EFFECTS OF GENOTYPE AND PICLORAM CONCENTRATIONS ON CALLUS INDUCTION AND PLANT REGENERATION FROM IMMATURE INFLORESCENCE OF SPRING BARLEY CULTIVARS (*HORDEUM VULGARE* L.)

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ABSTRACT

The effects of different picloram (4-amino-3,5,6-trichloropicolinic acid) concentrations (2.5, 5, 7.5 and 10 mg. l^{-1}) in the LS (Linsmaier and Skoog, 8) medium of immature inflorescences taken from four barley (Hordeum vulgare L.) cultivars were investigated.

The result of the study showed that callus induction rate, shoot induction rate, callus weight and plant regeneration from the immature inflorescence were significantly affected by the genotypes. There was a significant interaction between genotype and picloram concentrations. Depending on the genotypes, mean callus induction rate, mean shoot induction, mean callus weight (mg/petri dishes) and number of regenerates (per inflorescence segment) varied from 0.0-39.1%, 12.5-29.7%, 0.0-150.3 mg and 0.2-1.5 respectively.

Mean callus and mean shoot induction rates, mean callus weight and mean regeneration rate were also significantly influenced by the picloram concentrations. The segments cultured on the LS medium containing 7.5 mg. l^{-1} of picloram gave the highest values of callus induction rate (31.3 %), shoot induction rate (40.6%) and plant regeneration (1.2 regenerates per segment). In this study, only albino plantlets were obtained.

Keywords: barley, LS medium, picloram

Introduction

Barley belongs to genus *Hordeum* in the tribe *Triticeae* of the grass family (*Poaceae*) and is one of the first domesticated cereals. It is also considered as a model crop due to its morphological, physiological, and genomic characteristics. Barley ranks fourth in world cereals crop production and is mostly used for animal feed, brewing malts and human food. Barley is a short season crop with early maturing grain and is a salt-tolerant crop species. It is of considerable economic importance in salinity-affected arid and semiarid regions of the world, found in widely varying environments globally.

In contrast to dicotyledonous species, callus induction and plant regeneration is difficult for cereal crops and insufficient knowledge both on the plant and also its agronomy causes the difficulties for of its breeding. Furthermore, the breeding time is longer. A lot of studies are conducted to shorten this time by using biotechnological techniques. Lührs and Lörz (11) reported that *in vitro* culturing is an experimental tool in plant breeding only when efficient and reproducible plant regeneration systems are available.

It is possible to find out and select the hopeful new variants from the selected individuals and also propagate them faster by the *in vitro* techniques (2). Some of the big developments have been obtained by using both undifferentiated meristematic zones as an explant in *in vitro* cultures and also different plant growth regulators in the medium in last years. However, the factors affecting on the *in vitro* response are determined by these investigations. Optimal physiological condition of donor plants, determination of the most productive explants, medium composition and the induction conditions must be evaluated for any plant before its *in vitro* culture (9). During the *in vitro* culture of cereal crops mature or immature embryos, young leaves and inflorescences are frequently used as explants.

This study was carried out to determine the effects of different picloram (4-amino-3,5,6-trichloropicolinic acid) concentrations (2.5, 5, 7.5 and 10 mg 1^{-1}) on the segments of immature inflorescence taken and cultured on the LS-medium from four different barley (*Hordeum vulgare* L.) cultivars.

Materials and Methods

Seeds from four barley (*Hordeum vulgare* L.) cultivars (Kral-97, Beyşehir-98, Anadolu-98 and Larende were used as donor plants in the study. Seeds were germinated in the greenhouse. The donor plants were planted in a soil+manure+sand (2:1:1) mixture in a greenhouse. The plants were fertilized weekly with a 0.1% solution of a fertilizer mixture including 8% N+8% P_2O_5 and irrigated regularly during the whole investigation. When the second stem node and flag leaf were visible stages, tillers were harvested from the donor plants and placed into a glass bottle with a little amount of water. Shoots were surface sterilized in 70% ethanol for 1 minute, then, in a sodium hypochlorite solution (1.5% available chlorine) with 2 drops of tween-20 per 100 ml solution for 10 minutes and rinsed four times in sterile distilled water at the end of a cold pre-treatment

TABLE 1

Effect of different picloram concentrations on the callus induction rate (%) in barley (*Hordeum vulgare* L.)

Cultivars	Picloram				Mean
	2.5 mg l^{-1}	5 mg l^{-1}	7.5 mg l^{-1}	10 mg l^{-1}	
Kral-97	0.0 (0.6)[1*] c	25.0 (26.4) b	68.7 (63.5) a	62.5 (56.1) a	39.1 (36.6)[2]a
Beyşehir-98	0.0 (0.6) c	0.0 (0.6) c	0.0 (0.6) c	0.0 (0.6) c	0.0 (0.6) c
Anadolu-98	0.0 (0.6) c	0.0 (0.6) c	0.0 (0.6) c	0.0 (0.6) c	0.0 (0.6) c
Larende	0.0 (0.6) c	0.0 (0.6) c	56.3 (48.8) a	56.3 (48.8) a	28.1 (24.7) b
Mean	0.0 (0.6)[2] b	6.3 (7.0) b	31.3 (28.3) a	29.7 (26.5) a	67.2 (62.4)

*) Transformed values

1) Means followed by the same letter within a column and within a row are not significantly different by Duncan's Multiple Range Test at the $p \leq 0.05$ levels.

2) Means in each column or row followed by same letter are not significantly different by LSD-test at the $p \leq 0.05$ levels.

under +4°C for a night. Immature inflorescences with 10-20 mm length were isolated from the shoots and measured with the help of a scale placed onto the table of a binocular (**Fig. 1A**). Any inflorescence longer than 5 mm was cut to that length. Isolated inflorescences were placed into Petri dishes (60x15 mm) filled with 7 ml Linsmaier and Skoog (8) basal medium supplemented with different concentrations of picloram (4-amino-3,5,6-trichloropicolinic acid) concentrations (2.5, 5, 7.5 and 10 mg 1^{-1}) and solidified by 8 g^{-1} agar. Petri dishes were then sealed with parafilm. All cultures were incubated in dark at 25 ± 1°C. The number of explants producing callus or directly shoot and the number of shoots per explants were scored after four weeks of culturing. Callus was transferred to the growth regulator free LS regeneration medium after weighted. All cultures were incubated at 25 ± 1°C under white fluorescent light, irradiance of 2000 lx with 16 h photoperiod (4).

The data were analyzed by ANOVA using LSD and Duncan's Multiple-Comparison Test. Software MSTATC was used. Data given in percentages were subjected to Arcsin √x transformation for callus and shoot induction rate and also √x+0.5 transformation for regeneration rate before statistical analysis.

Results and Discussion

Seven-days after the induction, an expansion on the dissections of inflorescences segment and the other floral parts was observed (**Fig. 1B**). After sixteen-days, we observed callus and direct shoot formations from dissections of some segments and in the junction of spikelets and inflorescence axis (**Fig. 1C-D**). Twenty-one days after culture initiation, we observed growth of callus induction and direct shoot formation from flower primordial and leaf formation from outer glumes (**Fig. 1E-F**). After four-weeks of culture duration, some cultures showed full shoot formation. On the other hand, especially callus formed around the dissections of inflorescent segments showed better growth during the culturing (**Fig. 1G-H**). After six-weeks of culture initiation, regenerated albino plantlets were observed (**Fig. 1I**).

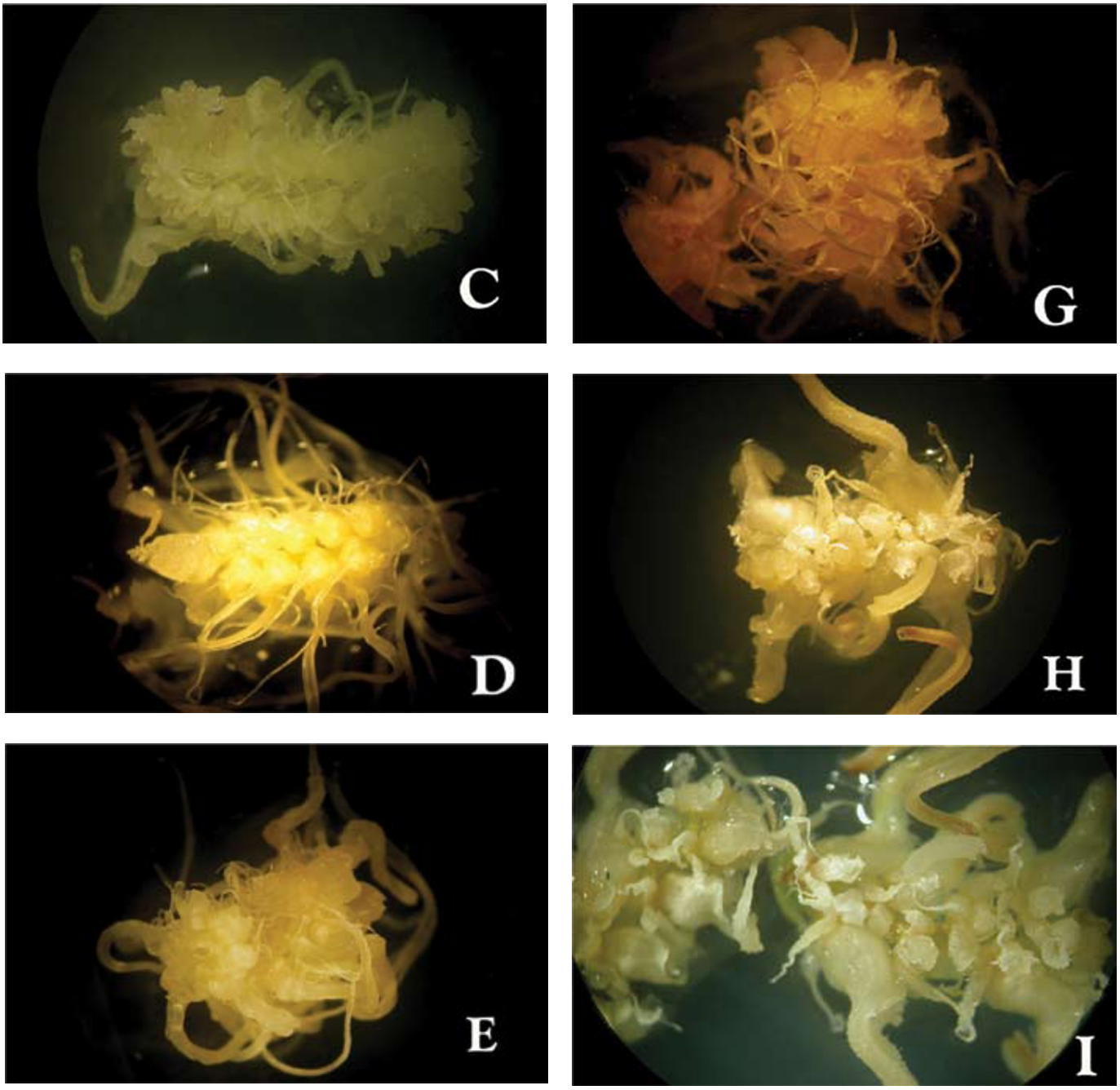

Fig.1. Callus induction and plant regeneration from immature inflorescence segments of barley (*Hordeum vulgare* L.) **(A)** Immature inflorescences with 10-20 mm length. **(B)** An expansion on the dissections of inflorescence segment seven-days later of the culture initiation. **(C)** Callus initiation sixteen-days later of the culture initiation. **(D)** Direct shoot formation sixteen-days later of the culture initiation. **(E)** Growth of callus twenty-one days later of the culture initiation. **(F)** Growth of shoot twenty-one days later of the culture initiation. **(G)** Callus formation after four-weeks of the culture initiation. **(H)** Shoot formation after four-weeks of the culture initiation. **(I)** Regenerated albino plantlets after six-weeks of the culture initiation

Our findings about callus and shoot induction from immature inflorescence segments of barley (*Hordeum vulgare* L.) are in corroboration with other studies such as in wheat, barley and tritordeum by Barro et. al. (3) in barley by Ruiz

TABLE 2

Effect of different picloram concentrations on the direct shoot induction rate (%) in barley (*Hordeum vulgare* L.)

Cultivars	Picloram				Mean
	2.5 mg l^{-1}	5 mg l^{-1}	7.5 mg l^{-1}	10 mg l^{-1}	
Kral-97	31.3 (33.8)*[1] ab	37.5 (37.5) a	50.0 (45.0) a	0.0 (0.6) d	29.7 (29.2)[2] a
Beyşehir-98	0.0 (0.6) d	25.0 (26.4) abc	43.8 (41.3) a	6.3 (7.9) cd	18.8 (19.0) ab
Anadolu-98	0.0 (0.6) d	25.0 (30.0) abc	25.0 (30.0) abc	0.0 (0.6) d	12.5 (15.3) b
Larende	0.0 (0.6) d	25.0 (30.0) abc	43.8 (41.3) a	12.5 (11.7) bcd	20.3 (20.9) ab
Mean	7.8 (8.9)[2] b	28.1 (31.0) a	40.6 (39.4) a	4.7 (5.2) b	81.3 (84.5)

*) Transformed values

[1)] Means followed by the same letter within a column and within a row are not significantly different by Duncan's Multiple Range Test at the $p \leq 0.05$ levels.

[2)] Means in each column or row followed by same letter are not significantly different by LSD-test at the $p \leq 0.05$ levels.

(17) and in maize by Redway et. al. (16). In our study, we only observed white collared succulent callus formation.

Effects of Genotype

The result of the study showed that callus induction rate from the immature inflorescence was significantly affected by the barley genotypes. There was also a significant interaction between genotype and picloram concentrations.

Mean callus induction rate differed according to the type of the cultivars and ranged from 0.0% to 39.1% (**Table 1**). The highest mean callus induction rate was obtained from the cultivar Kral-97. The immature inflorescences of Beyşehir-98 and Anadolu-98 cultivars could not produce callus induction.

In this study, we found that different barley cultivars produced different callus induction rate. These results are in agreement with studies on barley by Chernobrovkina et. al. (5), Ruiz (17) and Sharma et. al. (19) as well as some other studies on cereals, such as in secale by Popelka and Altpeter (15), Ward and Jordan (20) and Zimny and Lörz (22), in maize by Duncan et. al. (6), in wheat by Redway et al. (16).

Mean direct shoot formation rate differed according to type of the cultivars and ranged from 12.5% to 29.7% (**Table 2**). The highest mean direct shoot formation rate was obtained from the cultivars Kral-97. However, this is not statistically significant from cultivars Beyşehir-98 and Larende. The lowest mean direct shoot formation rate was obtained from the cultivar Anadolu-98.

Mean weight of callus formed from immature inflorescence of barley cultivars was different according to the type of clones (**Table 3**). Mean callus weight (mg/per Petri dishes) ranged from 0.0 mg to 150.3 mg with respect to cultivars. The highest mean callus weight was obtained from the cultivar Kral-97. However, this is not statistically significant from cultivar Larende. The immature inflorescences of Beyşehir-98 and Anadolu-98 cultivars could not yield callus weight. This variation trend in mean callus weight seemed similar to mean callus induction rate considering cultivars. Therefore, difference in mean callus weights was caused by difference in mean callus induction rate.

Plant regeneration among the four barley cultivars cultured from immature inflorescences changed significantly with respect to cultivars (**Table 4**). A total of 183 regenerates are obtained from 256 inflorescence segments of different cultivars. Mean regeneration ranged from 0.2 to 1.5. The highest mean plant regeneration (plantlets/per inflorescence) was obtained from the cultivars Kral-97. However, this is not statistically significant from Larende cultivars.

These results suggested that changing the medium composition could significantly change the percentage of callus induction, direct shoot induction, callus weight and plant regeneration. These results are in agreement with the study on barley by Barro et. al. (3).

Effects of Picloram Concentrations

There was a significant interaction between genotype and auxin concentrations.

Mean callus induction rate differed according to picloram concentrations and ranged from 0.0% to 31.3% (**Table 1**). Although the highest mean callus induction rate were obtained from 7.5 mg. l^{-1} picloram concentration, this is not statistically significant from 10 mg. l^{-1} picloram concentration.

The results showed that effects of picloram concentrations on the callus induction from explants were significantly different. The highest callus induction rate (68.7%) was obtained at 7.5 mg. l^{-1} picloram concentration on the Kral-97 cultivar. However, this is not statistically significant from 10 mg. l^{-1} picloram concentration on the Kral-97 cultivar and 7.5-10 mg. l^{-1} picloram concentration on the Larende cultivars.

TABLE 3

Effect of different picloram concentrations on the callus weight (mg/per Petri dishes) in barley (*Hordeum vulgare* L.)

Cultivars	Picloram				Mean
	2.5 mg l^{-1}	5 mg l^{-1}	7.5 mg l^{-1}	10 mg l^{-1}	
Kral-97	0.0[1] c	73.3 bc	195.0 ab	332.8 a	150.3[2] a
Beyşehir-98	0.0 c	0.0 c	0.0 c	0.0 c	0.0 b
Anadolu-98	0.0 c	0.0 c	0.0 c	0.0 c	0.0 b
Larende	0.0 c	0.0 c	210.3 a	256.5 a	116.7 a
Mean	0.0 [2] b	18.3 b	101.3 a	147.3 a	267

[1)] Means followed by the same letter within a column and within a row are not significantly different by Duncan's Multiple Range Test at the p≤0.05 levels.

[2)] Means in each column or row followed by same letter are not significantly different by LSD-test at the p≤0.05 levels.

TABLE 4

Effect of different picloram concentrations on the plant regeneration (plantlets/per inflorescence) in barley (*Hordeum vulgare* L.)

Cultivars	Picloram				Mean
	2.5 mg l^{-1}	5 mg l^{-1}	7.5 mg l^{-1}	10 mg l^{-1}	
Kral-97	0.3 (1.1)[1*] de	0.9 (1.4) cd	1.9 (1.8) ab	2.7 (2.1) a	1.5 (1.6)[2]a
Beyşehir-98	0.0 (0.5) g	0.3 (1.0) ef	0.7 (1.3) cde	0.1 (0.6) fg	0.3 (0.9) c
Anadolu-98	0.0 (0.5) g	0.3 (1.1) de	0.4 (1.1) de	0.0 (0.5) g	0.2 (0.8) c
Larende	0.0 (0.5) g	0.4 (1.2) d	1.4 (1.7) bc	1.9 (1.9) ab	1.0 (1.3) a
Mean	0.1 (0.6)[2]c	0.5 (1.2) b	1.2 (1.4) a	1.2 (1.4) a	3.0 (4.6)

[*)] Transformed values

[1)] Means followed by the same letter within a column and within a row are not significantly different by Duncan's Multiple Range Test at the p≤0.05 levels.

[2)] Means in each column or row followed by same letter are not significantly different by LSD-test at the p≤0.05 levels.

Similarly, mean direct shoot induction rate differed according to picloram concentrations and ranged from 4.7% to 40.6% (**Table 2**). The highest mean direct shoot induction was obtained from 7.5 mg. l^{-1} picloram concentration, which is not statistically significant from 5 mg. l^{-1} picloram concentration.

The effects of picloram concentrations on the direct shoot induction from explants were significantly different. The highest direct shoot induction rate was obtained at 7.5 mg. l^{-1} picloram concentration on the Kral-97, Beysehir-98 and Larende cultivars. However, this is not statistically significant from 5 mg. l^{-1} picloram concentration on the all cultivars. Differential responses of explants to the auxin concentrations may be attributed to endogenous hormone levels of explants. Wernicke et al. (21) reported that increased auxin concentrations prevented meristematic cell divisions, resulting in decreased reactions.

Mean callus weight (mg/per petri dishes) differed according to picloram concentrations and ranged from 0.0% to 147.3 mg (**Table 3**). Although the highest mean callus weight were obtained from 10 mg. l^{-1} picloram concentration, this is not statistically significant from 7.5 mg. l^{-1} picloram concentration.

The effects of picloram concentrations on the callus weight from genotypes were significantly different. The highest callus weight (mg/per petri dishes) was obtained at 10 mg. l^{-1} picloram concentration on the Kral-97 cultivar. However, this is not statistically significant from 7.5 mg. l^{-1} picloram concentration on the Kral-97 cultivar and 7.5-10 mg. l^{-1} picloram concentration on the Larende cultivar.

We also showed that the effect of picloram concentrations on the plant regeneration (plantlets/per inflorescence) were significantly different (**Table 4**). The highest mean regeneration (plantlets/per inflorescence) was obtained with 7.5-10 mg. l^{-1} picloram concentrations, which resulted in highest mean number of 1.2 regenerates.

The effects of picloram concentrations on the genotypes were significantly different. The highest plant regeneration was obtained at 10 mg. l^{-1} picloram concentration on the Kral-97 cultivar. However, this is not statistically significant from 7.5 mg. l^{-1} picloram concentration on the Kral-97 cultivar and 10 mg. l^{-1} picloram concentration on the Larende cultivar.

The result of the study showed that callus induction rate, shoot induction rate and plant regeneration from the immature inflorescence was significantly affected by the barley genotypes and changing the medium composition. Picloram significantly enhanced callus growth, however, embryogenic response and plant regenerability were low reported by Mendoza and Kaeppler (13). These results are in agreement

with the study on different explants, genotypes and changing the medium composition such as in barley by Barro et.al. (3), Chernobrovkina et. al. (5), King (7), Lupotto (10), Ruiz (17) and Sharma et. al. (19), in wheat by Ahloowalia (1), Machii et. al. (12), Mohmand and Nabors (14), Sears and Deckard (18), in secale by Ward and Jordan (20).

Conclusions

In vitro culture of barley (*Hordeum vulgare* L.) immature inflorescence, showed that genotype and picloram concentrations are important success factors. The segments cultured on the LS medium containing 7.5 mg. 1^{-1} of picloram gave the highest values of callus induction rate, shoot induction rate and plant regeneration.

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