EFFECTS OF GENOTYPE AND CONCENTRATIONS OF DICAMBA ON CALLUS INDUCTION AND PLANT REGENERATION FROM YOUNG INFLORESCENCES OF PERENNIAL RYEGRASS (*LOLIUM PERENNE* L.)

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ABSTRACT

*This study was carried out to determine the effects of genotypes and dicamba concentrations (2.5, 5, 7.5 and 10 mg^{-1}) on the callus induction and plant regeneration from the segments of young inflorescences cultured on the LS-medium in perennial ryegrass (**Lolium perenne L**).*

The result of the study showed that callus induction rate, callus weight per petri dish and plant regeneration from the young inflorescences were significantly affected by the genotypes. Depending on the genotypes, callus induction rate, callus weight mg/petri dish and number of regenerates per inflorescence segment varied from 20.3 %-67.2 %, 54.4-118.1, 0.797-2.719 respectively.

Callus and shoot induction rates, callus weight and regeneration rate were also significantly influenced by the dicamba concentrations. The segments cultured on the LS medium containing 5 mg^{-1} of dicamba gave the highest values of callus induction rate (77.1 %), shoot induction rate (66.6 %), callus weight (168.2 mg/ petri dish) and regeneration rate (3.458 regenerates per segment).

Introduction

Perennial ryegrass **(***Lolium perenne* L.) is a perennial forage grass that has a short life period, bunch-type grass, cultivated for fresh, hay, pasture and also for silage in the regions having a regularly rainfall in over all the world. Because of easy establishment, fast regrowth after cutting and also high feeding quality, it is an important species of the North Europe (1). Breeding perennial ryegrass **(***Lolium perenne* L.) cultivars with high forage yield and forage quality is of great importance for the animal nutrition. But, its complex cytology and insufficient knowledge both on the plant and also its agronomy cause the difficulties on working of its breeding. Furthermore, the breeding time takes longer. A lot of studies have been conducted to shorten this time by using biotechnological techniques in recent years. 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 explants in in-vitro cultures and also different plant growth regulators in the me-

dium in last years. On the other hand, the factors affecting on the in-vitro response were 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 plants before its in-vitro culture (18). During the in-vitro culture of grasses mature or immature embryos, young leaves and inflorescences are frequently used as explants. The investigations showed that the growth stage of the explants is the most important factor affecting the callus induction and plant regeneration (3, 5, 6, 7, 9, 10, 11, 23).

This study was carried out to determine the effects of genotype and dicamba concentration on the segments of young inflorescences cultured on the LS-medium in perennial ryegrass (*Lolium perenne* L).

Materials and Methods

Plants from the six different cultivars (Lasso, Tivolvi, Tetramax, Spira, Cheops and Tove) of perennial ryegrass (*Lolium perenne* L) were used as donor plant. One plant of each cultivars grown in the plots at the research area of Mustafa Kemal University was dug out from the soil. Each plant was regarded as a genotype. Four clones with three tillers from each genotype were planted in a soil+manure+sand mixture and placed in a greenhouse. The plants were fertilized weekly with a 0.1 % percent solution of a fertilizer mixture including 8 % N+8 % P_2O_5 and watered regularly during the whole investigation. When the shoots reached to the two nodes stage, they 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-80 per 100 ml solution for 10 minutes and rinsed four times in sterile distilled water at the end of a cold pretreatment under +4 °C for a night. Young inflorescences with 5-20 mm length were isolated from the shoots and measured with the help of a scale placed on to the table of a binocular. The inflorescence longer than 5 mm cut to the this length. Isolated inflorescences were placed into the petri dishes (60x15 mm) filled with 7 ml Linsmaier and Skoog (17) basal medium supplemented with different concentrations of Dicamba (3,6 dichloro-2 methoxybenzoic acid) (2.5, 5, 7.5 and 10 mg^{-l}) and solidified by 8 g^{-l} agar. Petri dishes were then sealed with para film. All cultures were incubated in darkness 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 culture. Callus was transferred to the growth regulator free LS (12) regeneration medium after weighted. All cultures were incubated at 25 ± 1 °C under white fluorescent light, irradiance of 2000 lx with 16 h photoperiod. Regenerated shoots were excised and rooted in half-strength LS medium. The rooted plantlets (8-10 cm in length) were then transferred to compost.

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 Öx transformation for callus and shoot induction rate, also Öx+0.5 transformation for regeneration rate before statistical analysis.

Results and Discussion

We observed that an expansion on the dissections of inflorescence segment, inflorescence axis and other floral parts one week after from the induction (**Fig. 1a**).

In 15th day, there appeared an extension on outer glumes of inflorescence segments as well as substantial swelling of flower primordial. Additionally, we observed a callus formation in dissections of some segments and in the junction of spikelets and inflorescence axis (**Fig. 1b**). Three weeks after culture started shoot formation from flower primordial and leaf formation from outer glumes (**Fig.1c**). After four- week 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.1.d**).

Our observations about callus and shoot induction from young inflorescence segments of perennial ryegrass are in corroboration with other studies such as in *Lolium multiflorum,by* Dale et al. (11), in *Lolium multiflorum, Lolium perenne, Festuca pratensis, Festuca arundunacea, Phleum pratense* and *Dactylis glomerata by* Dale and Dalton (10), in *Lolium multiflorum* by Kobabe and Franke (15) and in *Dactylis glomerata* by Hatipoglu (14). In our study, we only observed white collared succulent callus formation.

The Effects of Genotype

Callus induction rate differed according to genotype and ranged from 20.3% to 67.2% (**Table 1**). Inflorescence taken from the genotype 1, originated from the cultivar Lasso, produced statistically significant higher callus induction rate (%67.2) than genotype 3, originated from the cultivar Tetramax. Other genotypes were not significantly different from the genotype 1.

In this study, we found that different perennial ryegrass genotypes revealed different callus induction rate. These results are in agreement with the study by Hatipoglu on *Dactylis glomerata*. Similar findings are also observed on in vitro culturing of young inflorescences of various gramineae species (4, 12, 18, 19, 20, 21, 22).

Shoot formation rate varied between 53.1% and 32.8% with respect to genotypes used (Table 1.) although this difference is statistically insignificant.

Weight of callus formed from young inflorescence of perennial ryegrass was different according to genotypes (Table 1). Callus weight per petri dish ranged from 54.4 mg to 118.1 mg with respect to genotypes.This variation trend in callus weight seemed similar to callus induction rate considering genotypes (i.e. young inflorescence with higher callus induction rate resulted in higher callus weight). Therefore, differences in callus weights were caused by differences in callus induction rates.

Plant regeneration rate from young inflorescence of perennial ryegrass changed significantly with respect to genotypes used (Table 1).

A total of 732 regenerates were obtained from 384 inflorescence segments of six different genotypes. Average regeneration rate appeared to be between 0.797 and 2.719.

Our findings regarding different regeneration rates from varying perennial ryegrass genotypes are in corroboration with previous studies with diverse gramineae species (13, 15, 17, 19, 24).

The Effects of Dicamba Concentrations

Our findings showed that effect of dicamba concentrations on callus formation is significantly different (**Table 2**).

$5mg^{-l}$ concentration of Dicamba produced the highest callus formation rate of 77.1 %. However, this is not statistically significant from $2.5mg^{-l}$ dicamba concentration.

Dicamba concentrations higher or lower than $5mg^{-l}$ decreased callus induction rate.

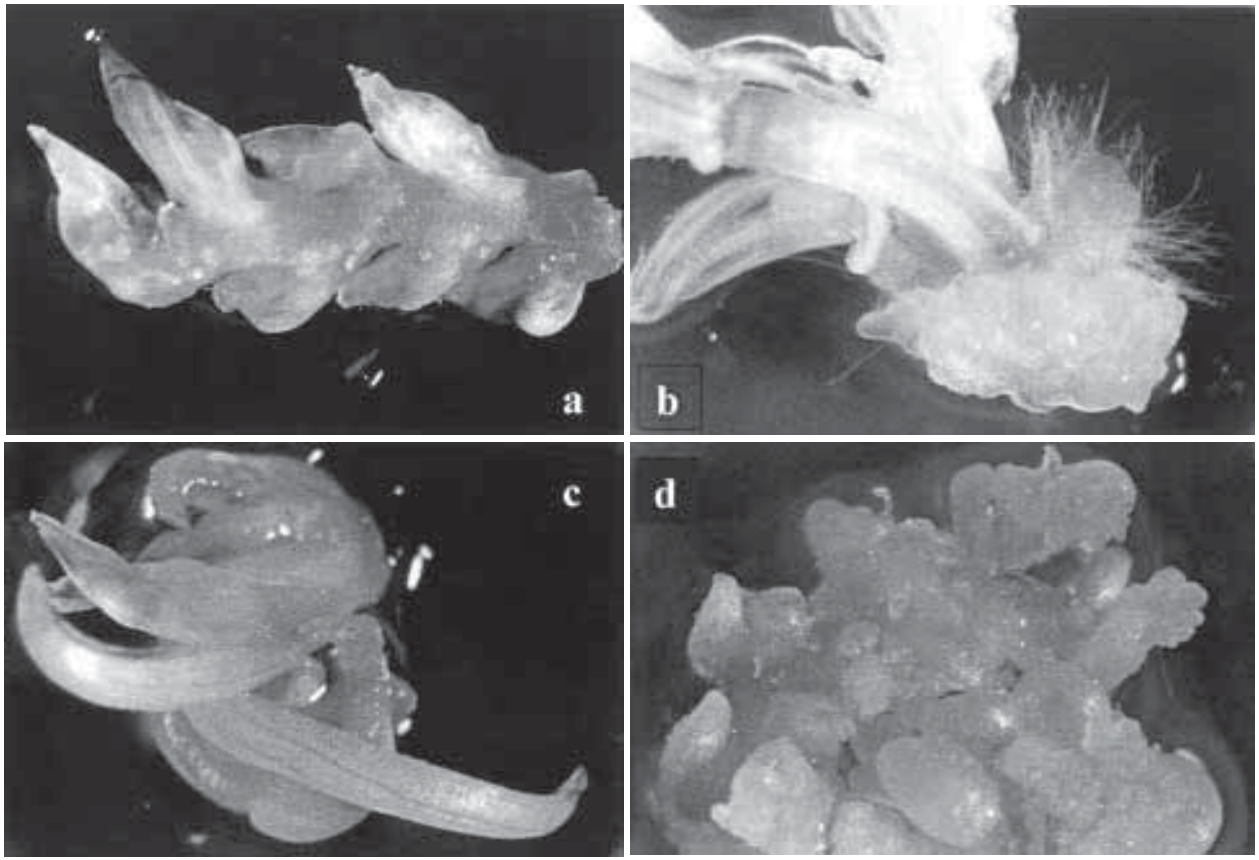

Fig.1. Callus induction and plant regeneration from inflorescence segments of perennial ryegrass. ***(a)*** An expansion on the dissections of inflorescence segment one week later of the induction. (b) Callus initiation two weeks later of the induction. (c) Shoot formation three weeks later of the induction. (d) Callus formation after four-weeks of culture.

TABLE 1

Effect of genotype on callus induction rate (%), shoot Induction rate (%), callus weight (mg/per petri dish) and regeneration rate (plantlets/per inflorescence) in inflorescence culture of perennial ryegrass (*Lolium perenne* L).

Genotype	Origin cultivar	Callus Induction Rate (%)	Shoot Induction Rate (%)	Callus Weight (mg/per petri dish)	Regeneration Rate (plantlets/per inflorescence)
1	Lasso	67.2 (59.821) a	53.1 (48.678)	106.8 ab	2.375 (1.616) $^{+}$ ab*
2	Tivolvi	53.1 (49.580) a	50.0 (45.00)	114.1ab	2.719 (1.726) a
3	Tetramax	20.3 (19.072) b	35.9 (32.956)	54.4c	0.797 (1.028) c
4	Spira	40.6 (36.670) ab	48.4 (42.259)	109.2ab	2.297 (1.526) ab
5	Cheops	54.7 (48.714) a	42.2 (37.607)	118.1a	2.109 (1.526) ab
6	Tove	48.4 (43.161) ab	32.8 (30.179)	68.7 bc	1.125 (1.167) bc

TABLE 2

Effect of Dicamba concentration on callus induction rate (%), shoot Induction rate (%), callus weight (mg/per petri dish) and regeneration rate (plantlets/per inflorescence) in inflorescence culture of perennial ryegrass (*Lolium perenne* L).

Dicamba Concentration	Callus Induction Rate (%)	Shoot Induction Rate (%)	Callus Weight (mg/per petri dish)	Regeneration Rate (plantlets/per inflorescence)
2.5 mg^{-l}	54.2 (49.30) ab	47.9 (43.15) ab	97.8 b	1.510 (1.376) $^{+}$ b*
5 mg^{-l}	77.1 (68.46) a	40.6 (35.77) bc	168.2 a	3.458 (1.925) a
7.5 mg^{-l}	37.5 (33.893) bc	66.6 (60.41) a	74.0 bc	1.740 (1.347) b
10 mg^{-l}	20.8 (19.685) c	19.8 (18.46) c	40.7 c	0.906 (1.079) b

Our findings about changes in callus induction rate with respect to Dicamba concentrations are in agreement with the results of Gland and Hatipoglu's (13) and Can et al. (8).

Different dicamba concentrations significantly affected callus weights (Table 2). Varying dicamba concentrations is critical factor influencing shoot induction from young inflorescences of *Lolium perenne* (Table 2).An increase in dicamba concentration from 7.5 to 10 mg^{-1} decreased shoot induction rate. The highest shoot induction rate (66.6%) was obtained from 7.5mg^{-1} dicamba concentration and the lowest one (19.8 %) from 10 mg^{-1} .

Dicamba concentration of 5 mg^{-1} produced the highest callus weight (average 168.2 mg per pedri dish) while the lowest callus weight (40.7 mg) was obtained from 10mg^{-1} dicamba. Deviation from 5mg^{-1} resulted in decline in callus weight as in callus induction rate.

Regeneration rate was significantly influenced by the dicamba concentrations (Table 2). The highest regeneration rate was obtained with 5mg^{-1} , which resulted in highest number of 3,458 regenerates. The other dicamba concentrations were not significantly different from each other in regeneration rate. Our findings regarding regeneration rates with respect to dicamba concentrations were in agreement with that of Lörz et al. (17) and Gland and Hatipoglu's.

Conclusion

In the investigation of the in vitro culture of *Lolium perenne* young inflorescences, it was concluded that genotype and dicamba concentration are important success factors. Our results suggest that LS medium with 5mg^{-1} dicamba can be successfully used for regeneration from inflorescences of perennial ryegrass.

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