



Effect of growth regulators on tissue culture parameters in rice (*Oryza sativa* L.)

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ABSTRACT

This study was conducted in Ankara University, Faculty of Agriculture, Department of Field crops, Biotechnology Laboratory. The objective of the present study was to determine the effects of growth regulators on tissue parameters in rice. In this study, mature embryos of three rice cultivars (Aromatik-1, Baldo and Karadeniz) and different growth regulators (2,4-Dichlorophenoxyacetic acid (2,4-D) and picloram) were used as material. For callus induction, mature embryos were placed with scutellum upwards on three different medium (hormone-free MS-0, MS + 2 mg/l 2,4-D and MS + 2.5 mg/l picloram) in sterile Petri dishes for two weeks at 25±10C in darkness. After incubation; obtained calli were transferred to hormone-free MS-0 medium for regeneration. According to results, the effect of growth regulators and genotypes on callus induction and plant regeneration in rice were found to be statistically significant

Keywords: rice, *oryza sativa* L., callus induction, 2, 4-D, picloram

Introduction

Rice (*Oryza sativa* L.) is the grain with the third-highest cultivated area, after wheat and maize. Rice can be grown in all types of soils with sufficient water holding capacity and suitable temperature, in Turkey its grown intensively especially in Marmara and Karadeniz Regions. However, domestic production is not enough for the internal consumption. In recent years, the amount of import has outpaced the amount of production. According to data of year 2012, approximately 880.000 tones of rice had been obtained from 118.720 ha area in Turkey (FAO, 2012). Rice breeders in Turkey should improve the yield and quality of rice to decrease the amount of import. In this context, beside the classical plant breeding, genetic engineering and biotechnological methods should also be utilized. However, plant breeders developed different types of rice cultivars successfully in recent years and showed that gene transferring techniques can be used as supporting

tool for classical plant breeding methods (Koyuncu et al. 2005). Recently, particle bombardment technique (Christou et al. 1991; Li et al. 1993; Christou 1997) and protoplast culture (Moura et al. 1997; Tsugawa and Suzuki, 2000) methods have also been used in the rice gene transfer studies.

On the other hand, plant tissue culture is the most important step of plant regeneration and gene transfer among modern methods. Embryogenic calli, rather than direct tissues such as shoot spines, immature inflorescences, roots and leaves are used for genetic transformation and regeneration of rice plants because the callus culture, compared with organogenesis, is much more suitable for the gene delivery and regeneration of transgenic rice plants (Ananthi et al. 2010).

As known, callus induction and plant regeneration potential are affected by the genotypes, carbohydrate metabolism-source, plant growth regulators, culture medium and conditions etc. In particular genotype,

and explants are important factors for a successful embryogenic callus induction and regeneration of the rice plants (Rueb et al. 1994). In the plant tissue culture studies, embryos are mostly used as source of explant in cereal crops. For embryo culture, mostly embryos obtained from mature and immature seeds are used. Mature embryos, which are always available without time limitation, are widely used rather than immature embryos according to Özgen et al., (1996).

In this study, the effects of some plant growth regulators such as 2,4-D and picloram, on callus induction and plant regeneration were observed and determined by using mature embryo culture method.

Materials and methods

In this study, 3 rice (*Oryza sativa* L.) genotypes, Aromatik-1, Baldo and Karadeniz were used as sources of mature embryos. A completely randomized design with three replications per seed group for each genotype was used. Rice seeds were dehulled mechanically and they were surface-sterilized with 70% (v/v) ethanol for 5 min., washed 3 times with sterile distilled water, immersed in commercial bleach (containing 5% sodium hypochlorite) for 30 min, and rinsed at least 7 times with sterile distilled water. Then, the seeds were imbibed in sterile distilled water for 2 h at 33°C in submarine. Afterwards, the embryos were separated from the endosperm in imbibed seeds and scutellum were placed on 3 types of culture media containing 20 g/l sucrose+ 4,43 g/l MS + 2 mg/l 2,4-D + 7 g/l agar, 20 g/l sucrose + 4,43 g/l MS + 2.5 mg/l picloram + 7 g/l agar and hormone-free MS medium 20 g/l sucrose + 4,43 g/l MS + 7 g/l agar and incubated for callus induction at 25±1°C for 14 days in darkness. Based on preliminary work and literature survey; 2 mg/l 2,4-D and 2,5 mg/l picloram doses were used in this study which have provided high callus induction and regeneration capacity in mature embryo culture of cereals (Raina et al, 1987; Barro et al, 1999; He and Lazzeri 2001) At the end of this stage, callus induction ratio (%) and callus weight (g) parameters were determined.

After the incubation, the calli were transferred to hormone-free MS medium for initiating root and shoot and maintained for 4 weeks at 25±1°C in 16-h light and 8-h dark photoperiod. After 4 weeks, by counting the regenerated calli regeneration capacity and culture efficiency data were obtained.

Petri dishes containing 10 embryos were considered the units of replication. All obtained data were subjected to statistical analyses using MSTAT statistical software and comparison of means was based on a LSD test (Düzgüneş et al. 1983)

Results

Callus induction

Callus formation from mature embryos started after 4-5 days of culture. At the end of 14 days, callus induction rate (%) and callus weight (g) data were obtained (Figure 1). Considering overall averages in examined parameters; the medium including 2,4-D gave higher callus weight and callus induction values than the medium containing picloram (Table 1). Our experimental results revealed that in the medium with 2,4-D, Aromatik-1 and Baldo cultivars gave higher callus induction frequency (100% and -100%) and callus weight (0,310 g and 0,403 g), respectively as compared to the medium with picloram. Also, it was observed that Karadeniz gave highest results in callus induction (100%) and callus weight (0,325 g) in medium containing picloram compared to 2,4-D (Table 2).

According to the results of the analysis of variance; Genotype x Hormone interaction was found statistically significant in terms of callus induction frequency ($P<0.05$) and callus weight ($P<0.01$) parameters. These effects indicated that used genotypes are affected differently by hormones.

Similarly, the correlation between callus induction and callus weight found statistically significant ($r=0,946$, $P<0.01$) (Table 3). This situation shows that, when callus weight is increasing regeneration capacity also increases significantly.

Plant regeneration

At the end of 14 days, green spots and shoots were observed after 3-4 days in the calli which were transferred to hormone-free MS-0 medium (Figure 2). The calli of Aromatik-1 developed in 2,4-D medium gave the highest regeneration capacity (80%). The calli of Baldo developed in picloram medium had higher results (95,3%) in regeneration capacity (Table 2). On the other hand, the calli of Karadeniz, developed in 2,4-D and picloram media gave the same regeneration capacity (93,3%) result (Table 2).

In terms of culture efficiency; the calli of Aromatik-1 and Baldo cultivars developed in 2,4-D medium gave higher results (80% and 86,7%, respectively), however the calli of Karadeniz developed in picloram medium gave the highest culture efficiency result (93,3%) (Table 2).

According to the results of the analysis of variance; Genotype x Hormone interaction was found statistically significant in terms of the regeneration capacity and culture efficiency ($P<0.01$). It means that the increment in regeneration capacity increases the culture efficiency significantly in the used cultivars. Additionally, the

correlation coefficients between regeneration and culture effect were found statistically significant ($r=0,990$, $P<0.01$) and they are presented at Table 3.

Discussion

According to the data of this research; it figured out that the active ingredient 2,4-D is more effective than picloram in terms of tissue culture parameters.

Also, the statistical performed showed significant Genotype x Hormone interaction and the increment of callus weight increases the regeneration capacity and it was seen that culture efficiency was increased parallelly. It can be concluded that using appropriate genotype and hormonal selection and its application can provide increase in tissue culture parameters in rice plant and enhance success.

Table 1. The effects of growth regulator on tissue culture parameters in 3 rice genotypes

Hormones	Callus induction frequency (%)	Callus weight (g)	Regeneration capacity ^a (%)	Culture Efficiency ^b (%)
Control	2,2	0,008	0	0
2,4-D	98,9	0,337	86,7	85,6
Picloram	90,0	0,304	74,5	68,9

^a Regenerated callus number/ Induced callus number x 100

^b Regenerated callus number/Cultured embryo number x 100

Table 2. The effects of 2,4-D and picloram on tissue culture parameters in mature embryos of rice genotypes

Genotypes	Hormones	Callus induction (%)	Callus weight (g)	Regeneration capacity (%)	Culture efficiency (%)
Aromatik	MS 0	0 c	0 e	0 c	0 c
	2,4-D	100 a	0,310 bc	80 a	80 a
	Picloram	86,7 b	0,234 d	35 b	33,3 b
Baldo	MS 0	6,7 c	0,024 e	0 c	0 c
	2,4-D	100 a	0,423 a	86,7 a	86,7 a
	Picloram	83,3 b	0,352 b	95,3 a	80 a
Karadeniz	MS 0	0 c	0 e	0 c	0 c
	2,4-D	96,7 a	0,276 cd	93,3 a	90 a
	Picloram	100 a	0,325 bc	93,3 a	93,3 a

Table 3. The correlation coefficients of mature embryogenic calli of rice genotypes

	Callus induction (1)	Callus weight (2)	Regeneration capacity (3)	Culture efficiency (4)
1	-	0,946 **	0,907 **	0,922 **
2	-	-	0,925 **	0,922 **
3	-	-	-	0,990 **
4	-	-	-	-

Significantly different from zero at ** $P<0,01$

Figure 1. The callus induction from mature embryos of rice genotypes after 14 days (a: Aromaik-1, b:Baldo, c: Karadeniz)

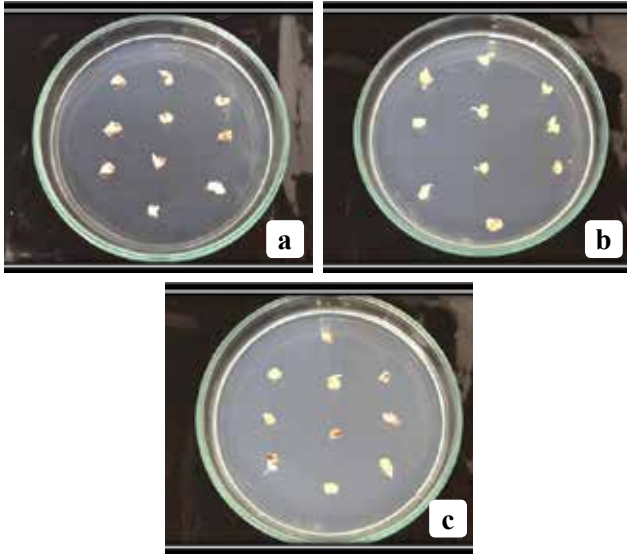
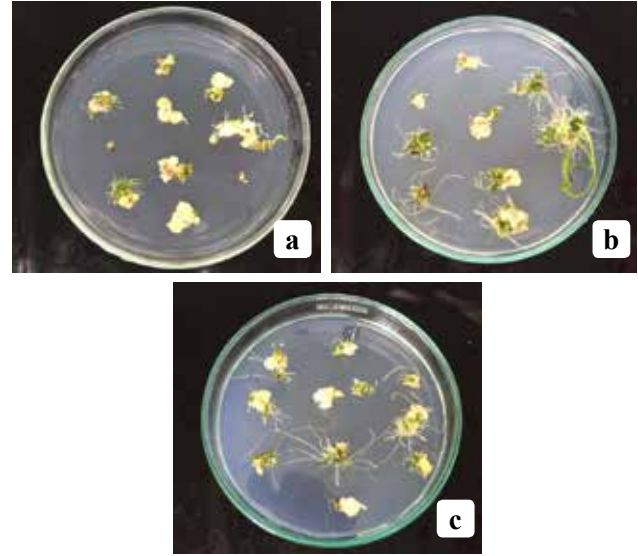


Figure 2. Plant regeneration in rice genotypes after 4 weeks (a: Aromatic-1, b:Baldo, c: Karadeniz)



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