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Full Length Research Paper

Influential effects of arabinogalactan-proteins on plant regeneration using calli derived from wheat mature embryos

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Arabinogalactan-proteins (AGPs) are classified in the group of proteoglycans and useful substrates for different part of plants. There are kind of effects of AGPs on the events of plant growing and improvement which are supported by embryogenesis and cell division. In this study, effect of AGP has been investigated on plant regeneration in wheat plants using calli derived from mature embryos. Calli developed were produced by cultivating mature embryo with some selected hormones and their combinations (auxin and cytokinin) and differences found in increasing of either calli number or weight statistically. Murashige and Skoog (MS) medium was used for AGP application in plant regeneration stage of this tissue culture study. AGP increased regeneration capacity and culture effects of genotypes significantly (P < 0.05). It can be said that AGP (10 mg/l) was found to be useful substance for wheat tissue culture to increase plant regeneration. However 10 mg/l of AGP did not make difference statistically between the capacity of plant regeneration in tetraploid and hexaploid genotypes.

Key words: Arabinogalactan-proteins (AGPs), callus production, plant regeneration, *Triticum* sp. L., tissue culture.

INTRODUCTION

In tissue culture of wheat (*Triticum sp.* L.), callus induction and plant regeneration rates generally belong to media compounds and type of explants (Fennel et al., 1996; Redway et al., 1990). Studies on immature embryos showed the best performance in various explants like flower, mesocotyle, seed, leaf, apical meristem, immature embryo, mature embryo and parts of mature embryo. On the other hand, immature embryos are not preferred as treatment materials by plant researchers often because of their limited time for culture studies in living cycle of a plant. However, mature embryo has been used as a plant material in many wheat tissue culture researches which can be stored and prepared easily each time from fresh seeds (Chen et al., 2006).

The main disadvantage of mature embryo is to produce low level of healthy and limited calli as explant in tissue culture. Therefore, production of plant regeneration in embryo culture has been affected negatively. Some of the endosperm-supported culture or culture media including auxins and cytokinins within various concentrations were used by researchers to increase the efficiency of culture conditions on mature embryo (Ozgen et al., 1998; Turhan and Baser, 2004; Chen et al., 2006). Besides, other various applications about oxalate oxidase activity, thidiazuron application and calli increase or modifications in culture medium have been discussed by Chen et al. (2006), Yaqubov et al. (2005) and Rahman et al. (2008) subsequently.

Arabinogalactan-proteins (AGPs) are classified in the group of proteoglycans and also found in various organs of plants such as roots, shoots, leaves, flowers and seeds (Showalter, 2001). The main part of its molecular structure, nearly 90% of that, has included carbohydrates which comprises compounds like arabinosyl and galactosyl as sugar constituents. The remaining part of the

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structure (10%) is protein and it includes some amino acids which are hydroxy proline/proline, alanine, serine and threonine. There are kind of effects of AGPs on the events which are supported by embryogenesis and cell division, plant growing and improvement (Majewska-Sawka and Nothnagel, 2000). However, it has been found that there is still limited remarkable knowledge on the effects of AGP on plant regeneration in wheat.

In this study, the effects of different combinations of hormones on calli production using explants of mature wheat embryos were investigated. The second part of the study focuses on the possibility of increasing the capacity of plant regeneration in wheat by supporting AGP with the MS (1962) regeneration media.

MATERIALS AND METHODS

Plant materials

Two different wheat genotypes were used, a tetraploid (AABB) wheat (*T. durum* Desf. cv. Mirzabey) and also a hexaploid (AABBDD) cultivar (*T. aestivum* L. cv. Ikizce-96) in this study. Seeds of genotypes were obtained from Central Field Crops Research Center in Ankara. This research has been done in the Plant Tissue Culture and Biodosimetry Laboratory in the Department of Biology, Süleyman Demirel University in 2009.

Surface sterilization of seeds

Dry dormant seeds were pre-treated in 70% of ethanol for 3 min and then surface sterilization was done with sterile distilled water 3 times, each for 30 s. In the second step of sterilization, seeds were soaked in commercial bleach (containing 5% of sodium hypocloride) adding 2 drops of Tween-20 for 20 min and washed again with sterile distilled water 3 times, each for 30 s.

After sterilization, seeds were imbibed in sterile water for 90 min at 33°C for easy seperations of embryo from endosperma following the procedure suggested by Özgen et al. (1998).

Preparation of media

Mature embryos were put on the MS media (Murashige and Skoog, 1962) with 20 g/l sucrose and 7 g/l agar and five different MS adding 5 mg/l 2,4-D (2,4-dichlorophenoxyacetic acid), 5 mg/l Dicamba and 1 mg/l kinetin as written below:

i. MS (control)
ii. MS + 5 mg/l 2,4-D
iii. MS + 5 mg/l Dicamba
iv. MS + 5 mg/l 2,4-D + 1 mg/l Kinetin
v. MS + 5 mg/l Dicamba + 1 mg/l Kinetin.

Media were arranged to pH level of 5.8 and separated to 9 cm petri dishes after autoclaved for sterilization.

Regeneration medium

Developed calli were then transferred to the regeneration medium for plant regeneration without adding any hormones. They were;

i. MS medium
ii. MS + AGP.

MS media (Murashige and Skoog, 1962) were prepared adding 20 g/l sucrose and 7 g/l agar at a pH level of 5.8. The following steps of this study for MS modification can be described as below:

1) 1400 mg/l KNO_3, instead of 1900 mg/l KNO_3
2) 300 mg/l NH_4NO_3, instead of 1650 mg/l NH_4NO_3
3) 975 mg/l glutamine,
4) 300 mg/l myo-inositol,
5) 10 mg/l AGP (in groups containing AGP) (Kasha et al., 2003).

This amount of AGP was added to other ingredients before sterilization. Media were autoclaved at 121°C and 1.1 kg/cm^2 pressure for 20 min and separated in 9-cm petri dishes.

Explant and calli procedure

In each petri dish belonging to control and hormone treated groups respectively, 20 embryos were put onto the medium taking into account the scutellum (upper part), wrapped with parafilm two times and incubated at 25±1°C in darkness for 14 days [Figures 2 (a, b) and 3 (a, b)].

After two weeks, each developed callus was then weighed and transferred to the fresh-prepared regeneration medium described above, also in 9-cm Petri dishes, wrapped with parafilm under sterilized conditions. Wrapped petri dishes containing calli were then kept at 25±1°C in 16-h photoperiod (1500 lux) for 4 weeks.

Regenerated calli were then transferred again to the MS standard regeneration medium and kept under the same conditions, at 25±1°C in 16-h photoperiod (1500 lux), for 4 weeks [Figures 2 (c, d) and 3 (c, d).

Acclimatization

Regenerated plantlets were transferred from regeneration medium to pots containing peat : sand (3:1) when they reached plant height of about 10 cm and kept in a plant growth chamber, at 21°C in 16-h photoperiod (12 klux) till they produced spikes (Figure 4).

Data analysis

Data were analyzed as follows:

$$\text{Callus induction efficiency} = \frac{\text{Number of calli}}{\text{Number of mature embryo}} \times 100$$

Weight of callus = Each of callus was weighed on their 14th day of culture.

$$\text{Plant regeneration efficiency} = \frac{\text{Number of plant regenerated}}{\text{Number of mature embryo}} \times 100$$

$$\text{Culture efficiency} = \frac{\text{Callus induction efficiency}}{\text{Plant regeneration efficiency}} \times 100$$

Plant number = Plant number transferred to soil.

Each petri plate containing 20 embryos was considered as single replication and treatment was conducted according to randomised complete design with 4 replications. SPSS 15.0 software programme was used for statistical analysis and means were compared with Duncan's Multiple Range Test (Duncan's Test) in each means of application of hormones and means between culture media groups.

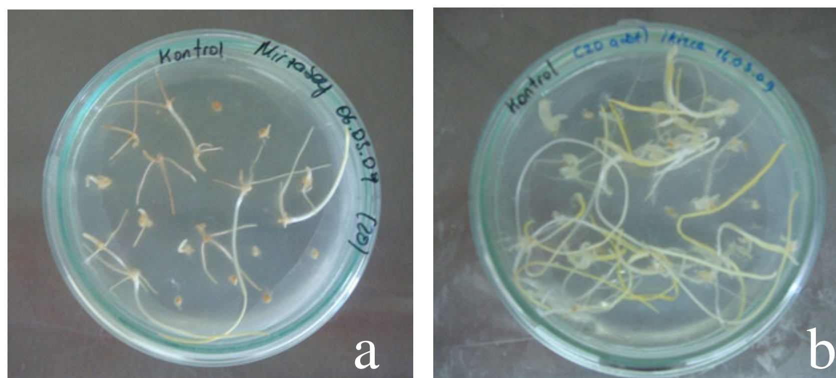

Figure 1. Root and shoot formation from mature embryos in media without auxin and cytokinin. a. Mirzabey, b. İkizce-96.

Table 1. Callus induction efficiency and mean weight of callus of wheat genotypes in different hormone treatments (14 days).

Genotype	Hormone treatments	Percentage of induced calli (%)	Weight of callus (mg)
Mirazabey	Control	0.00 ± 0.00 a*	00.0 ± 0.00 a
	2,4-D	68.75 ± 4.78 c	44.5 ± 2.5 c
	Dicamba	76.25 ± 4.78 d	68.2 ± 1.7 e
	2,4-D + Kinetin	83.75 ± 4.78 e	70.0 ± 4.0 e
	Dicamba + Kinetin	91.25 ± 2.50 f	81.6 ± 1.0 f
	Control	0.00 ± 0.00 a	00.0 ± 0.00 a
	2,4-D	61.25 ± 4.78 b	34.9 ± 4.5 b
Ikizce-96	Dicamba	73.75 ± 7.50 cd	68.5 ± 8.6 e
	2,4-D + Kinetin	77.5 ± 6.45 de	51.8 ± 1.8 d
	Dicamba + Kinetin	92.5 ± 2.88 f	82.4 ± 4.5 f

*Entries within column followed by the same letter are not significantly different according to Duncan's Test. (P < 0.05).

RESULTS AND DISCUSSION

Statistical analysis revealed that in this study, different combinations of some hormones were used to provide healthy calli from mature embryos of wheat species (*T. durum* Desf. cv. Mirzabey and *T. aestivum* L. cv. İkizce) except control group (Figure 1). Hormones, 2,4-D and Dicamba from auxins and kinetin from cytokinins were combined with MS medium to get more productive culture conditions for wheat genotypes. On the other hand, 10 mg/l AGP was put into modified MS regeneration medium to increase the amount of plant regeneration. The most effective group of hormone combination, dicamba + kinetin, increased the highest amount of calli in both genotypes significantly ($P < 0.05$) (Table 1). Also the production of plant regeneration capacity, culture efficiency and number of plants transferred to soil were found to be statistically different between hormone treatment groups in either MS or MS + AGP (10 mg/l) in both genotypes ($P < 0.05$) (Table 2). MS + AGP (10 mg/l) affected culture efficiency and numbers of plants transferred to soil better than MS regeneration medium in particularly auxin and cytokinin combinations (Table 2). However, there was no difference in these results between both genotypes (Table 2). On the other hand, callus production and plant regeneration gave comparable results for discussion in this study.

Callus induction

Mature embryos were induced calli about 2 - 3 days after culture and calli reached 0.5 - 1.0 cm sizes in media containing hormones in all treatment groups after 14 days of culture of embryos for inoculation (Figures 2a, 2b, 3a, and b). However, there was no callus in media without auxin and cytokinin. They produced shoot and root directly from embryo (Figures 1a and b).

Calli were counted and weighed after 14th day produced in media either unique hormone treatment or including auxin and cytokinin combinations (Table 1). Statistical analysis of results were given in Table 1. Percentage of callus induction was changed from 61.25 to 92.5% in all of treated groups with hormones. Dicamba + kinetin hormone combinations produced the highest callus induction in both genotypes Mirzabey and Ikizce-

Table 2. Plant regeneration on regeneration medium and medium + AGP, using callus induced with various hormone treatments in wheat genotypes and plant numbers transferred to soil.

Genotype	Regeneration medium	Hormone treatments for callus production	Regeneration capacity (%)	Culture efficiency (%)	Number of plants transferred to soil (n)
Mirzabey	MS	2,4-D	66.75 ± 3.50 a*	45 ± 0.00 ab	4 ± 0.00 ab
		Dicamba	70.83 ± 5.89 ab	55 ± 7.07 b-e	6 ± 2.82 a-d
		2,4-D+Kinetin	78.86 ± 3.37 cd	65 ± 0.00 e-h	7 ± 2.82 a-e
		Dicamba+Kinetin	81.14 ± 3.10 c-e	75 ± 0.00 h-j	9 ± 1.41 c-e
	MS + AGP	2,4-D	75.13 ± 2.53 bc	52.5 ± 3.53 a-d	5 ± 1.41 a-c
		Dicamba	83.48 ± 3.15 d-f	62.5 ± 3.53 d-g	7 ± 1.41 a-e
		2,4-D+Kinetin	85.41 ± 2.94 d-f	72.5 ± 3.53 g-j	9 ± 2.82 c-e
		Dicamba+Kinetin	86.11 ± 3.92 d-f	77.5 ± 3.53 ij	10 ± 2.82 de
İkizce-96	MS	2,4-D	70.97 ± 2.46 ab	42.5 ± 3.53 a	3 ± 1.41 a
		Dicamba	79.91 ± 1.89 c-e	60 ± 7.07 c-f	5 ± 1.41 a-c
		2,4-D+Kinetin	80.46 ± 2.67 c-e	62.5 ± 10.60 d-g	7 ± 0.00 a-e
		Dicamba+Kinetin	83.77 ± 0.62 d-f	77.5 ± 3.53 ij	8 ± 1.41 b-e
	MS + AGP	2,4-D	80.12 ± 4.53 c-e	50 ± 0.00 a-c	4 ± 0.00 ab
		Dicamba	86.05 ± 2.05 d-f	62.5 ± 10.60 d-g	8 ± 1.41 b-e
		2,4-D+Kinetin	87.05 ± 0.63 ef	67.5 ± 3.53 f-i	9 ± 2.82 c-e
		Dicamba+Kinetin	89.17 ± 0.41 f	82.5 ± 3.53 j	11 ± 1.41 e

*Entries within column followed by the same letter are not significantly different according to Duncan's Test ($P < 0.05$).

96, 91.25 and 92.5% respectively (Table 1). There was no remarkable difference between the results of tetraploid (Mirzabey) and hexaploid (Ikizce-96) genotypes however there was found significantly different results ($P < 0.05$) between hormone treatments in each genotype (Table 1). Papenfuss and Carmen (1987), Satyavathi et al., (2004) and also Bahieldin et al., (2000), have found similar results in their works related to the effects of dicamba and 2,4-D on aestivum wheat, durum wheat and three spring wheat genotypes respectively.

Both of dicamba and 2,4-D hormones combination with kinetin increased callus induc-tion efficiency. Similarly, Carman et al. (1988), reported the positive effects of kinetin in their tissue culture study for bread wheat.

It has been observed that the weight of callus changed from 34.9 to 82.4 mg (Table 1) and both value were taken from Ikizce-96, 2,4-D and dicamba + kinetin treatment groups respectively. Also Kilinç (2004), has mentioned that bread wheat has showed the best performance for callus weight with a dicamba (5 mg/l) treatment. In this study, amount of auxin used was 5 mg/l in each hormone treatment group.

Plant regeneration

Calli were transferred to two different regeneration media (MS culture media and MS + AGP) after they were produced in induction media including four different hormone treatments. Calli began differentiation and changed to green colour in AGP added media after 3 or 4 days. Statistical results of performances of genotypes in different regeneration media were given in Table 2. It can be observed that regeneration capacity has changed between 66.75 and 89.175% values in this study. The highest regeneration capacity was

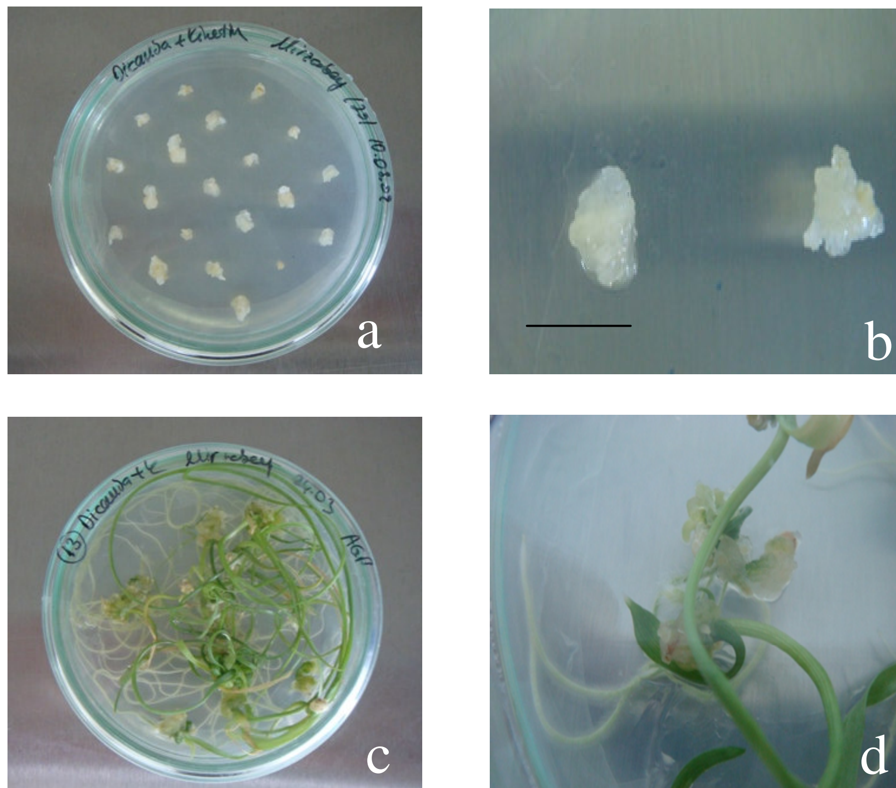

Figure 2. General view of callus production from Dicamba+Kinetin hormones in a 9-cm petri dish (a) 0.5 cm dimension of callus (b) plant regeneration on MS+AGP medium (c and d) derived from mature embryos of Mirzabey cultivar. Bar: 0.5 cm.

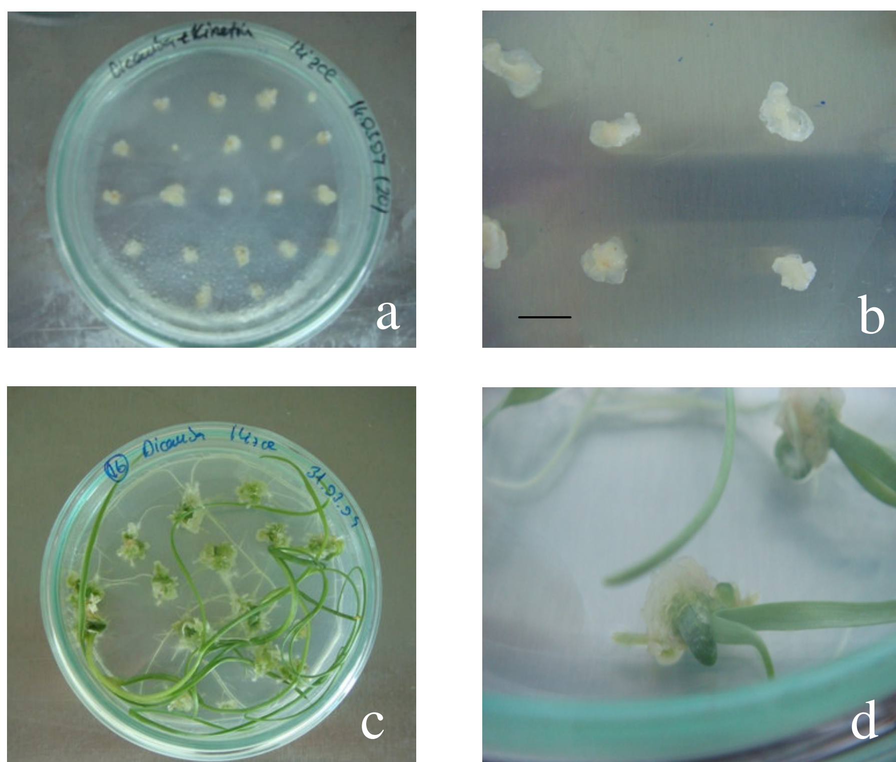

Figure 3. General view of callus production from Dicamba+Kinetin hormones in a 9-cm petri dish (a) 0.5 cm dimension of callus (b) plant regeneration on MS+AGP medium (c and d) derived from mature embryos of Ikizce-96 cultivar. Bar: 0.5 cm.

Figure 4. Plants transferred to soil were grown until spike development a.) Mirzabey, b.) İkizce-96.

found in dicamba + kinetin treatment group and MS + AGP regeneration medium of Ikizce-96 (89.17%) (Figure 2c), while the lowest value of plant regeneration was found in 2,4-D treatment group and MS regeneration medium of Mirzabey (66.75%) (Table 2). Different important results ($P < 0.05$) between regeneration media of genotypes were observed. Similar results have been found in previous studies in aestivum wheat genotypes, 12 different winter wheat genotypes and some of Irakian aestivum wheats stated by Chen et al. (2006), Ozgen et al. (1988) and Ahmet and Adak (2007) subsequently.

AGP increased the plant regeneration when it was supplemented to MS regeneration medium (Table 2). Letarte et al. (2006) showed that AGPs induced the embryogenesis in wheat. However, there were not any remarkable reports about plant regeneration of tissue culture media including AGP in wheat. In this research, statistically different results were observed for the plant regeneration in wheat; either tetraploid or hexaploid genotypes was increased by AGP supported MS media groups.

Culture effects were found significantly different ($P < 0.05$) between genotypes and also treatment groups (Table 2). There was found the lowest percentage in the group of 2,4-D and MS culture medium (42.5%) and also the highest percentage in the group of dicamba + kinetin and MS + AGP (82.5%) in Ikizce-96. Culture effect is an important parameter in tissue culture studies and Bi et al. (2007) and also Ozgen et al. (2001) used this evaluation in tissue culture of mature embryo of wheat.

Conclusion

As a result of this study, AGPs can be used in wheat tissue culture to increase plant regeneration. Because genotype effect was not observed in the production of plant regeneration even if limited number of genotypes were used in this treatment. This protein should be worked out in further regeneration researches with more details, supporting regeneration medium with different amount of AGPs.

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