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Wheat Immature Embryo Culture for Embryogenic Callus Induction

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Abstract: Four 2,4-D concentrations along with three embryo developmental stages were tested to determine the optimum morphogenesis of wheat immature embryo culture. Concentration of 2 mg L^{-1} 2,4-D was found to be optimum level for morphogenesis which was a good indicator of embryogenesis. Compact and nodular calli were observed in first and second developmental stages of 2 mg L^{-1} 2,4-D concentration. In addition, five callus initiation media were tested to determine effects of medium constituents on somatic embryogenesis of wheat (*Triticum aestivum* L.). The highest embryogenic callus formation was observed on MS+B5 medium (98.3%). Therefore, first and second developmental stages of 2 mg L^{-1} 2,4-D concentration along with MS+B5 medium is suitable for embryogenic callus production in wheat.

Key words: 2,4-D (2,4 dichloeophenoxyacetic acid), developmental stages, wheat

Introduction

First requirement for the successful application of biotechnology in crop improvement is to have an efficient plant regeneration from cultured cells and tissues. Immature embryos are the most responsive source to produce embryogenic callus and regenerate plantlets among other explants in culture. Therefore, the success of cell and tissue culture research depends upon reliable callus culture and plant regeneration procedures. The frequencies of callus induction and plant regeneration in tissue culture of wheat are commonly influenced by the explant source (Ozias-Akins and Vasil, 1982; Maddock *et al.*, 1983; Redway *et al.*, 1990), genotype (Sears and Deckard, 1982; Fennell *et al.*, 1995) and culture medium (Mathias and Simpson, 1986; Fennell *et al.*, 1995). Various explant sources, such as immature embryos (Ahloowalia, 1982; Sears and Deckard, 1982), immature inflorescences (Ozias-Akins and Vasil, 1982; Maddock *et al.*, 1983; Redway *et al.*, 1990), mature embryos (Ozgen *et al.*, 1998) have been used for callus culture in wheat. The highest frequencies of callus and plant regeneration have been obtained from the culture of immature embryos in wheat (Maddock, 1983; Eapen and Rao, 1985; Redway *et al.*, 1990). Immature embryos are, therefore, known to be the best explant for efficient regeneration from callus culture of wheat. Developmental stage of immature embryos has been reported as an important factor on embryogenesis (Shimada, 1978; Sears and Deckards, 1982; Ozias-Akins and Vasil, 1982; Maddock *et al.*, 1983; He *et al.*, 1988). The medium components also play an important role in wheat immature embryo culture. He *et al.* (1988) obtained the enhancement in the frequency of white callus upon increasing the concentration of macroelements from half-strength to full or double strength. Mathias and Simpson (1986) reported that the effect of genotype was much stronger than the presence of complex organic additives in the medium.

The objectives of this study was to optimize the immature embryo culture system for wheat by investigating different 2,4-D concentrations with developmental stages and five callus initiation medium.

Materials and Methods

A spring wheat genotype bob white was used throughout this study. Bob white was grown in growth chamber with a 16 h light (light intensity of 350 μmol s^{-1} m^{-2}) and 8 h dark photoperiod at 18/16 °C day: night temperature. Immature caryopses were collected 13-14 days post-anthesis. Immature caryopses were surface-sterilized in a 1.05% (v/v) sodium hypocloride for 2-3 min with periodic agitation and rinsed 5 times in sterile ddH_2O. Total 240 embryos were dissected aseptically and cultured on modified Murashige and Skoog medium to test the effects of embryo developmental stages and 2,4-D concentrations on morphogenesis. The stage of development of each embyo was determined using a stereo-microscope. Embryos at stages I, II and III described by He *et al.* (1986) were used as experimental material. Tested 2,4-D concentrations were 1, 2, 3 and 4 mg L^{-1}. Calli were weighted per 20 embryos after 1 month culture.

Six hundred and fourteen immature embryos were dissected aseptically and cultured on five callus initiation media (~120 embryos/medium) to test the effects of medium constituents on somatic embryogenesis of immature embryos. Five initiation media used in this study were 1) Murashige and Skoog (1962), no vitamins; 2) MS + wheat starch; 3) MS + (100 mg L^{-1}) casein hydrolysate + (150 mg L^{-1}) glutamin; 4) MS + Kao vitamins + coconut water; 5) MS + B5 vitamins. All five media were suplemented with 100 mg L^{-1} myo-inositol, 1 mg L^{-1} nicotinic acid, pyrodoxie HCl, thiamine and 2 mg L^{-1} 2, 4-D, 30 g L^{-1} sucrose, 6 g L^{-1} agar at pH 5.8. Explants were cultured at 25 °C in the dark and 30 days later, the callus induction rate and somatic embryo formation was measured. Callus development during induction and initiation was periodically monitored.

Results and Discussion

Effect of embryo developmental stages and 2,4-D concentrations: Four 2,4-D concentrations along with three embryo development stages were tested to determine the optimum morphogenesis of wheat immature embryo culture (Table 1).

Concentration of 1 mg L^{-1} 2,4-D produced compact calli, precocious germination and root development. Most embryos tended precociously germinate in the first developmental stage. The primary reason for precocious germination most likely was the lower level of 2,4-D (1 mg L^{-1}). Therefore, this concentration was found to be not suitable for embryogenesis.

Table 1: Effects of embryo developmental stages and 2,4-D concentrations on morphogenesis

2,4-D (mg L^{-1})	Developmental stages of embryos	Calli weight (mg)*	Characteristics
1	I	1408	compact calli; precocious germination
	II	1530	compact calli; rooting
	III	1250	root development
2	I	903	compact and nodular calli
	II	1151	compact and nodular calli
	III	1332	compact and nodular calli and rooting
3	I	779	a little bit hydrated calli; browning
	II	1044	a little bit hydrated calli
	III	1243	a little bit hydrated calli
4	I	853	highly hydrated calli; dead tissues
	II	1049	highly hydrated calli; dead tissues
	III	1090	highly hydrated calli

* Calli weight per 20 embryos 1 month after culture

Concentration of 2 mg L^{-1} 2,4-D was found to be optimum level for morphogenesis which was a good indicator of embryogenesis. Compact and nodular calli were observed in first and second developmental stages of 2 mg L^{-1} 2,4-D concentration. These two stages were equally determined as the best stages for morphogenesis. Third developmental stage at the same concentration of 2,4-D resulted in rooting as well as compact and nodular calli. Average calli was 1129 mg.

Table 2: Effects of medium constituents on somatic embryogenesis from *Triticum aestivum* (L.) cv. bob white immature embryos

Media	No. of embryos	Calli weight (mg)	No. of embryogenic calli (%)
MS[1)]	118	1027	95(80.5)
MS + Starch[2)]	120	1089	26(21.7)
MSCHG[3)]	120	1229	117(97.7)
MS + Kao + CW[4)]	140	1349	132(94.3)
MS + B5 vit[5)]	116	1358	114(98.3)

[1)] Murashige and Skoog (1962); [2)]MS + wheat starch; [3)]MS + casein hydrolysate + glutamine; [4)]MS + Kao vitamins + coconut water; [5)]MS + B5 vitamins.

As concentration of 2,4-D increased, characteristics of calli were dramatically changed. Little hydrated calli, tissue browning as well as dead tissues were observed. Mainly, little amount of hydrated calli in all developmental stages and tissue browning in only first developmental stage were observed in concentration of 3 mg L^{-1} of 2,4-D.

In 4 mg L^{-1} of 2,4-D concentration, highly hydrated calli was general phenomenon for all developmental stages. Dead tissues were observed in first and second developmental stages.

Developmental stage of immature embryos has been reported as an important factor on embryogenesis (Shimada, 1978; Sears and Deckards, 1982; Ozias-Akins and Vasil, 1982; Maddock *et al.*, 1983; He *et al.*, 1988). Ozias-Akins and Vasil (1982) found the embryos about 1 mm long to be suitable for the induction of scutellum callus. Sears and Deckard (1982) also reported that the optimum size for scutellum size for maximum callus formation appear to be about 1 mm diameter. They also observed that smaller embryos usually did not produce callus. However, Maddock *et al.* (1983) reported embryos with scutella smaller than 2 mm produced greater frequency of shoot-forming callus.

Effects of medium constituents on somatic embryogenesis: Immature embryos formed callus in varying degrees on all five media tested and percentage of embryogenic callus formation respect to their media type presented in Table 2. Somatic embryo formation from immature embryos is an indication of embryogenic capacity of cultured immatured embyos. Therefore, somatic embryo formation was monitored during cultured embryos in initiation medium. Both the composition of the callus initiation medium and concentration of 2,4-D along with the developmental stages tested played important roles for production of somatic embryos on initiation medium. Callus weight ranged from 1027 to 1358 mg. Callus initiation medium had an effect on embryogenic callus formation. The highest embryogenic callus formation was observed on MS + B5 vit. medium (98.3%). In contrast, MS medium had less embryogenic callus formation 80.5 %. The positive was also found relationship between calli weight and number of embryogenic calli. As calli weight increased, number of embryogenic calli were found to be higher among tested callus initiation media.

Overall, MS + B5 vit. initiation medium had the highest value for callus formation. This medium would be suitable in immature embryo culture system to produce explants for wheat transformation studies.

All five media are based on MS medium (Murashige and Skoog, (1962) and are suplemented with 2,4-D, a commonly used growth regulator in wheat tissue culture (Ahloowalia, 1982; Carman *et al.*, 1988; Elena and Ginzo, 1988). The five media differ in several ways. Hence, it is not possible to attribute successful embryogenesis to a single media component.

The medium components also play an important role in wheat immature embryo culture. He *et al.* (1988) obtained the enhancement in the frequency of white callus upon increasing the concentration of macroelements from half-strength to full or double strength. Mathias and Simpson (1986) reported that the effect of genotype was much stronger than the presence of complex organic additives in the medium. They found that the effect of coconut milk on the formation of shoots might be positive, neutral, or negative, depending on the genotype. The use of casein hydrolysate and glutamine in medium is conflicting. Maddock *et al.* (1983) reported that when casein hydrolysate and glutamine were used, shoot development from somatic embryos was very poor. Redway *et al.* (1990) reported that casein hydrolysate and glutamine were the major components of media that gave the highest percentages of callus formation. Ozias-Akins and Vasil (1982) found that casein hydrolysate was the only constituent, which completely suppressed precocious germination, but it also deleteriously affected the morphology of scutellum callus.

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