

(1) Induction frequency of pollen callus %

$$= \frac{\text{No. of pollen calli}}{\text{No. of anthers inoculated}} \times 100$$

(2) Differentiation frequency of green (or albino) plantlets %

$$= \frac{\text{No. of pollen calli giving green (or albino) plantlets}}{\text{No. of pollen calli transferred to regeneration medium}} \times 100$$

(3) Induction frequency of green plantlets %

$$= \frac{\text{No. of pollen calli giving green plantlets}}{\text{No. of anthers inoculated}} \times 100$$

Here the differentiation frequency indicates the regeneration ability of the pollen callus, and the induction frequency of green plantlets indicates the yield of green plantlets per 100 cultured anthers of each treatment. We calculate the green plantlet yield on the basis of the number of calli differentiating green plantlets instead of the number of individual plantlets because, according to the observation of ours in wheat (unpublished) and that of Yin et al. in rice (1981), the several plantlets regenerated from one piece of callus were of the same genotype with only a few exceptions. Among the calli differentiating green plantlets there were about 5% calli which also differentiated albino plantlets or green-albino chimeras. This type of calli was no longer counted in the number of calli differentiating albino plantlets.

Results

Genotypic variation of the response to high temperatures

It was found previously that high temperature could increase the induction frequency but at the same time decrease the regeneration ability of the pollen callus (301 Research Group 1977). We assumed that if the anthers were cultured at high temperature for only a short period and then transferred to a relatively low temperature or if the high-temperature-induced pollen calli were transferred as early as possible to the regeneration medium, perhaps we could not only get a high callus induction frequency, but also maintain the regeneration ability of the callus at a high level. In 1978 some experiments were carried out according to this assumption. But the results showed that the responses to high temperature treatments were not consistent and varied conspicuously with anther donor genotypes. Special response to culture temperature was observed in 'Xiaoyan 759'. All F_1 hybrids of the crosses with 'Xiaoyan 759' as one of the parents responded badly to high temperature treatments. In 1979 and 1980, in order to confirm this phenomenon, further experiments were carried out using materials from several crosses including F_1 hybrids, parental varieties, and sometimes also the pollen-derived lines from F_1 hybrid anthers. The temperature treatment was culture of anthers at 33 °C for eight days followed by culture at 25 °C (or 26 °C) using continuous culture at 25 °C (or 26 °C) as the control. The results are presented in Table 1. It can be seen that genotypic variation in response to high temperature treatment exists. Culture at 33 °C for a short period resulted in a significant increase in induction frequency of pollen callus, differentiation frequency of green plantlets and induction frequency of green plantlets for 'Kedong 58' and 'Jinghong 5', but a significant decrease in the same three parameters for 'Norin 10', 'Xiaoyan 759' and 'Huapei 1'. For 'Pitic 62'

Table 1. Genotypic variation in the response of wheat anther culture to culture temperature (1979 – 1980)

Genotype	Temperature for inducing callus (°C)	No. of anthers plated	Induction frequency of pollen callus (%)	% calli giving green plantlets	% calli giving albino plantlets	Induction frequency of green plantlets %
Field-grown winter wheat						
'Kedong 58'	25°	287	2.4	14.3	28.6	0.3
	33° 8d → 25°	288	27.4	31.6	24.1	8.7
'Kedong 58' ×	25°	288	16.3	44.7	10.6	7.3
'Norin 10' F_1	33° 8d → 25°	288	25.0	41.7	12.5	10.4
'Norin 10'	25°	282	7.1	30.0	15.0	2.1
	33° 8d → 25°	288	1.7	20.0	0	0.3
Field-grown spring and semi-winter wheat						
'Pitic 62'	25°	218	25.7	35.7	25.0	9.2
	33° 8d → 25°	252	15.9	60.0	5.0	9.5
'Pitic 62' ×	25°	252	22.6	28.1	26.3	6.3
'Huapei 1' F_1	33° 8d → 25°	252	17.9	33.3	17.8	6.0
'Huapei 1'	25°	252	7.5	10.5	10.5	0.7
	33° 8d → 25°	252	3.2	0	25.0	0
'Jinghong 5'	25°	288	6.9	25.0	25.0	1.7
	33° 8d → 25°	288	16.0	43.5	32.6	6.9
'Jinghong 5' ×	25°	180	112.0	38.6	37.1	43.3
'Xiaoyan 759' F_1	33° 8d → 25°	216	26.4	64.9	15.8	17.1
Pollen line	25°	288	2.8	12.5	37.5	0.3
2533-1	33° 8d → 25°	288	2.8	12.5	12.5	0.3
Pollen line	25°	240	43.3	13.5	39.4	5.8
2531-10	33° 8d → 25°	288	47.6	12.4	37.2	5.9
'Xiaoyan 759'	25°	288	23.6	16.2	44.1	3.8
	33° 8d → 25°	288	15.3	2.3	68.2	0.3
Greenhouse-grown spring and semi-winter wheat						
'Orofen'	26°	204	3.0	17.0	17.0	0.5
	33° 8d → 26°	208	17.0	12.5	28.0	2.0
'Orofen' ×	26°	252	52.0	16.0	32.0	8.0
'Xiaoyan 759' F_1	33° 8d → 26°	252	12.0	11.0	22.0	1.0
'Xiaoyan 759'	26°	222	6.0	0	47.0	0
	33° 8d → 26°	177	0	0	0	0

Table 2. Effects of culture temperature on the induction frequency and regeneration ability of pollen calli (1) (greenhouse-grown spring and semi-winter wheat in 1980)

Genotype	Temperature for inducing callus (°C)	No. of anthers plated	Induction frequency of pollen callus (%)	% calli giving green plantlets	% calli giving albino plantlets	Induction frequency of green plantlets %
'Jinghong 5'	22.5°	300	2.0	0	16.7	0
	26°	302	5.6	5.9	4.2	0.3
'Pitic 62'	22.5°	300	0.7	0	50.0	0
	26°	244	11.9	3.4	31.0	0.4
'Ciano'	22.5°	272	1.8	40.0	0	0.7
	26°	305	8.9	44.4	11.1	3.9
'Huapei 1'	22.5°	261	0.4	0	100.0	0
	26°	306	6.2	5.3	10.5	0.3
'Xiaoyan 759'	22.5°	315	14.9	4.3	25.1	0.6
	26°	315	41.0	2.3	41.9	1.0
'Xiaoyan 759' ×	22.5°	165	21.8	2.8	5.6	0.6
'Xiaoyan blue grain F_1	26°	176	36.4	9.4	14.1	3.4

high temperature treatment resulted in a decrease in induction frequency of pollen callus but an increase in differentiation frequency of green plantlets, thus the final yield of green plantlets was similar to the control. Based on these results we might call 'Kedong 58', 'Jinghong 5' and 'Orofen' the high temperature type, 'Norin 10', 'Huapei 1' and 'Xiaoyan 759' the low temperature type, and 'Pitic 62' the intermediate type. Thus among the four crosses listed in Table 1, three were 'high temperature type × low temperature type', the other was 'intermediate type × low temperature type'. It may be seen in Table 1 that the responses of F_1 hybrids of two crosses with 'Xiaoyan 759' as one of the parents inclined towards the low temperature type parent 'Xiaoyan 759', that of 'Pitic 62' × 'Huapei 1' inclined towards the intermediate type parent 'Pitic 62', and that of 'Kedong 58' × 'Norin 10' was intermediate between the high and low temperature type parents. The responses of two pollen lines, 2533-1 and 2531-10, derived from anther culture of an F_1 hybrid of 'Jinghong 5' × 'Xiaoyan 759' were also intermediate between both parents. In a word, all the F_1 hybrids and pollen lines, no matter what crosses they were from, reflected the influences of their respective parents, indicating that the response of different materials to culture temperature was a heritable character. And furthermore the expressions of the F_1 hybrids also indicated that this character was not monogenic but might be a multigenic, quantitative character because, if it was monogenic, the responses of F_1 hybrids of the three

Table 3. Effects of culture temperature on the induction frequency and regeneration ability of pollen calli (2) (1981)

Genotype	Temperature for inducing callus (°C)	No. of anthers plated	No. of calli	Induction frequency of pollen callus(%)	Calli giving green plantlets		Calli giving albino plantlets		Induction frequency of green plantlets (%)
					No.	%	No.	%	
Greenhouse grwon spring wheat									
'Jinghong 5'	26°	282	9	3.2	2	22.2	4	44.4	0.7
	28°	232	19	8.2	2	10.5	8	42.1	0.9
	30°	229	41	17.9	9	22.0	12	29.3	3.9
	30° 8d → 26°	266	18	6.8	3	16.7	5	27.8	1.1
'Pitic 62'	26°	244	34	13.9	2	5.8	10	29.4	0.8
	28°	200	36	18.0	9	25.0	2	5.6	4.5
	30°	289	70	24.2	20	28.6	15	21.4	6.9
	30° 8d → 26°	248	23	9.3	5	21.7	1	4.3	2.0
'Ciano'	26°	318	36	11.3	20	55.6	1	2.8	6.3
	28°	275	70	25.5	29	41.4	5	7.1	10.5
	30°	326	114	35.0	24	21.1	29	25.4	7.4
	30° 8d → 26°	320	104	32.5	58	55.8	13	12.5	18.1
'Huapei 1'	26°	310	24	7.7	1	4.2	1	4.2	0.3
	28°	308	29	9.4	0	0	4	13.8	0
	30°	267	39	14.6	0	0	4	10.3	0
	30° 8d → 26°	267	23	8.6	1	4.3	4	17.4	0.4
Field grown winter wheat									
'Kedong 58'	26°	257	19	7.4	2	10.5	3	15.8	0.8
	28°	259	51	19.7	6	11.8	16	31.4	2.3
	30°	256	115	44.9	12	10.6	50	43.5	4.8
	30° 8d → 26°	299	59	19.7	9	14.6	10	17.3	2.9
'Norin 10'	26°	214	9	4.2	1	11.1	0	0	0.5
	28°	261	16	6.1	2	12.5	1	6.3	0.8
	30°	263	5	1.9	1	20.0	0	0	0.4
	30° 8d → 26°	256	13	5.1	1	7.7	1	7.7	0.4
40333 × 78-5268F_1	26°	310	56	18.1	8	14.6	2	2.9	2.6
	28°	303	92	30.4	9	10.0	9	9.2	3.0
	30°	267	145	54.3	9	6.5	38	26.3	3.5
	30° 8d → 26°	303	109	36.0	9	8.4	12	11.2	3.0
50106 × 78-5268F_1	26°	303	55	18.2	12	21.8	14	25.5	4.0
	28°	300	101	33.7	11	10.9	29	28.7	3.7
	30°	297	135	45.5	19	14.1	41	30.4	6.4
	30° 8d → 26°	260	68	26.2	10	14.7	8	11.8	3.8

'high × low' crosses should be the same, being either all low or all high temperature type, or else being all intermediate type, if there was no dominance present. But it was not the case, their responses varying with different crosses.

The response to various temperatures ranging from 22.5 °C to 32 °C

Since there was significant genotypic variation in the response of wheat anthers to culture at 33 °C for a short period, we wanted to know (i) if there was genotypic variation in response to other culture temperatures too, (ii) what was the culture temperature most suitable for induction of pollen callus in large quantity and with good quality. Thus the effects of various temperatures between 22.5 °C and 32 °C were tested in detail from 1980 to 1982.

Response to culture temperature below 26 °C. A simple experiment was carried out to test the response to culture temperature below 26 °C, with only one temperature treatment (22.5 °C) being used with 26 °C as a control. The experimental materials included high temperature types (such as Jinghong 5') and low temperature types (such as 'Xiaoyan 759' and 'Huapei 1'). As shown in Table 2 the tendencies of the responses of all the six genotypes, whether a high or low temperature type, were the same, no significant genotypic variation being observed, i.e., the responses at 26 °C were all better than at 22.5 °C. The induction frequencies of pollen callus or pollen plantlets at 26 °C were almost all several times higher than those at 22.5 °C. The green plantlet differentiation frequencies of a majority of the materials were also higher at the former temperature than at the latter. However the albino plantlet differentiation frequencies did not show regular changes.

Responses to continuous culture at various temperatures between 26 °C and 32 °C. The experiments on the effects of this range of temperatures were carried out four times from 1981 to 1982. The results are presented in Tables 3, 4 and 5. In the experiments shown in Table 3, three constant temperatures, 26 °C, 28 °C and 30 °C were tested, using four greenhouse-grown spring wheats and four field-grown winter wheats as materials, among them one spring variety ('Jinghong 5') and one winter variety ('Kedong 58') were of the high temperature type, and one spring variety ('Huapei 1') and one winter variety ('Norin 10') were of the low temperature type. The callus induction frequencies of almost all materials, whether a high or low temperature type, increased regularly with increasing culture temperatures within the range from 26 °C to 30 °C, with only 'Norin 10' showing no regular changes. For the high temperature type, the ability of pollen callus to regenerate green plants did not decrease with the increase of temperature, and for certain material like 'Pitic 62' it even significantly increased, so that their final yields of green plantlets all increased with increasing culture temperatures, reaching their peaks at 30 °C (Tables 3, 4 and 5). However for the low temperature type 'Huapei 1', green plantlets were obtained only at around 26 °C (Tables 1, 2 and 3), and for 'Norin 10', the suitable culture temperature seemed to be 28 °C. Other materials ('Ciano', 40333 × 78-5268 F_1 and 50106 × 78-5268 F_1) presented themselves as an intermediate type, their green plantlet yields did not vary much among different temperature treatments,

Table 4. Effects of culture temperature on the induction frequency and regeneration ability of pollen calli (3) (greenhouse-grown spring wheat in 1982)

Genotype	Temperature for inducing callus (°C)	No. of anthers plated	No. of pollen calli	Induction frequency of pollen callus (%)	% calli giving green plantlets	% calli giving albino plantlets	Induction frequency of green plantlets (%)
'Jinghong 5'	28°	273	30	11.0	3.3	23.3	0.4
	30°	238	35	14.7	14.3	28.6	2.1
	32°	257	33	12.8	0	36.4	0
'Ciano'	28°	266	42	15.8	59.5	2.4	9.4
	30°	264	37	14.0	35.1	18.9	4.9
	32°	284	21	7.4	28.6	23.8	2.1
'Pitic 62'	28°	226	22	9.7	18.2	31.8	1.8
	30°	234	20	8.5	40.0	30.0	3.4
	32°	196	9	4.6	11.0	44.4	0.5
'Huapei 1'	28°	259	14	5.4	0	35.7	0
	30°	252	31	12.3	0	0.3	0
	32°	261	25	9.6	0	16.0	0

Table 5. Effects of culture temperature on the induction frequency and regeneration ability of pollen calli (4) (1981)

Genotype	Temperature for inducing callus (°C)	No. of anthers plated	No. of pollen calli	Induction frequency of pollen callus (%)	Calli giving green plantlets		Calli giving albino plantlets		Induction frequency of green plantlets (%)
					No.	%	No.	%	
Field grown spring wheat									
'Jinghong 5'	28°	251	127	50.5	30	23.5	59	46.5	11.9
	30°	264	174	65.9	33	19.1	84	48.5	12.6
	30° 8d → 28°	263	168	63.9	25	14.8	73	43.3	9.4
	32° 8d → 28°	217	162	74.7	65	40.1	55	33.8	30.0
'Pitic 62'	28°	262	125	47.7	55	43.6	14	11.4	20.8
	30°	267	191	71.5	81	42.6	14	7.5	30.5
	30° 8d → 28°	259	164	63.3	79	47.9	13	8.0	30.3
	32° 8d → 28°	220	117	53.2	82	70.3	4	3.4	37.4
'Ciano'	28°	267	137	51.3	92	66.9	10	7.5	34.3
	30°	221	254	114.9	159	62.5	49	19.2	71.9
	30° 8d → 28°	266	173	65.0	103	59.7	28	16.3	38.9
	32° 8d → 28°	255	179	70.2	131	73.1	10	5.5	51.3
Field grown winter wheat									
'Kedong 58'	28°	312	62	19.9	7	10.6	13	20.3	2.1
	30° 8d → 26°	304	87	28.6	16	18.7	27	30.8	5.4
	30° 8d → 28°	261	129	49.4	27	20.6	27	20.8	10.2
'Norin 10'	28°	306	14	4.6	1	7.1	4	28.6	0.3
	30° 8d → 26°	310	30	9.7	8	26.7	2	6.7	2.6
	30° 8d → 28°	314	11	3.5	3	27.3	1	9.1	1.0
50512×	28°	223	29	13.0	3	11.4	7	23.1	1.3
78-5268 F_1 [a]	30° 8d → 26°	304	92	30.3	13	14.1	8	8.7	4.3
	30° 8d → 28°	302	84	27.8	14	16.7	20	23.8	4.6

[a] Spikes were stored at 3 °C for 24 h prior to inoculation

although the abilities of their pollen calli to differentiate green plantlets somewhat decreased with increasing culture temperatures. It is worth noticing that in the three experiments shown in Tables 3, 4 and 5, the responses of 'Pitic 62' to 30 °C were always better than to 28 °C and 26 °C, suggesting that 'Pitic 62' was actually a high temperature type.

In Table 4, the responses to 28 °C, 30 °C and 32 °C were compared, using four greenhouse-grown spring wheats as materials. It may be seen that when the culture temperature was raised from 30 °C to 32 °C the induction frequencies and green plantlet regeneration abilities of the pollen calli of all the materials, whether a high or low temperature type, decreased to various extents, and their final green plantlet yields, which depended upon these two factors together, were sharply decreased.

The differentiation frequency of albino plantlets did not regularly vary with the temperature changes. It tended to increase with increasing culture temperature from 26 °C to 32 °C in a small majority of cases (Tables 3, 4 and 5). But it was interesting that for the variety 'Ciano' it did increase quite regularly with the increase of culture temperature in a wide range from 22.5 °C to 32 °C.

Responses to short period high temperatures. In the above, we have stated the response to culture at 33 °C for eight days followed by 26 °C. In 1981, three other short period high temperature treatments were also tested, namely, culture at 30 °C for eight days followed by 26 °C or 28 °C and culture at 32 °C for eight days followed by 28 °C, using continuous culture at 26 °C, 28 °C and 30 °C as controls. The results (Tables 3 and 5) showed that the induction frequencies of pollen callus and green plantlets in culture at 30 °C for eight days followed by 26 °C were generally higher than those in continuous culture at 26 °C, lower than those in continuous culture at 30 °C, and approximately equivalent to or in some cases higher than those in continuous culture at 28 °C. For the intermediate and high temperature type, the effects of culture at 32 °C for eight days followed by 28 °C were all better than those of continuous culture at 28 °C, but approximately equivalent to those of continuous culture at 30 °C. However so far as the regeneration abilities of pollen

Table 6. The induction frequency and regeneration ability of the pollen calli derived from different culture temperatures, different batches and different genotypes

Genotype	Temperatur for inducing callus (°C)	No. of anthers plated	Induction frequency of pollen callus (%)			% calli giving green plantlets			% calli giving albino plantlets		
			1st batch	2nd batch	3rd batch	1st batch	2nd batch	3rd batch	1st batch	2nd batch	3rd batch
'Jinghong 5'	28°	251	7.2	39.0	4.4	33.3	23.4	9.1	33.3	50.0	36.4
	30°	264	24.2	38.3	3.4	24.1	16.6	11.1	41.4	53.4	44.4
	32° 8d→28°	217	24.0	45.2	5.5	45.8	40.0	16.7	34.2	36.6	8.3
'Pitic 62'	26°	244	7.0	6.1	0.8	5.9	6.7	0	23.5	40.0	0
	28°	200	9.5	8.0	0.5	36.8	12.5	0	5.3	6.3	0
	30°	289	15.2	8.7	0.3	40.9	8.0	0	27.3	12.0	0
'Ciano'	26°	318	5.0	6.0	0.3	56.0	57.9	0	0	5.3	0
	28°	275	13.8	10.5	1.1	55.3	27.6	0	5.3	10.3	0
	30°	326	30.7	3.1	1.2	18.0	60.0	0	27.0	20.0	0
50106×	26°	303	13.5	4.3	0.3	26.8	7.7	0	29.3	15.4	0
78-5268 F_1	28°	300	30.0	2.7	1.0	10.0	25.0	0	32.2	0	0
	30°	297	42.1	3.4	0	14.4	10.0	0	31.2	20.0	0

calli were concerned culture at 32 °C for a short period was significantly better than continuous culture at 30 °C, the differentiation frequency of green plantlets being higher, but that of the albino ones being lower. The effects of culture at 30 °C for eight days followed by 28 °C seemed to be intermediate between the effects of culture at 30 °C for eight days followed by 26 °C and those of culture at 32 °C for eight days followed by 28 °C.

In the short period high temperature treatments somatic calli originating from anther filaments were sometimes observed. However as has been reported in a previous paper (Ouyang et al. 1973) these filament derived somatic calli were significantly different from the pollen derived ones in many characteristics, and thus were very easy to distinguish: they were located at the cut ends of filaments, were not as compact in texture as pollen calli, emerged earlier than pollen calli, and by the time when we transferred the first batch of pollen calli to regeneration medium they usually had become brown, and looked to be in a decaying stage. And even though the fresh filament-derived calli were transferred to regeneration medium, generally they would not differentiate shoots.

Effects of different culture temperatures and genotypes on the emerging time of pollen callus

It was observed in a series of experiments that the higher the culture temperature, the earlier and more concentrated the emerging time of pollen callus. At relatively low culture temperatures the pollen callus emerged rather later and the emerging time lasted longer. However this also varied with different genotypes. Data of four genotypes are shown in Table 6 as representatives. The pollen calli were counted and transferred to regeneration medium in three batches on the 26th, 38–40th and 50th day in culture respectively. It may be seen that the pollen calli of 50106×78-5268 F_1 emerged earliest, a great majority of them concentrated in the first batch. Especially at 30 °C, 92.5% of the calli came from first batch, and there were no calli in the third batch. In contrast with 50106×78-5268 F_1, the pollen calli of 'Jinghong 5' emerged latest, the majority of them coming from the second batch, and there were still considerable number of calli in the third batch. Especially at relatively low temperature, the number of calli of the third batch was almost similar to that of the first batch. The situations of 'Ciano' and 'Pitic 62' were intermediate between those of 50106×78-5268 F_1 and 'Jinghong 5'.

The plantlet regeneration abilities of the pollen calli of the first and second batches were approximately similar, although the green plantlet regeneration abilities in the first batch were higher than in the second batch in many cases. The plantlet regeneration abilities of the third batch were much lower than those of the former two batches except that in Jinghong 5 the albino plantlet regeneration abilities of the third batch were approximately similar to those of the former two batches.

From all the results mentioned above it may be summarized that for all genotypes, in the temperature range from 26 °C to 32 °C, the main emerging period of pollen callus is from the 20th to the 40th day after anther inoculation.

Discussion

The response of wheat anther culture to culture temperature is quite sensitive. Temperature difference of only 2 to 3 centigrade degrees would lead to conspicuous and even striking changes in the induction fre-

quencies of pollen callus and pollen plantlets, especially at temperatures higher than 30 °C or lower than 26 °C. Only the so called intermediate type was insensitive to temperature changes between 26 °C and 30 °C.

The response to culture temperature is also rather complicated. This was first shown in the genotypic variation in the response. We can even further classify the various genotypes into the so called high temperature, low temperature and intermediate type. In each type there were also different cases. For example, 'Jinghong 5', 'Kedong 58' and 'Pitic 62' were all of the high temperature type, having high yields of green pollen plants at high temperature, yet the high green plantlet yields of 'Jinghong 5' and 'Kedong 58' were mainly due to the higher yields of pollen callus, and those of 'Pitic 62' were mainly due to the higher abilities of the pollen calli to regenerate green plantlets (Tables 1, 2, 3 and 4). In a word, the response of anther culture to culture temperature was quite diverse. However there are two points which should be mentioned: (i) The genotypic variation in the response to culture temperature could only be observed in continuous culture at temperatures between 26 °C and 30 °C, or in culture at 33 °C for eight days followed by 25 °C (or 26 °C), and could not be significantly observed in continuous culture at temperatures higher than 30 °C or lower than 26 °C. (ii) The classification of various genotypes into high temperature, low temperature and intermediate type is only relative. Actually the culture temperatures (26 °C–28 °C) required by the so called low temperature type are not of low temperature in the common sense. Furthermore, certain genotypes sometimes responded differently in different years or under different growth conditions of anther donor plants. For example, the high temperature type 'Pitic 62' performed as intermediate type in the summer of 1979 (Table 1); and the variety 'Ciano' had highest green plantlet yield at 28 °C when grown in the greenhouse (Table 4), but had highest green plantlet yield at 30 °C when grown in the field (Table 5). It should be particularly mentioned here that for quite a few genotypes the suitable temperatures required in culture in the summer of 1980, unusually, were significantly lower than in other years. The appearance of this phenomenon was very likely due to the different physiological states of the anthers resulting from different growth conditions of anther donor plants. The physiological state of the anther might influence the response of the anther to culture temperature to some extent. Both the great diversity of the response of anther culture to culture temperature and the possible existence of the influence of growth conditions of anther donors on the response are in accordance with the viewpoint that the response to culture temperature may be a multigenic, quantitative character.

Although there are genotypic variation and probably also the influence of growth conditions of anther donors in the response to culture temperature as mentioned above, it is obvious that the suitable temperature for pollen callus induction lies between 28 °C and 30 °C except for a few genotypes which seemed to require culture temperatures below 28 °C. The relatively low temperature requirement of some of these genotypes such as 'Xiaoyan 759' and its F_1 hybrids may be related to the influence from *Agropyron elongatum*.

The data in the present paper reveal that an increase in culture temperature did not lead to a decrease in the green plantlet regeneration abilities of pollen calli for most genotypes (mainly the high temperature type) in the range from 26 °C to 30 °C, and in some cases even lead to an increase of the regeneration abilities, if only the pollen calli were transferred to regeneration medium in time as in the present work. Given the timely transfer of callus to a regeneration medium, further application of culture at a high temperature for a short period followed by lower temperature seemed to have no significant advantage over the continuous culture at suitable temperatures. Yet this problem needs further studies.

From a comparison between culture at high temperature for a short period and culture at continuous constant temperature it can be seen that the beneficial effect of the appropriately high temperature (not beyond 30 °C) on the induction of pollen callus for the high temperature type may occur during the whole process from the initiation of induction to subsequent growth and development of the pollen callus. For example, the difference between the culture at 30 °C for eight days followed by 26 °C and the continuous culture at 26 °C is only 4 centigrade degrees higher in the former than in the latter during the first eight days in culture, however, for the high temperature type the green plantlet yields in the former case were conspicuously higher than in the latter case, indicating the beneficial effect of an appropriately high temperature in the first eight days of culture (Table 3). The difference between the culture at 30 °C for eight days followed by 26 °C and the continuous culture at 30 °C is only 4 centigrade degrees lower in the former than in the latter case during the culture period eight days after inoculation, however the green plantlet yields of the high temperature type in the former case were conspicuously lower than in the latter case, indicating the beneficial effect of an appropriately high temperature in the culture period eight days after inoculation (Table 3). According to the observations of Zhu (1978) and Tseng (1978), in the first eight days of anther culture the *T. aestivum* microspores divided and formed equal cells or cells with multiple equal free nuclei, thus showing the initiation of sporophytic development.

Hence the effect of an appropriately high temperature might be mainly in enhancing the initiation of sporophytic development of the microspores in the first eight days of culture, and mainly in enhancing the further growth and development of the pollen grains with multiple equal cells or equal free nuclei into embryoids or calli in the culture period eight days after anther inoculation.

It may also be observed from the data of this paper that the yields of pollen callus and green pollen plants varied greatly with genotypes and growth conditions of anther donors. In general, the yields of green plantlets of spring wheats seem to be higher than those of winter wheats; and those of the field-grown materials are much higher than those of the greenhouse-grown materials. The highest green pollen plantlet yield was observed in the summer of 1981 in a field-grown Mexican spring wheat 'Ciano' in which there were 71.9 pollen calli giving green plantlets from every 100 cultured anthers (Table 5).

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