J. Appl. Genet. 38(4), 1997, pp. 393-406

Influence of genetic and environmental factors on anther culture response of wheat

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Abstract. The influences of genetic and environmental factors on the anther culture responses of wheat were investigated. Significant differences for callus induction, plant regeneration, and green plant percentages were observed when the nucleus of *Triticum aestivum* L. cv. Selkirk was transferred to ten alien cytoplasms by substitution backcrosses. In most cases, the alien cytoplasms decreased anther culture responses, but sometimes they were as good as or better than the *T. aestivum* cytoplasm. Significant within-genotype variation for anther culture responses were observed for wheat varieties Chris, Yecora Rojo, WA7176 and Edwall, indicating genetic heterogeneity in the present commercial cultivars, and potential for improving anther culture responses by in vitro prescreening. When five genotypes (Chris, Pavon 76, Butte 86, WA6916, and Edwall) were cultured across three (potato-4 liquid, 100 g L^{-1} ficoll-supplemented, and 6 g L^{-1} agar-solidified) induction media, the liquid and ficoll-containing media were 10 to 15 times more productive than the agar-solidified medium. Whereas, the ficoll medium was not significantly different from the liquid medium. Several low concentration starch media appeared promising to replace current induction media. The starch media sustained the high-callus-induction properties of the liquid medium, while improving callus aeration similar to that observed on solid media, resulting in markedly higher plant regeneration and green plant percentages.

Key words: callus response, doubled haploids, induction media, plant regeneration, *Triticum aestivum*.

Received: July 1997.

College of Agriculture and Home Economics Research Center, Washington State University Project No. 1568 and 3571. This investigation was supported in part by the Washington Technology Center.

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Introduction

The potential for use of doubled haploid plants in genetic studies and plant breeding is already well recognized. A major doubled haploid system is currently being exploited in wheat. The efficiency of the wheat anther culture system depends on successes in callus induction, plant regeneration, green plant percentage, as well as on spontaneous or artificial chromosome doubling. These components of anther culture response are influenced by both genetic and environmental factors. The understanding and effective control of these factors can markedly improve haploid production.

The strong influence of genetic controls on callus induction, plant regeneration, and green plant percentage are well known in wheat (HENRY, De-BUYSER 1985, TUVESSON et al. 1989), rice (*Oryza sativa* L.) (QUIMIO, ZAPATA 1990), barley (*Hordeum vulgare* L.) (KNUDSEN et al. 1989, POWELL 1988), and other species (CHARMET, BERNARD 1984, TOMES, SMITH 1985, UHRIG 1985). Genetic effects have accounted for up to 85% of the total variation observed in wheat and barley anther cultures (KNUDSEN et al. 1989, TUVESSON et al. 1989, LARSEN et al. 1991), and these effects can be additive (DEATON et al. 1987, SZAKACS et al. 1988) or non-additive (POWELL 1988, AGACHE et al. 1989, TUVESSON et al. 1989), depending upon the species and probably the genotypes within a species. Genetic variations controlled by cytoplasm were also significant in anther culture systems (LAZAR et al. 1984, POWELL 1988, EKIZ, KONZAK 1991c). But, the genetic effects caused by cytoplasm were not detected in other studies based on reciprocal differences (DUNWELL et al. 1987, AGACHE et al. 1989, QUIMIO, ZAPATA 1990, LARSEN et al. 1991). The apparent inconsistency is most likely due to the limited range of genotypes cultured in some studies, because the expression of reciprocal differences is determined by the range of cytoplasmic and nuclear genetic variation among the parents. This has been demonstrated by anther culture experiments using alloplasmic lines, in which a *T. aestivum* nucleus was transferred to alien cytoplasms by substitution backcrosses. In those studies, significant cytoplasmic effects (SAGI, BARNABAS 1989, EKIZ, KONZAK 1991a) and nuclear × cytoplasmic interactions were observed (EKIZ, KONZAK 1991b, c).

Among the culture medium components affecting culture responses, the types and concentration of sugars and hormones are major factors that determine the yield of haploid plants (ARMSTRONG et al. 1987, ORSHINSKY et al. 1990, KONZAK, ZHOU 1991, BALL et al. 1992). In particular, physical conditions of the culture media also showed marked influences on culture ability (JONES, PETOLINO 1988, ZHOU et al. 1991). In prior haploid production systems, anthers were cultured on agar-solidified induction media for callus

initiation. Recent studies showed that callus induction was 10 to 20 times greater for anthers cultured on liquid potato-4 (P4) versus agar-solidified induction media (UHRIG 1985, ZHOU, KONZAK 1989). This increase was probably due to the higher conductivity of liquid medium and the better contact between medium and cultured tissues. However, some studies found that the calli produced on liquid-induction media had a low regeneration ability, and also produced more albino plants (JONES, PETOLINO 1988, KASHA et al. 1989). In liquid-induction media, some calli sink to the bottom of petri plates, where an anaerobic condition may impair the normal development of the chloroplasts in calli. Therefore, several laboratories have vigorously searched for medium components able to sustain the properties of liquid medium while preventing calli from sinking. Ficoll and dextran possess such properties, and these have been used in several anther culture experiments (JONES, PETOLINO 1988, KASHA et al. 1989, ZHOU et al. 1992). However, ficoll is very expensive, and the benefits of ficoll as well as of dextran are not consistent between studies. Reviewed herein are studies aimed toward understanding the nuclear and cytoplasmic inheritance of androgenesis, and studies to assess the influence of medium physical conditions on anther culture responses in wheat.

Material and methods

Eight genotypes of spring wheat, and 10 alloplasmic lines of wheat were used in these studies. The plants were grown in a greenhouse with day/night temperatures at 25/15C°. The photoperiod was 16 hours with a light intensity of 300 µmol m^{-2} s^{-1}, supplemented by high-pressure sodium lamps. Th sampling and pretreatment of the spikes and anther culture methods were the same as previously described by ZHOU and KONZAK (1989) and KONZAK and ZHOU (1991).

Potato-4 (P4) induction media were used throughout these studies. The P4 media contained 90 g L^{-1} sucrose, 1.5 mg L^{-1} 2,4-D, and 0.5 mg L^{-1} kinetin. Ficoll or gelling agents were sometimes added to the induction media as indicated in each experiment. Calli induced on the media were transferred to an MS regeneration medium for plant initiation (KONZAK, ZHOU 1991). The regeneration medium contained 30 g L^{-1} sucrose and 2.5 g L^{-1} gelrite gellan gum, but no plant growth regulator.

Experiment 1 (alloplasmic lines)

Ten alloplasmic lines carrying a *T. aestivum* nucleus were developed by repeated backcrosses using wheat variety Selkirk as male parent. The numbers of backcrosses varied from 9 to 13 generations (Table 1). The material was developed by Dr. S.S. MEAN and kindly provided by his colleague Dr. Ken KO-

FOID, Department of Crop and Weed Sciences, North Dakota State University, Fargo, North Dakota. The 10 alloplasmic lines and the recurrent male parent, variety Selkirk, were cultured to assess cytoplasmic effects on anther culture responses. These same materials also were studied by EKIZ and KONZAK (1991a,b).

Experiment 2 (genotypic and within-genotype variation)

Four genotypes, Chris, Yecora Rojo, Edwall, and WA7176, were cultured to investigate the genetic control of their differential anther culture-responses. Eight plants from each genotype were randomly selected and cultured to assess within genotypic variation. From each plant 2 to 3 spikes were cultured separately and treated as replicates. The genotypic and within-genotype variations were analysed according to a nested experiment design.

Experiment 3 (physical conditions of induction media)

A factorial experiment with five genotypes and three induction media was conducted. Anthers from the five genotypes (Chris, Pavon 76, Butte 86, Edwall, and WA6916) were cultured on P4 liquid, 100 g L^{-1} ficoll-supplemented, and 6 g L^{-1} agar-solidified induction media to determine the induction medium effects. Each treatment had 4 replicates with 60 to 80 anthers per replicate. The concentration of ficoll (100 g L^{-1}) was lower than most previous studies (200 g L^{-1}) because higher concentrations of ficoll were found deleterious (ZHOU et al. 1992). Since the solid medium was less productive, 2 to 4 times more anthers were cultured in order to ensure enough calli was available on which plant regeneration could be calculated.

Experiment 4 (gelling agents in induction media)

Five starches (wheat starch, obtained courtesy Dr. S. BAENZIGER, Univ. of Nebraska, referred to as commercial wheat starch; wheat starch, Sigma S-2760; potato starch, Sigma S-2630; corn amylose, Sigma A-7043; and corn amylopectin, Sigma A-7780) and three gelling agents (Agar, Sigma A-1296; Agarose, Seaplaque; and Gelrite gellan gum, MERCK 463070-00) were used in induction media. The concentration of starches was 3%, and those of agar, agarose, and gelrite were 0.2%. Selection of the concentrations was based on previous experiments for obtaining maximum conductivity while retaining a solid medium. Four replicates were included in each treatment, with 60 anthers per replicate.

The calculation of callus induction was based on the number of calli per 100 cultured anthers; plant regeneration was calculated as the percentage of calli that produced green or albino plants; whereas green plant percentage was calculated as the number of calli that produced green plants divided by

the total number of regenerated calli. The experiments were conducted either according to a randomized complete-block design (experiments 1, 2, and 4) or a factorial design (experiment 3). The data were analyzed using the G124 procedure of SAS/PC (SAS 1988). Significant differences between treatments or genotypes were determined by the protected LSD test at the 0.05 probability level.

Results

Alloplasmic lines

When the nucleus of *T. aestivum* cv. Selkirk was transferred to the ten alien cytoplasms by substitution backcrosses, significant differences in callus induction ($P \leq 0.01$), plant regeneration ($P \leq 0.05$) and green plant percentage were observed among the lines (Table 1). Five of the alloplasmic lines significantly decreased callus induction, and the other five were not significantly different from variety Selkirk. Only one alloplasmic line significantly increased regeneration, whereas the other nine were not significantly different from the *T aestivum* cytoplasm. None of these alloplasmic lines showed significant differences from the nucleus donor parent in terms of green plant percentage, but significant differences among the alloplasmic lines were observed. The yields of green plants per 100 cultured anthers for these alloplasmic lines were either significantly lower than or remained the same as the nucleus donor parent. It was noteworthy that *Aegilops juvenalis, Haynaldia villosa, T. macha* (PI140191 and PI190923), and *T. turgidum* showed equivalent responses for all anther culture components as the *T. aestivum*, thus indicating potential for exploiting these cytoplasms in breeding and haploid production.

Genotypic and within-genotype variations

Genotype differences for anther culture responses were predominant (Table 2). Chris was a highly responsive genotype to anther culture. On average, 28 green plants were produced from 100 anthers. Yecora Rojo had a low callus induction (23 calli/100 anthers), but regeneration of plants from the calli and green plant percentage were high. In contrast, Edwall and WA7176 had a higher callus induction than Yecora Rojo, but then green plant percentages were very low. It was obvious from these results that callus induction, plant regeneration and green plant percentage are genetically controlled independently of anther culture components. The multiple product of the three components determines the yield of green plants. It is interesting that genetic variation within genotype also was significant for callus induction ($P \leq 0.01$)

Table 1. Anther culture responses of alloplasmic lines obtained by the transfer of the nucleus of *Triticum aestivum* cultivar Selkirk into alien cytoplasms through repeated backcrosses

Type of cytoplasm	Backcross	Calli/anthers	Green plants	Albino plants	Calli/100 anthers*	Regen.calli* (%)	Green plants* (%)	Green plants/100 anthers*
Ae. juvenalis (Thell.)Eig	9	313/320	72	69	98 a	46 bc	50 a	23 ab
Ae. cylindrica Host	10	113/320	13	47	35 c	50 ab	21 b	4 c
Ae. variabilis Eig.	13	165/320	25	33	52 bc	36 c	43 ab	8 c
Ae. squarrosa vs. *strangulata*	19	127/320	38	29	40 c	51 ab	59 a	12 bc
Ae. uniaristata Vis.,	10	93/320	17	17	29 c	37 c	49 a	5 c
Ae.ventricosa Tausch	13	72/320	16	29	23 c	64 a	38 ab	5 c
Haynaldia villosa Schur.	9	259/320	72	51	81 ab	49 bc	58 a	23 ab
T. macha Dek. & Men. (PI140191)	17	352/320	77	89	110 a	46 bc	45 a	24 a
T. macha Dek. & Men. (PI190923)	9	352/320	77	74	102 a	47 bc	50 a	24 a
T. turgidum L.	9	247/320	63	72	77 ab	56 ab	51 a	20 ab
T. aestivum cv. Selkirk		337/320	64	95	105 a	47 bc	42 ab	20 ab

Ae. = *Aegilops*, *T.* = *Triticum*.

* Means in the same column followed by the same letter are not significantly different at $P \leq 0.05$.

Table 2. Culture responses and analysis of variance for the four wheat genotypes and plant variation within the genotypes

Culture responses

Genotype	Plants cultured	No. of anthers	No. of calli	Green plants	Albino plants	Calli/100 anthers	Regen. calli	Green plants	Green plants/100 anthers
Chris	8	1046	1468	295	139	139±37	30±12	68±15	28±12
Yecora Rojo	7	966	235	68	83	23±11	66±18	42±29	6±6
Edwall	8	1043	1017	7	544	98±48	55±6	1.4±2.3	0.7±1.1
WA7176	8	1396	1696	21	815	119±55	49±7	2.9±3.2	1.5±1.6

Analysis of variance

Source of variation	df	Callus induction	Plant regeneration	Green plant
Genotype	3	4.591**	0.345**	1.879**
Within genotype	28	0.274**	0.018*	0.026
Error	43	0.122	0.09	0.026

*, ** Significantly different at $P \leq 0.05$ and $P \leq 0.01$, respectively.

and plant regeneration ($P \leq 0.05$) (Table 2). Using 'Yecora Rojo as an example, among the seven plants tested, callus induction varied from 4.4 ± 2.6% to 30 ± 11.7% calli/100 anthers; regenerable calli varied from 46.7 ± 18.9% to 83.1 ± 23.6%; and green plant percentages varied from 23.6 ± 20.6% to 76.2 ± 22.8%. The within-genotype variation for green plant percentage was not statistically significant for this experiment, but the actual range of green plant percentage was over 3-fold for different plants of Yecora Rojo. A similar pattern of variation was also observed for Chris, Edwall and WA7176 (data not presented).

Physical conditions of induction media

Among the three induction media, the liquid and ficoll-containing media produced significantly ($P \leq 0.01$) more calli than the agar-solidified medium for all five genotypes (Table 3). Liquid medium was more productive than the ficoll medium in all cases, but the differences were not significant for some genotypes. Calli produced on the liquid medium were not necessarily less-regenerable than those from the agar-solidified medium as expected. But, ficoll addition significantly ($P \leq 0.05$) increased plant regeneration in four out of five genotypes, and increased the green plant percentage in two out of five genotypes. The liquid and ficoll-containing media produced significantly more green plants per 100 anthers than the agar-solidified medium, but the liquid and ficoll media were not significantly different in most cases. Significant interactions between genotype and induction media also were observed for callus induction ($P \leq 0.01$), plant regeneration ($P \leq 0.01$), and green plant yield ($P \leq 0.01$). In other words, the physical conditions of induction media showed differential influences on anther culture responses for different genotypes (Table 3). But, the interpretations of these interactions was difficult, and the implications of the interactions cannot be generalized.

Gelling agents in induction media

Liquid medium markedly increased callus induction. However, liquid medium reduced callus differentiation ability and increased albino plant percentage in some studies. The objective of this experiment was to develop media which sustain the high callus production on the liquid medium while preventing calli from sinking. A low concentration of starch and gelling agents was chosen, so that the media were marginally solidified. In all but one case, the semi-solid media significantly reduced callus induction ($P \leq 0.01$), as well as plant regeneration from the calli ($P \leq 0.05$). However, the semi-solid medium significantly increased green plant percentages ($P \leq 0.05$). In terms of green plant

Table 3. Culture response of anthers of five wheat genotypes on three induction media

Genotype	Treatment	No. of anthers	No. of calli	Green plants	Albino plants	Calli/ 100 anthers*	Regen. callus* (%)	Green plants* (%)	Green plant/ 100 antherts*
Chris	Solid	1200	397	40	24	33.1 b	16.1 b	63.1 b	3.3 b
	Liquid	320	452	164	56	141.3 a	49.3 a	75.5 a	51.3 a
	Ficoll	320	356	147	41	111.3 a	53.0 a	78.3 a	45.9 a
Pavon76	Solid	786	557	40	65	69.1 c	21.0 b	38.0 b	5.2 b
	Liquid	240	903	168	246	376.3 a	46.3 a	40.4 ab	70.0 a
	Ficoll	240	391	109	67	162.9 b	44.1 a	57.9 a	45.4a
Butte86	Solid	880	34	1	3	4.0 b	9.9 a	16.7 a	0.1 b
	Liquid	240	178	11	36	74.2 a	24.9 a	22.3 a	4.6 a
	Ficoll	240	99	5	12	41.3 ab	22.4 a	28.3 a	2.1 ab
WA6916	Solid	1100	363	8	76	33.7 c	23.8 b	8.4 a	0.8 a
	Liquid	240	423	4	127	176.3 a	29.7 b	2.6 a	1.7 a
	Ficoll	240	228	16	104	95.0 b	54.4 a	12.9 a	6.7 a
Edwall	Solid	560	93	18	18	16.6 c	23.6 b	9.2 a	0.4 a
	Liquid	240	394	16	147	164.2 a	40.4 ab	8.8 a	6.7 a
	Ficoll	240	243	12	91	101.3 b	46.5 a	10.2 a	5.0 a

*Means in the same column followed by the same letter are not significantly different at $P \leq 0.05$.

Table 4. Influence of induction medium gelling agents on anther culture response of Pavon 76

Treatment	Content ($g\ L^{-1}$)	Calli/anthers	Green plants	Albino plants	Calli/100 anthers*	Regen. callus* (%)	Green plant* (%)	Green plants/anthers*
Wheat starch (Com)	30	509/240	88	49	212.1 bcd	26.4 bcd	62.7 a	36.7 ab
Wheat starch (Sig)	30	396/240	73	36	165.0 def	29.0 bc	68.5 a	30.4 ab
Potato starch	30	582/240	84	54	242.5 bc	23.7 cde	60.5 a	35.0 ab
Corn amylose	30	247/240	50	31	102.9 f	33.1 ab	60.5 a	20.8 b
Corn amylopectin	30	640/240	75	61	266.7 ab	21.3 de	54.3 a	31.3 ab
Agarose	2	488/240	56	31	203.3 bcd	17.7 e	60.5 a	23.3 b
Gelrite	2	455/240	85	56	189.6 cde	31.3 bc	60.9 a	35.4 ab
Agar	2	300/240	49	32	125.0 ef	29.3 bc	62.1 a	20.4 b
Control	Liquid	769/240	102	214	320.4 a	40.8 a	31.2 b	42.5 a

*Means in the same column followed by the same letter are not significantly different at $P \leq 0.05$.

yield, the liquid medium produced the highest green plant percentage, but commercial wheat starch and potato starches, and gelrite-containing media were as good as the liquid medium. This experiment was repeated several times by varying concentrations of the starches and gelling agents. The relative ranks of the three anther culture components were variable between experiments. But, the final yields of green plants were quite consistent, indicating that starch could potentially replace agar and ficoll as an induction medium component.

Discussion

Substitution backcrosses are often used to compare responses of the nucleus of a genotype when present in different cytoplasms. After repeated backcrosses, the nucleus of the recurrent parent is theoretically restored in each of the test cytoplasms even though some residuals of the nucleus from the original female parents may still remain. It was assumed in the present study that: (1) the cytoplasmic factors of the female parent remained stable throughout backcrossing; (2) there was no introduction of cytoplasmic factors through the pollen; and (3) the nuclear genes of the cytoplasm donors were completely eliminated in the test lines. If these assumptions are correct, it was clear that the influence of cytoplasm on anther culture ability was significant. Among the 10 alien cytoplasms tested, most showed significant influences on one or two anther culture components. Two additional series of alloplasmic lines, with *T. aestivum* cv. Siete Cerros 66 or Penjamo 62 nuclei, were also tested by EKIZ and KONZAK (1991a). Results from those experiments indicated that *T. dicoccum pseudomacrotherum*, *T. monococcum* ssp. *aegilopoides*, *T. dicoccum khapli*, *T. turgidum persicum fuliginosum*, and *T. turgidum turanicum notabile* cytoplasms showed positive influences on all three components of anther culture response, or did not negatively affect any component while increasing one or two of them. The yields of green plants per 100 anthers, therefore, were 2 to 6 times higher than *T. aescivum* cv. Siete Cerros 66 or Penjamo 62. Significant nucleus × cytoplasm interactions also were observed between the three nuclei and the alien cytoplasms (EKIZ, KONZAK 1991a). These results indicated a potential for use of alien cytoplasms to improve haploid production of wheat. The significant influence of cytoplasm also indicated that extensive cytoplasmic variation for anther culture components may occur within *T. aestivum*, and has been demonstrated in further studies (EKIZ, KONZAK 1991b,c). The presence of cytoplasmic variation determines the allocation of genetic

resources in making crosses. Sorting of cultivars based on the female parentage may provide useful information for determining the association of cytoplasm genetic factors with anther culture responses, and may have pertinence to other breeding traits.

High random variations and lack of reproducibility also are inheritent problems in tissue culture experiments. In some studies, the random variation is as large as the treatment or genotype effects. This variation is known to be influenced by environmental factors, but within-genotype variation (heterogeneity) as detected in the current experiments may be a factor responsible for some of the error variation. Such genetic heterogeneity may be extensively distributed within cultivars, depending on the selection history of individual cultivars and specific traits. The probable cause among public cultivars, is the fact that most are comprised of composites of lines derived from selections made originally at the F_3 or F_4 generation. Since current breeding schemes have not placed selection pressure on anther culture ability, prescreening of some genotypes may markedly improve tissue culture responses, through within genotype selection alone.

Genetic improvement to enhance anther culture response appears promising as a method to construct breeding populations within which anther culture can be more efficiently exploited. However, environmental factors related to donor plant growth conditions and culture media are not yet under experimental control. Of special interest are the physical properties of induction media, such as the conductivity and osmolality of solid media, and the density, osmolality and viscosity of liquid media. Results presented here indicate that the types of starch and gelling agents determine the physical conditions of the medium, affecting the productivity of anther cultures. Previous experiments showed that induction media with a high concentration of starch produced very few calli (ZHOU, KONZAK, unpublished data). At a starch concentration of 2 to 3%, a pool of liquid medium was formed around the anthers and calli, probably due to enzymatic digestion of the solidified starch media. The liquid medium in this pool may provide a better buffered environment and improved access to air for the anther and callus tissues, whereas the solid base beneath the liquid pool prevents the tissues from sinking. This observation amplifies previous studies in which starch functioned as a carbon source after being enzymatically digested by the cultured tissues (SORVARI, SCHIEDER 1987). Thus, starch offers promise as a replacement for agar as a gelling agent, and an inexpensive substitute for ficoll to improve medium aeration. Moreover, ficoll not only increases medium density, preventing calli from sinking, but also increases medium viscosity and osmolality. The increased medium viscosity had a ne-

gative influence on anther culture productivity, whereas the effects of ficoll on medium osmolality interacted with sugar concentration (ZHOU et al. 1992). High molecule weight dextran (KASHA et al. 1989) and high density cellulose (ZHOU, unpublished data) also were tested as substitutes for ficoll, but with limited success. Thus, some types of starch at low concentrations may offer unique properties as the medium component/gelling agent for use in anther culture.

Notes. This work was performed in connection with the FAO/IAEA Coordinated Research Programme on "The Use of Induced Mutations in Connection with Haploids and Heterosis in Cereals". Scientific editing of the paper was undertaken by Perry GUSTAFSON (Columbia, Missouri, USA) and Mirosław MALUSZYNSKI (FAO/IAEA, Vienna, Austria).

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