EFFECT OF INFLORESCENCE LENGTH ON THE CALLUS INDUCTION AND PLANT REGENERATION IN ORCHARDGRASS (*DACTYLIS GLOMERATA* L.)

N. Çeliktas[1], E. Can[1], R. Hatipoglu[2]
Mustafa Kemal University, Faculty of Agric., Dept. of Field Crops, Hatay, Turkey[1]
Cukurova University, Faculty of Agric., Dept. of Field Crops, Adana, Turkey[2]

ABSTRACT

This study was carried out to determine the effect of inflorescence length on callus induction and plant regeneration in Orchardgrass (Dactylis glomerata L.). In the study, the inflorescences with different length (2-25 mm) isolated from the ecotype with a good in-vitro regeneration capacity were cultured on LS medium supplemented with 5 mg^{-l} 2,4-D. Both callus induction and directly shoot regeneration from the inflorescences of orchardgrass were observed during the investigation. Callus induction rates varied from 50 % to 84.5 % depending on the inflorescence length. The highest callus weight per inflorescence was obtained from the inflorescence with 11-15 mm length. The inflorescences being longer than 15 mm produced lower callus weight than those being shorter than 15 mm. The highest regeneration rate (4.4 plantlets per inflorescence) was obtained from the inflorescences with the 11-15 mm length. Increasing the inflorescence length up to 20 mm decreased the regeneration rate. Considering the results of study, it was concluded that the inflorescences being yellowish in color and 6-20 mm length could be used as explant for an acceptable regeneration rate.

Introduction

Orchardgrass (*Dactylis glomerata* L.) is a perennial forage grass cultivated for fresh, hay, pasture and also for silage. It is a natural species of the Europe, North Africa and the warm regions of Asia (14). However, it has been carried all over the world and cultivated any climatically region. Breeding orchardgrass 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 are 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. 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 affected on the in-vitro response are determined by these investigations. Optimal physiological condition of donor plants, determination of the most productive explant, medium composition and the induction conditions must be evaluated for any plants before its in-vitro culture (13). During the in-vitro culture of grasses mature or immature embryos, young leaves and inflorescences are frequently used as explants. The investigations are showed that the growth stage of

the explant is the most important factor affecting the callus induction and plant regeneration (6, 2, 1). When the young inflorescences are used as an explant, the length of it is the determiner of the growth stage.

This research was conducted to find out the optimum inflorescence length of Orchardgrass (*Dactylis glomerata* L.) for obtaining the high in-vitro response.

Materials and Methods

In this research, the regenerates of a highly regenerable Orchardgrass (*Dactylis glomerata* L.) ecotype selected by its in-vitro regeneration capacity among the collected clones from the natural pastures of southern part of Turkey were used as donor plants.

The donor plants 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 irrigated 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 pre-treatment under +4 °C for a night. Young inflorescences with 2-25 mm length were isolated from the shoots and measured with the help of a scale placed on to the table of a binocular. Isolated inflorescences were placed into the petri dishes (60x15 mm) filled with 7 ml Linsmaier and Skoog (12) basal medium supplemented with 5 mg^{-1} 2,4-D and solidified by $10g^{-1}$ agar. Petri dishes were then sealed with parafilm. 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 explant 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 Duncan's Multiple-Comparison Test. Software MSTATC was used. Data given in percentages were subjected to Arcsin $\sqrt{x}$ transformation before statistical analysis.

Results and Discussion

The Effect of Inflorescence Length on The Callus Formation

A swelling has been observed on the cutting edge of inflorescences, inflorescences axes and the flower tissues one week later of the incubation. Flower segments and glumes start to get longer and flower primordial significantly swelling 15 days of the culture. Furthermore callus induction was observed on the cutting edge of the other segments and the connection parts of the spikelet to the rachis axes. 3 weeks later of the culture shoot regeneration from the flower primordial and metamorphosis of the upper glumes to leaves were observed. Callus formed from the inflorescences with 2-10 mm length and those being longer than 20 mm was not embryogenic, soft, juicy, has a white color and not compact. Inflorescences being longer than 20 mm constitute shapeless callus only from their top and also directly shoot regeneration was not observed from this type of inflorescences. On the other hand, callus formed especially from the rachis axis and flower segments of inflorescences with 11-20 mm length was compact, yellowish and embryonic structure.

At the end of the four weeks of incubation, some of the petri dishes covered with a lot of shoots. Beside this, great amount of

TABLE 1

Mean frequencies of callus induction, callus weight and shoot formation rate for different inflorescences length in (%) Orchardgrass (*Dactylis glomerata* L.)

Inflorescences Length	Number of Cultured Inflorescences	Callus Induction Rate (%)	Callus Weight (mg/per inflorescence)	Shoot Formation Rate (%)
2-5 mm	28	57.0 (49.0) c	36.8 c	17.5[1] (24.5)[2] b
6-10 mm	34	68.0 (55.7) b	89.5 b	34.1 (35.0) ab
11-15 mm	33	84.5 (67.0) a	186.1 a	43.05 (40.8) a
16-20 mm	37	77.8 (61.9) ab	182.8 a	27.2 (31.3) ab
21-25 mm	32	50.0 (45.0) c	73.2 b	0.01 (0.57) c

[1]) Means with the same letter for each inflorescence length are not significantly different by Duncan's Multiple Range Test at the $p \leq 0.05$ levels. [2]) Transformed values.

embryogenic callus and significantly embryoid formation were observed. These observations are in harmony with the findings of Chen et. all (4) and Hatipoğlu (8) from the inflorescences culture of same species. Furthermore the observations of Dale et. all (5) and Kobabe & Franke (10) on embryogenic and non-embryogenic callus formation from the young inflorescences of *Lolium multiflorum* are in similarity with our findings. The reason of the direct shoot regeneration from the flower primordial during the in-vitro culture is explained with the hormonal imbalance caused by a supplementation of exogenous growth regulators to the medium or a production of wounding hormones as a result of excision (4).

Callus induction rate, callus weight and the percentage of shoot regeneration belong to different inflorescence length groups of Orchardgrass (*Dactylis glomerata* L.) are given in **Table 1**. Callus induction rate varied between 50.0 % and 84.5 % depending on the inflorescence length. When the inflorescence even shorter or longer than 11-15 mm length, a reduction was observed on this rate. The result of the study showed that the inflorescence with 11-20 mm length is the best explant type of the Orchardgrass (*Dactylis glomerata* L.). Can et all. (3) previously reported that they were obtained a callus induction rate of 39.4 % from the inflorescences with 5-20 mm length of different Orchardgrass (*Dactylis glomerata* L.) ecotypes This is rather low than the rate we obtained. This difference can be arisen from the usage of the regenerates of highly in vitro responsive ecotype as donor plants in this study.

While the inflorescences with 11-15 mm length were produced the highest callus weight (186.1 mg/per inflorescence), the callus weight produced by the inflorescences with 16-20 mm length was not statistically different than this value. A reduction on the callus weight was recorded when the inflorescence length even shorter or longer than 11-20 mm. These results are exposed that the optimum explant type for callus weight is 11-20 mm length (Table 1). The findings on callus weight obtained from the inflorescences with different length are close similarity with the result of the study conducted on different inflorescence length of hairy bluestem (*Hyparrhenia hirta* (L.) Stapf.) by Hatipoğlu (9).

The results of the study showed the similarity between the occurrence of callus induction and plant regeneration rates depending on the inflorescence length. The inflorescences showing more induction rate showed high regeneration capacity than the others. Furthermore, directly shoot regeneration was not observed when the inflorescences being longer than 20 mm were used as explant.

TABLE 2

Mean frequencies of green plantlets for different inflorescences length in Orchardgrass (*Dactylis glomerata* L.).

Inflorescences Length	Number of Cultured Inflorescences	Regeneration Rate (plantlets/inflorescence)
2-5 mm	28	1.4 b[1]
6-10 mm	34	3.2 a
11-15 mm	33	4.4 a
16-20 mm	37	3.9 a
21-25 mm	32	0.8 c

[1]) Means with the same letter for each inflorescence length are not significantly different by Duncan's Multiple Range Test at the $p \leq 0.05$ levels.

The Effect of Inflorescence Length on Plant Regeneration

The chlorophyll occurrence and the color changing to green on the shoots were observed when they were transferred to the light after incubation for 5 weeks in the dark.

The in-vitro regeneration rates obtained from the different inflorescences were given in **Table 2.**

The highest regeneration rate (4.4 plantlets / inflorescences) was obtained from the inflorescences with 11-15 mm length. The regeneration rate drastically decreased when the inflorescences being longer than 20 mm length were used. A reduction on the regeneration rate was recorded when the inflorescence length even shorter or longer than 6-20 mm. The findings on changing of the regeneration rates depending on the inflorescences length are in harmony with the results of the study conducted by George and Eapen (7), Hatipoğlu (9) and Kostina et all (11) with different grass species.

The observations and the obtained data showed that the inflorescences with 6-20 mm length and the light yellow color could be used as explant in the in vitro culture of Orchardgrass (*Dactylis glomerata* L.).

REFERENCES

1. **Ahn B.J., Huang F.H., King J.W.** (1985) Crop. Sci., **25**, 1107-1109.

2. **Botti C., ve Vasil I.K.** (1984) Can. J. Bot., **62,** 1629-1635.

3. **Can E., Tükel T., ve Hatipoğlu R.** (1994) Congress of Field Crops, Aegean University, Agric. Faculty. Vol. **2,** p. 254-256. İzmir-Turkey.

4. **Chen C.H., Songstad D.D., Boe A.A.** (1985) Proc. S.D. Acad. Sci., **64,** 77-81.

5. **Dale P.J., Thomas E., Brettel R.I.S., Wernice W.** (1981) Plant Cell Tis. Org. Cult., **1,** 47-55.

6. **Dale P.J., Dalton S.J.** (1983) Z. Pflanzenphysiol., **111,** 39-45.

7. **George L., Eapen S.** (1990) Euphytica, **48,** 269-274.

8. **Hatipoğlu R.** (1991) Dissertation an der Universitaaet Hohenheim.

9. **Hatipoğlu R.** (1992) J. Agric. Fac. Cukurova University, **7(3),** 153-166.

10. **Kobabe G., ve Franke B.** (1988) Pflanzenzüchtung, **14,** 129-134.

11. **Kostina G.I., Larina T.V., Efremova I.G.** (1997) Plant Breed. Abs., Vol. **67,** No.1, p.316.

12. **Linsmaier E.M., Skoog F.** (1965) Physiol. Plant., **18,** 100-127.

13. **Lörz H., Gobel E., Brown P.** (1988) Plant Breeding, **100,** 1-25.

14. **Whyte R.O., Moir T.R.G., Cooper C.P.** (1975) Grasses in Agric. FAO Agric. Studies No. 42, Rome.