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Asian Journal of Plant Science and Research, 2013, 3(1):115-119

ISSN : 2249-7412
CODEN (USA): AJPSKY

Comparıson of amıno acıds for theır effıcıency on regeneratıon ın wheat embryo culture

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ABSTRACT

Regeneration ability and regenerant length of Triticum durum Desf. "Ç-1252" and "Kunduru 1149" and Triticum aestivum L. "Bolal" and "İkizce 96" cultivars investigated in this study. Mature embryos using as an explant source for callus production. Embryo derived calli from four wheat cultivars were cultured with MS + serine, alanine, glutamine or arginine (0.5 mM) to test their efficiency of regeneration rate and regenerant length. After surface sterilization of seeds, mature embryos seperated from seeds and transferred to MS medium containing 2 mg/l 2,4-D and 1,5 mg/l. Cultures were incubated at 25°C in dark for callus production. At the end of three weeks calli were transferred to hormone free MS regeneration medium containing various amino acids. There were significant differences in percentage of callus induction and regeneration capacity on the different amino acid medium. Amino acid applications increased the regeneration rate ($P<0.01$) and regenerant length ($P<0.05$) with varying degrees in almost all genotypes. These results indicate that genotype and medium had significant effects on regeneration capacity of cultivars.

Keywords: Embryo culture, Wheat, Amino acid, Regeneratin rate, Regenerant length.

INTRODUCTION

Development of efficient plant regeneration protocol from either single cell or organized tissue is a important of many commercially important crops like wheat. Because wheat, among the food crops, a common source of energy and proteins for the world population.

It is well known that the frequencies of callus induction and plant regeneration in wheat tissue culture completely depend on medium composition [13,6] and explant sources [21,16]. For explant source; Immature embryos have some disadvantages as explants such as their availability is limited in a period of growing time in the year [20]. Due to mature embryos does not have such problems, they are favorable explant source in wheat tissue culture.

For media composition, amino acids are important supplement and widely used in tissue culture systems [12,1]. Amino acids prove an organic form of nitrogen (reduced state), which are readily metabolized by plant cells, stimulating faster cell growth and development [8]. Therefore amino acids have been used *in vitro* cultures of several species to enhance somatic embryogenesis and regeneration [15,9,7]. Therefore, the additional amino acids appear to have the potential to enhance to some extent the roles of suitable nitrogen source.

The present study was initiated to assess the regeneration ability for two durum wheat i.e. "Ç 1252", "Kunduru 1149" and two bread wheat varieties i.e. "Bolal", "İkizce 96"on different amino acids medium. To establish a highly efficient plant regeneration system for wheat, the effects of four different amino acid on plant regeneration rate and regenerant length from mature embryo cultures were evaluated.

MATERIALS AND METHODS

Plant materials

Callus culture was initiated from both mature seeds of two *Triticum durum* Desf. "Kunduru-1149" and "Ç-1252" and two *Triticum aestium* "İkizce-96" and "Bolal" genotypes. Mature seeds immersed in 70% ethanol for 10-15 min and rinsed with sterile distilled water. Then the grains were placed in 30% Clorox solution (1.5% v/v sodium hypochlorite) having a few drops of Tween-80 for 30 min with continuous shaking. After that, grains were rinsed 5-6 times with sterile distilled water.in order to remove excess of the chemical. 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*. [14]. Embryos were then isolated aseptically by cutting from the top of the grain with a sharp scalpel blade and placed in MS callus induction medium.

Callus induction and regeneration

Stocks were prepared and stored in refrigerator. Vitamin stock was also prepared in which thiamine, nicotinic acid, pyridoxine and glycine were added. Stocks of 2,4-D and Kinetin were also prepared. These stocks were used for fresh medium preparation. 30 g/L of sucrose as carbon source and 7 g/L of agar as gelling agent were added to the medium and its pH was adjusted at 5.8. The mature embryos were aseptically removed from the imbibed seeds and inoculated in MS medium which containing 1mg/l 2,4-D + 1,5 mg/lKin. Inoculated culture vessels were incubated at 25±1°C in continuous darkness for 3 weeks. Induced calli were transferred to hormone free MS regeneration medium containing of L-Serine, L-Glutamine, L-Alanine and L-Serine (0.5 mM). The cultures were maintained at 25±1°C under a 16/8-h (light/dark) photoperiod.

Experimental design

The experiment was established with a randomized complete block design. Each Petri plate containing 20 embryos was considered to be an experimental unit. Data were collected on a per plate basis. Callus induction were estimated. For regeneration only percentage was estimated. Also measured plant length of regenerants in all application. Each treatment was replicated four times.

Statistical analyses

For each experiment, the data were obtained as follows: proportion of callus induction = (The number of calli ÷The number of mature embryo)×100%. Proportion of plants regeneration = (The number of plantlets regenerated ÷ The number of mature embryos) × 100%. Genotypes and amino acid type were considered fixed effects, for the analysis of plant regeneration rate and regenerant length. Analysis of variance (ANOVA) was performed using the general linear model (GLM) procedure in SPSS15.0. Means were compared with Duncan's multiple range test (Duncan's test).

RESULTS

Genotype affected callus production of "Ç 1252", "Bolal", "İkizce 96" ve "Kunduru 1149" cultivars significantly. Also, genotype, amino acid and genotype x aminoacid interaction affected the regeneration capasity and regenerant length of cultivars significantly (Table 1).

Table 1. Variance analysis showing the effect of genotypes and amino acids on callus induction, regeneration rate and regenerant length

Source of variance	Calli production			Regeneration rate		Regenerant length	
	df	MS	F value	MS	F value	MS	F value
Genotypes	3	1215,473	7,399**	2694,095	15,930**	13,657	2,768**
Amino acids	4	-	-	937,321	5,542**	7,209	1,461*
Genotypes x amino acids	12	-	-	437,763	2,588**	13,730	2,783**

*Significant at the *0.05 and **0.01 level of probability.*

Generally, callus induction was observed from mature embryos on 10-12th day after inoculation. Calli induced from mature embryos were white, soft and spongy (Figure 1). "Ç 1252" 92.63%, "Bolal" 72.66%, "İkizce 96" 82.00% and "Kunduru 1149" 79.16% produced calli from mature embryos in MS medium containing 1mg/l 2,4-D + 1,5 mg/l kinetin (Figure 2). Regeneration rate of genotypes also paralleled with the amount of callus production. The highest regeneration rate was found in "Ç 1252" cultivar with 74.98%, the lowest regeneration rate was found in "Bolal" cultivar with 40.20% ($P<0.01$). There was no significant differences in "Kunduru 1149" and "İkizce 96" cultivars in terms of calli production and regeneration rate (Table 2).

Figure 1. Embryo-derived calli in Kunduru 1149 cultivar.

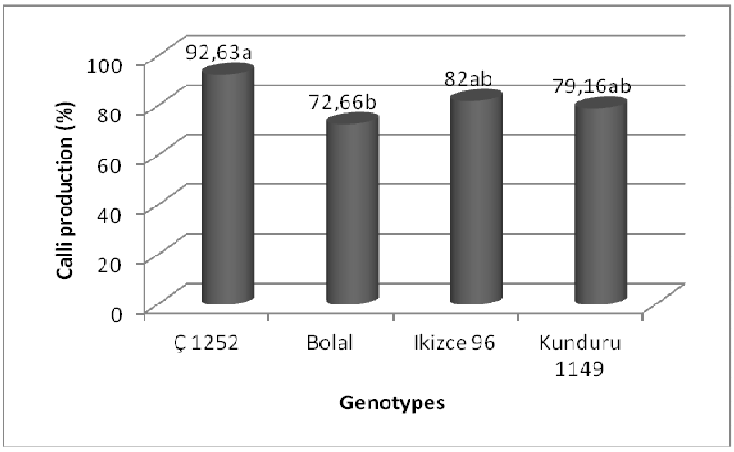

Figure 2. Calli production of wheat genotypes

Amino acid applications significantly increased the rate of regeneration in almost all genotypes. In "Ç 1252" cultivar regeneration rate was found 74.98% in control group. This rate increased significantly with all amino acids application except with serine application (Table 2). The highest increasing of regeneration rate was observed in arginine application (P<0.01).

All amino acid applications improved of regeneration rate significantly in "Bolal" cultivar (P<0.01) (Table 2). While the rate of regeneration 40.20% in control group, this rate increased to 67.08% with arginine application (P<0.01) (Figure 3a).

Serine and glutamine applications increased the regeneration rate in "İkizce 96" cultivar but this increase was not found significantly (Table 2). In "Kunduru 1149" cultivar regeneration rate was found 54.46% in control group. This rate increased significantly with alanine, glutamine and arginine applications (P<0.01) (Table 2). But the highest increasing was observed in alanine application (P<0.01) (Figure 3b).

Table 2. Effect of various amino acids on the regeneration rate of wheat genotypes

Genotypes	Regeneration Rate (%)				
	Amino acids				
	Control	Serine	Alanine	Glutamine	Arginine
Ç 1252	74,98^{a-d}±9,91	49,54^{def}±12,45	86,98^{abc}±12,02	94,43^{ab}±7,68	100^{a}±0
Bolal	40,20^{f}±6,00	53,19^{c-f}±24,98	54,54^{c-f}±7,86	51,84^{c-f}±12,82	67,08^{b-f}±16,28
Ikizce 96	57,40^{c-f}±8,48	57,57^{c-f}±13,11	51,85^{c-f}±3,20	60,00^{c-f}±17,32	52,12^{c-f}±16,91
Kunduru 1149	54,46^{c-f}±3,89	42,88^{ef}±16,41	75,55^{a-d}±18,25	72,21^{a-e}±11,65	58,88^{c-f}±11,57

In control group, the longest regenerants was observed in İkizce 96 and Kunduru 1149 cultivars (P<0.05) (Table 3). All amino acid applications increased in a significant degree of regenerant length in "Ç 1252" cultivar (P<0.05) (Table 3). Glutamine was found the most effective amino acid for regenerant height (Figure 3c). However, we have not found significant differences between other amino acid applications.

Figure 3. Effect of amino acids on regeneration ability of wheat genotypes a) Effect of arginine application on regeneration rate in "Bolal" cultivar b) Effect of alanine application on regeneration rate in "Kunduru 1149" cultivar c) Effect of glutamine application on regenerant length in "Ç 1252" cultivar.

Amino acid applications increased significantly the length of regenerants in "Bolal" cultivar ($P<0.05$). Length of regenerant found in control group 3.36 cm. This rate increased to 8.16 with glutamine application, maximally ($P<0.05$) (Table 3). Glutamine and arginine increased the length of regenerant with varying degress in "İkizce 96" cultivar but this increase was not found significant. Alanine application increased the regenerant length from 4.76 cm to 5.03 cm and this effect was found significant ($P<0.05$) (Table 3).

The best result was found with alanine application in "Kunduru 1149" cultivar. Regenerant length increased from 4,30 cm to 7,08 cm with alanine application and this increase was found significant ($P<0.05$). Other amino acids were also increased the length of regenerant but this increase was not found significant (Table 3).

Tabke 3. Effect of various amino acids on the regenerant length of wheat genotypes

Genotypes	Length of Regenerants (cm)				
	Amino acids				
	Control	Serine	Alanine	Glutamine	Arginine
Ç 1252	$3,40^{bc}\pm3,21$	$4,33^{abc}\pm1,72$	$4,87^{abc}\pm2,13$	$5,97^{abc}\pm0,75$	$4,56^{abc}\pm2,93$
Bolal	$3,36^{bc}\pm1,40$	$5,33^{abc}\pm3,28$	$6,30^{abc}\pm0,8$	$8,16^{a}\pm2,58$	$5,23^{abc}\pm2,09$
Ikizce 96	$4,76^{abc}\pm2,04$	$2,46^{c}\pm1,36$	$5,03^{abc}\pm1,70$	$4,13^{abc}\pm2,12$	$4,90^{abc}\pm3,21$
Kunduru 1149	$4,30^{abc}\pm2,08$	$5,66^{abc}\pm1,88$	$7,08^{ab}\pm2,80$	$4,48^{abc}\pm3,36$	$5,76^{abc}\pm1,97$

DISCUSSION

In spite of many methods have been developed for regeneration of plants in wheat mature embryos callus cultures, the rate of the regenerated plants is still relatively low. In this study, we observed that the rate of regenerated plants differed between the four genotypes and demonstrated that different amino acid types could affect the differentiation of wheat callus.

The highest regeneration frequency obtained in the present study is for *T. durum* cultivars. Chen *et al.* [3] reported an average regeneration capacity which is much lower than the present study for mature culture in their study. For *T. durum* cultivar "Ç 1252", the highest regeneration frequency obtained in this study is 100% in arginine medium and 94,43% in glutamine medium. For *T. aestivum* cultivar "Bolal" the highest regeneration frequency obtained in this study is 67,08% in arginine medium (Table 2). Delporte *et al.* [4] reported that 75% of the genotypes tested presented a regeneration frequency of less than 30% but only a few genotypes revealed a regeneration rate of up to 60%. Our results of regeneration capasity are higher than this report (Table 2).

Amino acids have been found critical to regeneration rate in plant tissue culture medium. Regeneration percentages were higher either in the presence of serine, alanine, glutamine or arginine up to maximum 100% as compared to control (Table 2).

In this study, the increase of regeneration rate occured maximum in arginin application in Ç 1252 and Bolal cultivars ($P<0.01$) (Table 2). The length of regenerant also increased significantly with arginin application in all genotypes tested ($P<0.05$) (Table 3). Arginine is one of the essential amino acids considered the main precursor of polyamines [2]. Polyamines are involved in the control of cell cycle and cell division in plants [19]. In the present study, arginine may promote the regeneration the differentiation of dividing cells.

Glutamine has been shown to improve somatic embryogenesis in both monocots and dicots viz. rice, maize, wheat, soybean, chickpea [10,18,17]. In chickpea, glutamine (400 mg l^{-1}) has been shown to improve somatic embryogenesis [18]. In present study, the rate of regeneration increased significantly with glutamine application in "Ç 1252", "Bolal" and "Kunduru 1149" cultivars (Table 2). Regenerant length also increased significantly with glutamine application in "Bolal" cultivar (Table 3).

Alanine, the next simplest amino acid after glycine, has a methyl group ($-CH_3$) as its side chain. We determined that regeneration rate and regenerant length increased significantly by the addition of this amino acid to the culture medium in "Ç 1252", "Bolal" and "Kunduru 1149" cultivars (Table 2-3). Serine, contain aliphatic hydroxyl groups. Serine can be thought of as a hydroxylated version of alanine. These hydroxyl groups on serine make them much more hydrophilic (water loving) and reactive than alanine. However, we have not observed any increase in regeneration rate with serine application except "Bolal" cultivar (Table 2). In addition, serine application increased significantly the length of regenerant in "Bolal" and "Ç 1252" cultivars (Table 3).

In this study, genotype and medium had significant effects on callus induction and regeneration capacity of *T.aestivum* and *T. durum* cultivars. Similar results found by other researchers [5,11]. We determined that amino acid applications generally increased the capasity of *T. durum* cultivars. But we found that each amino acid effected to each genotype with different degrees. We thougt that this is not only related to genotypic differences but also differences in the structure of amino acid.

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