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Effects of support medium on embryo and plant production from cultured anthers of soft-red winter wheat (*Triticum aestivum* L.)

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Abstract. The present study was designed to examine the effects of media support on the frequency of embryo and plant production from cultured anthers of soft-red winter wheat. Approximately twice as many embryos were produced when anthers were cultured in a liquid as compared to an agar-solidified medium. Upon transfer to regeneration medium, a significantly lower percentage of the embryos produced in liquid regenerated plants. The addition of activated charcoal to an agar-solidified medium resulted in a considerable increase in embryo production, however, plant regeneration from embryos produced on charcoal-containing medium was significantly lower than those produced on agar only. Embryo production frequencies ranged from 2.4–13.2 and 2.5–32.2 embryos per 100 anthers on media with and without charcoal, respectively. Plant regeneration frequencies from embryos produced in the presence of activated charcoal ranged from 0–5.5% as compared to 0–39.1% from embryos produced in the absence of charcoal. More than twice as many embryos produced on Ficoll-containing liquid medium regenerated plants when compared to embryos produced in liquid only. The results from this study suggest that cultural modifications designed to maximize embryo production must take into account the quality of the resulting embryos as they relate to plant regeneration.

Introduction

The production of haploid wheat using anther culture dates back to 1973 [8]. Since that time, a great deal of progress has been made with respect to the recovery of haploid plants such that the technique is now being applied to breeding programs. The development of Jinghua No. 1, a winter wheat variety, in only six years is testimony to the usefulness of this procedure [5]. The release of the new variety, Florin, developed via anther culture in only seven years, is further evidence of the potential value of the technique to wheat improvement [2]. The major limitation to the widespread application

of anther culture to wheat breeding is related to the relatively low frequencies of anther response and plant regeneration across a broad spectrum of elite germplasm [10].

Strategies to improve anther response frequencies include modifications of various cultural parameters; including the composition of the culture medium. One medium-related factor which has been shown to influence the efficiency of haploid plant recovery involves the matrix used for medium support [11]. Significantly greater embryo and plant production was achieved when anthers were floated in a liquid medium as opposed to being supported on a solidified medium [6, 13]. These improvements, however, were realized in a very limited germplasm.

With further refinements in cultural capabilities, anther culture should gain wider acceptance as a wheat breeding tool. There is, however, still a need for the development of procedures which result in efficient haploid production across a broad spectrum of commercially-used germplasm. The present study was designed to examine the effects of media support on the frequency of embryo and plant production from cultured anthers of various genotypes of soft-red winter wheat.

Materials and methods

Plant growth

Seed was sown in 25 × 150 mm culture tubes containing 15 g of Kronolite (Edgecombe Enterprise International Inc., Decatur, IL) moistened with de-ionized water. After one week at 28 °C with a 16/8 hour light/dark photoperiod (60 micromoles/m^2/sec), tubes were transferred to a growth chamber that was maintained at 4 °C with constant light (10 micromoles/m^2/sec). Following a ten week vernalization period, plants were transplanted into 10 cm pots containing Ball Mix 2 (Ball Seed Co., Chicago, IL) and watered daily, alternating tap water with a fertilizer solution containing 24 mg/l N, 62 mg/l P_2O_5, and 28 mg/l K_2O.

Plants were transferred to growth chambers maintained with a 16/8 hour light/dark photoperiod and 24 °C/18 °C day/night temperatures. Light in the chambers was supplied by a mixture of fluorescent and incandescent lamps (1000 micromoles/m^2/sec). Relative humidity was maintained at 55%. Under these growth conditions, spikes could be collected between 4 and 8 weeks after the plants were placed in the growth chambers.

Cultural procedures

Tillers containing spikes judged to be at the appropriate stage of development were cut and surface sterilized by first removing the outer sheaths and then immersing in a 10% v/v commercial bleach solution for 15 minutes followed by one sterile H_2O rinse. Anthers were excised from the outermost florets of each spikelet under a dissecting microscope. Microspore stages were cytologically determined after squashing anthers in acetocarmine. Anthers containing microspores ranging from mid- to late uninucleate stages were plated (30–50 per dish) onto an induction medium consisting of N6 basal salts [1] with 2 mg/l 2,4-D, 1 mg/l kinetin and 100 g/l sucrose adjusted to pH 5.8. Dishes were sealed with strips of Parafilm and placed in plastic boxes covered with aluminum foil. After 7 days in the dark at 28 °C, the plates were transferred to clear plastic boxes and incubated under fluorescent lamps (60 micromoles/m^2/sec) with a 16/8 hour light/dark photoperiod. After one month, the total number of embryos produced was determined. Embryos were then lifted from anthers and placed onto modified MS medium [7] containing 1 mg/l indole-3-acetic acid, 1 mg/l kinetin, 146 mg/l glutamine, 30 g/l sucrose, and 8 g/l agar (Gibco, Phytagar) adjusted to pH 5.8 [9]. After one month the percentage of embryos producing green plants was determined.

Experimental design

Four separate experiments were completed and analysed statistically. The experiments were set up as completely random designs with individual spikes used as replications. Analysis of variance was performed on total embryos produced per 100 anthers cultured and percentage of embryos producing green plants. A square root transformation of the data did not significantly alter the relative sizes of pertinent sums of squares and was thus not used. Comparison among means were made via the Least Significant Difference (LSD) multiple range test using the harmonic mean of the number of replications in each treatment [12].

Experiment 1

Anthers were inoculated onto either induction medium solidified with 6 g/l agar (T. C. Agar, Hazleton Research Products, Lenexa, KS) or the same medium without a solidifying agent. A total of 4234 anthers from 109 spikes of the cultivar Scotty were used in this experiment.

Experiment 2

Five treatments were set up in this experiment. Four of the treatments involved inoculation of anthers onto solidified induction medium 6 g/l agar; 6 g/l agar + 5 g/l activated charcoal; 6 g/l agarose (Type 1, Sigma Chemical Company, St. Louis, MO); 2 g/l Gelrite (Kelco, San Diego, CA). The remaining treatment consisted of the same induction medium without a solidifying agent. A total of 9409 anthers from 261 spikes of the cultivar Scotty were used in this experiment.

Experiment 3

Emphasis in this experiment was on evaluating the effects of activated charcoal on a broad spectrum of genotypes which would be typically found in a commercial breeding program. Eleven different three-way crosses (Table 3) made up of currently available adapted soft-red winter wheat varieties as well as advanced experimental material were used. Anthers were inoculated onto agar-solidified induction medium with or without the addition of 5 g/l activated charcoal. A total of 11 980 anthers from 330 spikes were used in this experiment.

Experiment 4

The final experiment was designed to examine the effects of the addition of Ficoll to liquid medium for overcoming the problem of embryo survival. Anthers were floated on induction medium with or without the addition of 100 g/l Ficoll (Type 400, Sigma Chemical Company, St. Louis, MO). A total of 3711 anthers from 103 spikes of the cultivar Scotty were used in this experiment.

Results

Experiment 1

Approximately twice as many embryos were produced when wheat anthers were cultured in a liquid as compared to an agar-solidified medium (Table 1). In general, a higher frequency of multiple-response anthers were observed in liquid as compared to solidified medium and the embryos were usually smaller and often sank in the medium. Upon transfer to regeneration medium a significantly lower percentage of the embryos produced in liquid regenerated plants (Table 1). Overall plant recovery rates were 2.7 and 2.0 plants per 100 anthers cultured for the solidified and liquid media, respectively.

Table 1. Liquid vs. semi-solid effects on embryo production and plant regeneration from cultured anthers of soft-red winter wheat.

Medium support	Anthers plated	Embryos per 100 anthers	Plants per 100 embryos
Semi-solid (agar)	2,191	5.7	47.3
Liquid	2,043	11.3	18.0
LSD (0.05)		4.8	13.3

Experiment 2

There was no significant differences in embryo production from anthers plated onto medium containing various solidifying agents, however, the addition of activated charcoal to an agar-solidified medium resulted in a considerable increase in embryo production (Table 2). As with the liquid treatment, however, the embryos produced on charcoal-containing medium tended to be smaller than those produced in the absence of charcoal and arise on multiple-response anthers. Plant regeneration from embryos produced on medium containing activated charcoal was significantly lower than those produced on agar only (Table 2).

Table 2. Effects of alternative solidifying agents on embryo production and plant regeneration from cultured anthers of soft-red winter wheat.

Medium support	Anthers plated	Embryos per 100 anthers	Plants per 100 embryos
Agar	1,827	6.6	28.7
Agarose	1,917	4.1	21.7
Gelrite	1,911	8.0	26.3
Agar + charcoal	1,906	46.4	3.6
Liquid	1,846	21.1	11.8
LSD (0.05)		9.5	9.9

Experiment 3

Increased embryo production and reduced plant recovery in the presence of activated charcoal was consistent across a broad spectrum of germplasm (Table 3). Embryo production frequencies ranged from 2.4–13.2 and 2.5–32.2 embryos per 100 anthers on media with and without activated charcoal, respectively. Although significantly more embryos were produced in the presence of activated charcoal, plant recovery rates were considerably lower (Table 3). Plant regeneration frequencies from embryos produced in the

Table 3. Charcoal effects on embryo production and plant regeneration from cultured anthers of various genotypes of soft-red winter wheat.

Genotype	Agar			Agar + charcoal		
	Anthers plated	Embryos per 100 anthers	Plants per 100 embryos	Anthers plated	Embryos per 100 anthers	Plants per 100 embryos
Scotty/FL7223A-3-3-A2//UAS566100	540	13.2	23.0	540	16.1	0.6
NAPB78-171/Hillsdale//UAS566100	504	9.8	27.5	504	32.2	5.5
Roland/FL791-G161//UAS566100	468	7.3	36.1	432	11.6	0
FL7223A-3-3-A2/UAS7//UAS566100	432	12.1	15.1	396	7.8	1.8
UAS6/M0300//UAS566100	468	3.0	39.1	432	7.2	0
UAS6/FL74265-10-A2-B1-01//UAS566100	648	8.2	17.5	648	11.0	0
UAS6/Hillsdale//UAS566100	360	2.5	0	360	2.5	0
UAS8/FL74265-10-A2-B1-01//UAS566100	432	5.8	28.0	396	16.5	1.6
M0299/Roland//UAS566100	288	2.4	11.1	288	4.9	0
B7321/FL301//UAS566100	324	3.1	0	288	6.6	0
FL791-G161/I3548//UAS566100	288	7.0	31.9	288	9.7	3.0
Overall	4752	7.3	22.6	4572	12.4	1.3

LSD (0.05) = 6.8 and 13.4 for embryos per 100 anthers and plants per 100 embryos, respectively, for comparisons among genotype × media means.
LSD (0.05) = 2.8 and 5.5 for embryos per 100 anthers and plants per 100 embryos, respectively, for comparisons among media means.

presence of activated charcoal ranged from 0–5.5% as compared to 0–39.1% from embryos produced in the absence of charcoal. Averaged over all 11 crosses, plant recovery frequencies were 1.6 and 0.2 plants per 100 anthers plated on medium with and without activated charcoal, respectively.

Experiment 4

The effects of Ficoll addition to a liquid induction medium are presented in Table 4. Although no significant difference was observed for embryo production, the increased solution density resulting from Ficoll addition enabled the anther-derived embryos to float on the surface of the medium. More than twice as many embryos produced on Ficoll-containing liquid medium regenerated plants when compared to embryos produced in liquid only (Table 4). Plant recovery frequencies of 3.9 and 1.8 plants per 100 anthers were observed from media with and without Ficoll, respectively.

Table 4. Ficoll effects on embryo production and plant regeneration from cultured anthers of soft-red winter wheat.

Medium support	Anthers plated	Embryos per 100 anthers	Plants per 100 embryos
Liquid	1,839	11.2	16.0
Liquid + Ficoll	1,872	10.7	36.3
LSD (0.05)		ns	10.1

Discussion

The present study indicated that embryo production from cultured anthers of soft-red winter wheat can be influenced by the support medium on which the anthers are placed. More embryos were produced in liquid than solidified medium and the addition of activated charcoal increased anther responsiveness. This is in general agreement with Lazar et al. [6] who also found liquid to be superior to solidified medium for embryo induction in cultured anthers of the spring wheat cultivar Chris. In contrast to the present study, however, activated charcoal was found to be an ineffective addition to solidified medium [6]. This may have been related to the fact that a potato extract-based medium was used in the previous study whereas the present study used a completely defined medium.

A lower percentage of the embryos produced in liquid and charcoal-containing medium proceeded to regenerate plants when compared to those

produced on agar only. These results are consistent with those of Wei [13] who reported reduced plant recovery from liquid-derived cultures but are in contrast to other published data [3, 6] where the physical nature of the induction medium did not directly affect plant regeneration. These results suggest that cultural modifications designed to maximize embryo production must take into account the quality of the resulting embryos as they relate to plant regeneration.

In the present study, the addition of Ficoll to liquid medium resulted in a significantly higher percentage of embryos regenerating plants upon transfer to an appropriate medium. Embryo submersion, commonly encountered in liquid anther cultures, was avoided by increasing the density of the medium. Similar effects were observed with barley anther cultures [4] and have been attributed to improved aeration required for embryo growth and development. Thus, the advantage of improved embryo production in liquid induction medium can be exploited without the accompanying reduction in plant regeneration by including Ficoll in the medium.

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