The Effect of Cultural Factors on Anther Culture in Spring Wheat (Triticum aestivum)

Tae-Jin Kang[1] and Deok-Chun Yang[2]*

[1] Institute of Basic Sciences, Chonbuk National University, Jeonju 561-756, Korea.
[2] Dept. of oriental medicinal materials & processing, College of Life Science, KyungHee University, Suwon 449-701, Korea.

ABSTRACT

Effects of shaking, medium consistency and anther density on polyhaploid production in two wheat cultivars, Pavon and Chris, were studied using a modified 85D12 medium. Pavon produced more calli in shaking and more albino plants than Chris. However, Chris produced threefold more green plants than Pavon in non-shaking treatment. More calli and green plants were derived from non-shaking treatment than those from shaking treatment. Anthers were cultured on both liquid and semi-solid 85D12 media, using two anther densities, 48 and 96 anthers per plate. Although Pavon generally produced more calli and albino plants than Chris, Chris produced more green plants than Pavon. More green plants were derived from semi-solid medium than those from liquid medium. A factor that may affect plant regeneration from anthers is the length of time on initiation medium. Most of the calli for both genotypes were transferred during the first two time periods. Fertility, as measured by seed set, was determined for all surviving regenerated plants. About 24% of Chris and Pavon anther-derived green plants in the experiment of medium consistency and anther density produced seed.

Keywords **:** Pavon, Chris, wheat, shaking treatment, anther culture, fertility

INTRODUCTION

The development of anther culture technology leading to homozygous diploids has proven to be one of the most effective methods of haploid plant production. However, the techniques for haploid production are - especially in crop plants - not very productive (Hu, 1986; Ouyang et al., 1987), although considerable progress has been made (Lazar et al., 1985; Ouyang et al,. 1983; Armstrong et al., 1987). Therefore, it's too risky to use anther culture method for a practical application in comparison with conventional breeding. To improve success in microspore regeneration, principally, two approaches may be possible: either a study of physiological factors such as *in vitro* culture conditions (Dunwell, 1982) or an investigation of the genotypic factors involved. Genotypic differences in wheat anther culture have been reported by many workers (Ouyang et al., 1973; Picard and deBuyser, 1975; Heszky and Mesch, 1976; Schaeffer et al., 1979; Dube, 1984; Lazar et al., 1987). Although a particular set of cultural conditions may be successful for some cultivars, the same conditions may not elicit a satisfactory response from others.

Corresponding author : **Deok-Chun Yang**, E-mail : dcyang@khu.ac.kr

Principally, two approaches may be possible: either a study of physiological factors, such as *in vitro* culture conditions (Dunwell, 1982), or an investigation of the genotypic factors involved. The manipulation of culture procedures and donor plant environments for specific genotypes could increase the range of responding cultivars. The effect of donor plant growth conditions on pollen plant production has been clearly demonstrated (Sunderland, 1971; Foroughi-Wehr et al., 1976; Keller and Stringam, 1978).

Plant suspension cultures, in a number of laboratories, are grown on horizontal rotary shakers, operating at speeds that maintain the cells and cell aggregates moving throughout the body of the culture medium. Apparently, the shaking promotes gaseous exchange, preventing the development of gradients of soluble nutrients in the culture medium and promoting cell separation (Rajasekhar et al., 1971). A high initial population density (10000 cells/ml) was required to grow plant cells in a mineral-salt liquid culture medium. The initial population density could be lowered to 1000 to 2000 cells/ml if conditioned medium, casein hydrolysate, or amino acids were added to the culture medium (Stuart and Street, 1971). The inability of plant cells to grow at a low population density may be caused by excessive diffusion of metabolic intermediates into the medium, diluting them in the cell to a level below that required for survival (Ham, 1973).

In this paper, anthers from two spring wheat cultivars, Chris and Pavon, were cultured in various combinations of the cultural factors in an attempt to identify an optimum set of conditions. In addition, these genotypes were compared.

MATERIALS AND METHODS

Two spring wheat (*Triticum aestivum*) genotypes, Chris and Pavon, were used in this study. The method for anther culture was performed as previously described (Kang, 1996). Wheat spikes were surface sterilized with 5% bleach for 5 min. Forty eight anthers taken out from a spike were placed on a modified 85D12 initiation medium supplemented with 2 mg/L 2,4-D and 1.0 mg/L NAA (Liang et al., 1987) in the dark condition. After 21 days, macroscopic embryoids/calli were counted and transferred to regeneration media (R85D12) as they became visible, which was from 35 to 70 days after culture initiation. Green and albino plantlets were counted, and green plantlets were transplanted to peat pots containing vermiculite. After 14 to 21 days, plants were transplanted into soil and grown to maturity in the greenhouse. Mature plants were scored as sterile or fertile according to their growth condition.

The following experiments were conducted. 1) Effect of Shaking : The initiation plates were maintained on a still platform or an orbital shaking platform (29 to 30 rpm) to test the shaking effect. The shaking and non-shaking treatments were placed side by side. This experiment was conducted under room conditions (about 24℃) until calli were transferred to R85D12 medium. These regeneration plates were placed in the incubator as previously described. 2) Effect of Medium Consistency and Anther Density : Semi-solid I85D12 medium was prepared by adding wheat starch (50g/L) to compare the effect with the liquid medium. Two anther densities, 48 and 96 anthers per plate, also were tested. The anthers from two spikes were placed in a single plate for the 96-anther density.

RESULTS AND DISCUSSIONS

Effect of shaking

This experiment was carried out under room conditions for callus production, which was cooler (24 ℃) than the desired incubator temperature (29℃). The shaking and non-shaking treatments were placed side by side. Regeneration plates containing calli or plantlets

were placed in the incubator. The frequency of callus or plant production in this experiment was less than that of the other experiment, presumably due to the lower temperature during the anther culture.

Pavon produced more calli in shaking and more albino plants than Chris (Figure 1). However, Chris produced about threefold more green plants than Pavon in non-shaking treatment. More calli and green plants were derived from non-shaking treatment than those from shaking treatment, whereas no difference was found in albino plant production between shaking and non-shaking treatment. This result is not consistent with a previous report (Rajasekhar et al., 1971) suggested that shaking promotes gaseous exchange, preventing the development of gradients of soluble nutrients in the culture medium and promoting cell separation. It is difficult to make a decision that non-shaking treatment is better than shaking treatment because of non-optimal cultural conditions in temperature, humidity and light. Therefore, further experiment with optimal condition should be performed.

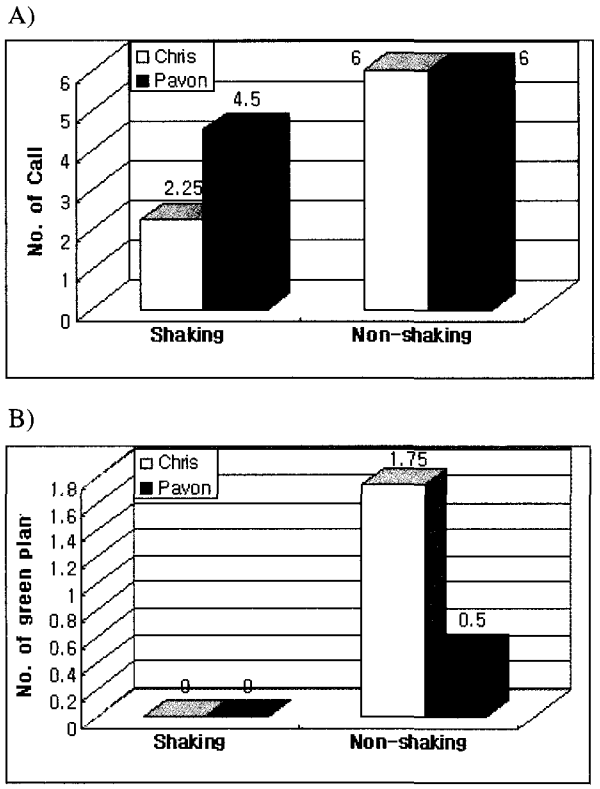

Fig. 1. The influence of A) genotype and B) shaking treatment on callus and plant production.

Effect of medium consistency and anther density

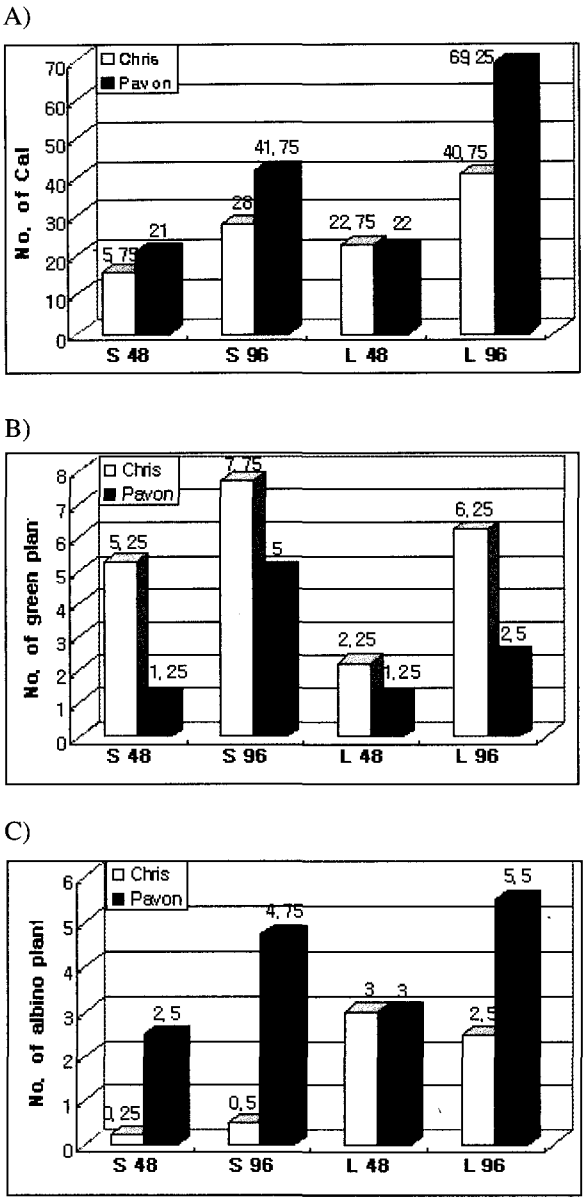

Fig. 2. The influence of genotype and medium condition semi-solid and liquid media and anther density treatment on callus and plant production. S 48: Semi-solid medium containing 48 anthers, S 96: Semi-solid medium containing 96 anthers, L 48: Liquid medium containing 48 anthers, L 96: Liquid medium containing 96 anthers

Table 1. The influence of transfer time to regeneration medium on callus and plant production from the experiment of medium consistency and anther density

	Calli		Green Plants		Albino Plants	
Days	Chris	Pavon	Chris	Pavon	Chris	Pavon
35-45	217	470	70(32%)	25(12%)	7(3%)	60(13%)
46-55	199	140	11(6%)	14(7%)	19(10%)	3(2%)
56-65	10	6	5(50%)	1(17%)	0	0
Total	426	616	86	40	26	63

Calli, green, and albino plants were produced from anthers cultured on both liquid and semi-solid 85D12 media, using two anther densities. Although Pavon produced more calli and albino plants than Chris except L 48 medium, Chris produced more green plants than Pavon (Figure 2). Chris produced almost twofold more green plants than Pavon; that is, Chris averaged 5.4 green plants per plate compared to 2.5 green plants per plate for Pavon. In contrast, Pavon produced nearly threefold more albino plants than Chris, 3.9 albino plants per plate for Pavon compared to 1.6 for Chris. More calli and albino plants were produced in the liquid medium than in the semi-solid medium. However, more green plants were derived from semi-solid medium than those from liquid medium.

A factor that may affect plant regeneration from anthers is the length of time on initiation medium. To examine this possibility, data were collected from the experiment of medium consistency and anther density regarding the time that each callus/embryoid was transferred to regeneration medium. The data were grouped into three consecutive time periods and examined for possible relationships between time of transfer and production of green and albino plants (Table 1). Most of the calli for both genotypes were transferred during the first two time periods. Caution must be used in interpreting this information as these experiments were not designed to examine these relationships.

Transfer time of Pavon calli to regeneration medium influenced regeneration of green or albino plants in Pavon calli. Almost 5% of Pavon calli transferred during the earliest time period produced green plants (Table 2). This percentage continually increased to about 17% for the last time period, whereas less percentage of albino plants was found in the latter time periods. Therefore, it suggests that longer time period to transfer to regeneration medium should be better to produce green plants in Pavon.

In Chris, the earliest time period produced high percentage, 32%, of green plants. Although the highest percentage was found in the last period, the number of calli was too small to exactly count. No albino plants were produced from Chris calli transferred during the last period, whereas 7% of the calli transferred during the first two periods produced albino plants in Chris and Pavon. Although the data showed some difference to produce green plants derived from callus depending on time period to transfer on regeneration medium, caution must be used in interpreting this information as this experiment was not designed to examine these relationships. The influence to produce plants might be affected by changes in media components, media osmotic pressure, or components excreted by the cells. Perhaps one or more of these factors affects the regenerability of calli, but this would need to be confirmed and extended by additional experiment.

Fertility, as measured by seed set, was determined for all surviving regenerated plants (Table 2). The proportion of anther-derived Chris and Pavon plants

Table 2. Fertility of surviving green plants regenerated from anthers for each of the experiments

Experiment	Number of Plants Observed	Seed Set		
		F[a]	F[b]	PF[c]
		Chris		
Shaking	2	0	2	0
Medium Consistency and anther density	58	11	47	0
		Pavon		
Shaking	2	0	2	0
Medium Consistency and anther density	25	7	17	1

[a] Fertile (More than 10 seeds per spike)

[b] Sterile (No seed per spike)

[c] Partially fertile (10 or fewer seeds per spike)

that were fertile generally ranged from 0 to 28%. Combining plants from the experiment of medium consistency and anther density, about 24% of Pavon and Chris plants was fertile. The reason for these sterile plants is not known, but possible reasons include aneuploidy or stresses during regeneration.

REFERENCE

Armstrong TA, Metz SG, Mascia PN (1987) Two regeneration systems for the production of haploid plants from wheat anther culture. Plant Sci. 51: 231-237

Chu CC, Hill RD (1988) An improved anther culture method for obtaining higher frequency of pollen embryoids in *Triticum aetsivum* L. Plant Sci. 55:175-181

Dube SD (1984) Genotypic differences for callus formation in immature anthers of wheat *Triticum aestivum*. Crop Improv. 11:64-65

Dunwell JM (1982) Development of zygotic barley embryos in vitro. In Sen SK, Giles KL. (eds.), Proc Int. Symp. Plant Cell Culture in Crop Improvement. Bose Institute, Calcutta.

Foroughi-Wehr B, Mix G (1979) In vitro response of *Hordeum vulgare* L. anthers cultured from plants grown under different environments. Environ. Exp. Bot. 19:303-309

Ham RG (1973) Dilution plating and nutritional considerations. A. Animal cells. In Kruse PF, Patterson MK (eds.), Tissue culture methods and applications. Academic Press, New York, pp. 254-261

Heszky L, Mesch J. (1976) Anther culture investigations in cereal gene bank collection. Z. Pflanzenzucht. 77:187-197

Hu H (1986) Wheat: Improvement through anther culture. In Bajaj YPS (ed.), Biotechnology in Agriculture and Forestry. Vol. 2. Springer-Verlag, pp. 55-72

Kang TJ (1996) Influence of Medium Components on Anther Culture of Spring Wheat. Korean J. Breed. 28: 366-372

Keller WA, Stringam GR (1978) Production and utilization of microspore-derived haploid plants. In Thorpe TA (ed), Frontiers of Plant Science. University of Calgary Press, Calgary, pp. 113-122

Lazar MD, Chen THH, Scoles GJ, Kartha KK (1987) Immature embryo and anther culture of chromosome addition lines of rye in chinese spring wheat. Plant Sci. 51:77-81

Lazar MD, Schaeffer GW, Baenziger PS (1985) The

physical environment in relation to high frequency callus and plantlet development in anther culture of wheat (*Triticum aestivum* L.) cv. Christ. J. Plant Physiol. 121:103-109

Liang GH, Xu A, Tang H (1987) Direct generation of wheat haploids via anther culture. Crop Sci. 27:336-339

McGregor LM, McHughen A (1990) The influence of various cultural factors on anther culture of four cultivars of spring wheat (*Triticum aestivum* L.). Can. J. Plant Sci. 70:183-191

Ouyang JW, He DG, Feng GH, Jia SE (1987) The response of anther culture to culture temperature varies with growth conditions of anther-donor plants. Plant Sci. 49:145-148

Ouyang JW, Hu H, Chuang CC, Tseng CC (1973) Induction of pollen plants from anthers of *Triticum aestivum* L. cultured in vitro. Sci.Sin. 16:79-95

Ouyang, JW, Zhou SM, Jia SE (1983) The response of anther culture to culture temperature in *Triticum aestivum*. Theo. Appl. Genet. 66:101-109

Picard E, DeBuyser J (1975) New results on anther culture in vitro of wheat (*Triticum aestivum* L.). Effects of thermic shock and the position of the anther on the spike. C. R. Acad. Sci. Paris. 281:127-130

Rajasekhar EW, Edwards M, Wilson SB, Street HE (1971) Studies on the growth in culture of plant cells. J. Exp. Bot. 22:107-117

Schaeffer GW, Baenziger PS, Worley J (1979) Haploid plant development from anthers and in vitro embryo culture of wheat. Crop Sci. 19:697-702

Stuart R, Street HE (1971) Studies on the growth in culture of plant cells. X. Further studies on the conditioning of culture media by suspensions of *Acer Pseudoplatanus* L. J. Exp. Bot. 22: 96-106

Sunderland N (1971) Anther culture: a progress report. Sci. Progr. 59:527-549

(Received Aug. 15, 2003)
(Accepted Aug. 25, 2003)