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Some Conditions for the Best Callus Induction in Common Bermudagrass [*Cynodon dactylon* (L.) Pers. (California Origin)]

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Abstract: Common Bermudagrass, a member of poaceae family, is a native of eastern Africa. It is widely used on landscape, where a close-mown is needed. In this experiment the best media for callus induction in common Bermudagrass was investigated using different explants including leaf, root from germinated seeds and seed after sterilization. Murashige and Skoog basal medium containing 30 g L^{-1} sucrose and 8.4 g L^{-1} agar, 10, 20, 30 and 40 μM 2,4-D along with control (without 2,4-D) was used for callus induction. For selecting the best environmental conditions for callus induction, the petri-dishes were placed at 20, 25 and 30°C in completely dark or 16/8 h light/dark photoperiods. Results showed the highest callus induction percentages and least days to visible callus induction in seed explants. Increasing the temperature and 2,4-D concentration raised the early callus induction percentage. Root explants showed the same results, except that highest 2,4-D concentration inhibited the callus induction. Leaf explants were not successfully produced the callus. Light/dark and completely dark were the best treatments for callus induction in seed and root explants, respectively.

Key words: Bermudagrass · callus induction · tissue culture

INTRODUCTION

Common Bermudagrass [*Cynodon dactylon* (L.) Pers.] is one of important warm-season turfs from temperate to tropical regions of the world and a valuable pasture and excellent fodder grass, staying green during hot weather [1, 2]. It is a member of Poaceae family, Eragrostoideae sub-family and Chlorideae tribe is a native of eastern Africa [3, 4] and was introduced into the United States in the mid 1700s [5]. It is widely used on lawns, roadsides, parks, school grounds, athletic fields, golf courses and other areas where a close-mown, dense turf is desired [6]. Bermudagrass is widely distributed between the latitudes of 45°N and 45°S [7] and well-adapted to the tropical and subtropical climates of the United States. It is less tolerant to cold temperature than zoysiagrass and buffalograss, which extend into the cool humid region. Its area of adaptation extends into the southern and central section of the transition zone but it easily winter-kills in the northern transitions zone [6]. Bermudagrass is sensitive to cool temperature and will stop growing, lose its chlorophyll and take on a brown-tan color when soil temperature fall below 10°C. The plant remains in this winter-dormant condition until soil temperatures at the 4-in. depth rise and remain above 10°C. Root and rhizome growth will substantially increase when soil temperature reach 15 to 20°C, with optimum growth occurring in soil temperatures of 24 to 29°C [6]. Plant regeneration from embryogenic callus is only and also the most important method for turfgrass tissue culture [8-9]. Mature seeds are the most convenient explants to use [10]. Callus induction from mature seeds has been reported for *Festuca rubra* L. [11, 12], *Cynodon* L.C. Rich. sp. [13,14], *Lolium perenne* L. [15], *Festuca arundinacea* Schreb. [16] and *Poa pratensis* L. [17]. Callus induction and plant regeneration from young inflorescence of common and hybrid Bermudagrass was first reported by Ahn *et al.* [13, 14]. Artunduaga *et al.* [18] tested three common Bermudagrass varieties and found one yielded 90% albino plants. Chaudhury and Qu [9] reported improved somatic embryogenesis and green plantlet regeneration from young inflorescence culture of turf-type common Bermudagrass and a hybrid. Early and in a large mass callus production is an important goal for genetic transformation and protoplast isolation and fusion in any plant. The purpose of the present study was to find the suitable 2,4-D concentration supplemented with culture medium for callus induction using leaf, root and seed explants and the effect of different temperatures with light/dark and completely dark treatments on efficient callus production.

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MATERIALS AND METHODS

Dehulling and surface sterilization: Seeds of *Cynodon* were dehulled by 50% sulfuric acid for 20 min [19]. Seeds were continuously shaken in cold acid, rinsed at least three times with distilled water and placed under tap water overnight. Then, the seeds were surface-sterilized in 70% ethanol for 1 min, followed by 100% laundry bleach (5.25% sodium hypochlorite) for 20 min and then rinsed six times with sterilized distilled water. In the case of leaf and root explants, after dehulling and surface-sterilizing the seeds the same as previous method, they were germinated root on petri-dishes for 20 days. Leaf and root explants were cut from germinated seeds.

Culture: For callus induction, Murashige and Skoog (MS) basal medium containing 30 g L^{-1} sucrose and 8.4 g L^{-1} agar supplemented with following growth regulators was used. According to preliminary experiments, 10, 20, 30 and 40 μM 2,4-D with control (without 2,4-D) was used for callus induction media.

The pH of medium was adjusted to 5.8 prior to autoclaving at 1.5 atm and 121°C for 20 min. The sterilized media were divided into 10-cm sterile plastic petri dishes and 10 seeds; root or leaf explants were cultured on them. Root and leaf explants were cut to 1.0 and 0.5 cm length, respectively.

Environmental conditions: In callus induction experiment, cultures were kept at 20, 25 and 30°C in complete dark or light/dark conditions with 16/8 h light/dark photoperiods with 140 μmol m^{-2} s^{-1} light from cool white fluorescent lamps [20].

Data recording: The days to visible callus induction in *in vitro* seed, root and leaf culture of *Cynodon* was recorded after 4 weeks; number of seeds that induced callus formation was scored and corrected according to seed viability, to determine the Callus Induction Percentage (CIP). The number of calli that regenerated shoots and number of shoots produced per explant were counted 4 weeks after their transfer to regeneration media.

Statistical analysis: Five replications were used in each treatment with 20 explants in each. All experiments were repeated at least two times. A completely randomized design with factorial arrangements was used for all the experiments. Analysis of variance was carried out using SPSS software. Means were separated using Tukey's test at P= 0.01.

RESULTS

Results showed that kind of explant is very important regard to number of days to visible callus induction and CIP in *Cynodon*. Seed explants showed highest CIP and least days to visible callus induction, but leaf explants did not produce callus. Root explants induced a good callus mass which was significantly lower than that of seed explants (Fig. 1). Different temperatures affected the days

Table 1: Days to visible callus induction in *in vitro* seed, root and leaf cultures of *Cynodon* at different conditions

		Explants					
		Seed		Root		Leaf	
Temperature	Concentration of 2,4-D (μM)	Light/ dark	Dark	Light/ dark	Dark	Light/dark	Dark
20°C	0	-†	-	-	-	-	-
	10	5.4 k-m§	9.0 f-i	-	-	-	-
	20	4.8 l-n	9.6 fg	19.6 a	15.6 b	-	-
	30	5.4 k-m	9.4 f-h	19.8 a	16.0 b	-	-
	40	5.4 k-m	9.4 f-h	-	-	-	-
25°C	0	-	-	-	-	-	-
	10	4.4 m-o	6.4 j-l	-	-	-	-
	20	4.0 m-o	7.8 g-j	16.6 b	15.0 bc	-	-
	30	4.4 m-o	7.6 h-j	16.6 b	12.0 de	-	-
	40	5.0 l-n	7.4 ij	-	-	-	-
30°C	0	-	-	-	-	-	-
	10	3.4 no	8.0 g-j	-	-	-	-
	20	3.2 no	7.2 i-k	15.4 b	13.4 cd	-	-
	30	2.8 o	9.0 f-i	15.4 b	10.4 ef	-	-
	40	5.0 l-n	10.2 ef	-	-	-	-

†No callus observed, §Data followed by the same letters are not significantly different according to Tukey's test at P= 0.01

Table 2: Comparison between callus induction percentages of *Cynodon* at different conditions after 4 weeks

		Explants					
		Seed		Root		Leaf	
Temperature	Concentration of 2,4-D (µM)	Light/ dark	Dark	Light/ dark	Dark	Light/dark	Dark
20°C	0	0.0 t†	0.0 t	0.0 t	0.0 t	0.0 t	0.0 t
	10	38.6 s	35.4 s	0.0 t	0.0 t	0.0 t	0.0 t
	20	50.2 pq	42.2 r	71.6 l	78.4 k	0.0 t	0.0 t
	30	68.8 lm	66.2 m	81.4 g-k	84.0 fg	0.0 t	0.0 t
	40	92.0 b-d	88.4 e	0.0 t	0.0 t	0.0 t	0.0 t
25°C	0	0.0 t	0.0 t	0.0 t	0.0 t	0.0 t	0.0 t
	10	43.2 r	36.2 s	0.0 t	0.0 t	0.0 t	0.0 t
	20	61.8 n	58.0 o	78.8 jk	80.0 h-k	0.0 t	0.0 t
	30	88.8 de	87.0 ef	82.2 g-j	83.4 gh	0.0 t	0.0 t
	40	99.0 a	93.2 bc	0.0 t	0.0 t	0.0 t	0.0 t
30°C	0	0.0 t	0.0 t	0.0 t	0.0 t	0.0 t	0.0 t
	10	52.6 p	46.8 q	0.0 t	0.0 t	0.0 t	0.0 t
	20	71.8 l	69.8 l	79.8 i-k	80.6 g-k	0.0 t	0.0 t
	30	93.4 bc	90.2 c-e	83.2 j-i	82.4 g-i	0.0 t	0.0 t
	40	99.6 a	94.2 b	0.0 t	0.0 t	0.0 t	0.0 t

† Data followed by the same letters are not significantly different according to Tukey's test at $P = 0.01$

Fig. 1: Effect of different explants on callus production 12 days after culturing on 30 µM 2,4-D at 30°C. Explants from left: seed, root and leaf

to visible callus induction and CIP in both root and seed explants and the condition of 30°C resulted in more callus production than the other temperatures (Table 1 and 2). Furthermore, light/dark or dark conditions affected the number of days to visible callus induction and CIP. These conditions had different effects on seed and root explants, that seed explants had greater callus production in light/dark condition, however in root explant greater callus production happened in dark condition.

In seed explants, concentration of 2,4-D affected the number of days to visible callus induction and CIP. In control explants (without 2,4-D application) no callus observed, however, with increase 2,4-D concentration the number of days to visible callus induction reduced and CIP increased (Tables 1 and 2). Although, in root explants, any callus was not observed in control and with increase in 2,4-D concentration the number of days to visible callus induction reduced and CIP increased up to 40 µM 2,4-D that no callus observed in this concentration (Tables 1 and 2).

DISCUSSION

Bermudagrass is an recalcitrant species in tissue culture. Moreover, the information regarding the effects of various plant growth regulators as culture medium supplements on Bermudagrass tissue culture is lacking. The requirement of exogenous plant growth regulators probably depends on the specific endogenous hormone levels [21]. Improved regeneration rates by including low levels of BA in combination with certain auxin in callus induction media have been reported in other grass species [17, 21-23]. In one experiment the effects of five culture medium supplements on tissue culture responses were

investigated with immature embryos and mature seeds of a tall fescue cultivar, 'Coronado', as explant tissues. For both explants, calli induced on 6-benzylaminopurine (BAP)-containing medium had significantly improved regeneration ability [10]. Our study showed that the explant type is important for callus induction and root and seeds are explants that can produce callus and leaf is not a suitable explant for producing callus. In the other experiment [20] callus of *Cynodon* was obtained by hypocotyls and seeds. Ahn *et al.* [4] reported embryogenesis and regeneration of Bermudagrass from callus induced from young inflorescences on N6 medium with 1 mg L^{-1} 2,4-D while Artunduaga *et al.* [18] reported a combination of 3.0 mg L^{-1} 2,4-D and 200 mg L^{-1} CH in MS medium induced the highest embryogenesis and regeneration from similar explant types for a common Bermudagrass cultivar. Artunduaga *et al.* [18] also reported a high percentage of albino plants among the regenerates.

Furthermore, when temperature rose, callus production increased. This result showed that Bermudagrass for having best callus induction needs high temperature condition. As well, Bermudagrass naturally is sensitive to cool temperatures and will stop growing, lose its chlorophyll and take on a brown-tan color when soil temperature fall below 10°C [3]. We showed better callus induction on seed explants in light/dark condition. Similarly, Salehi and Khosh-Khui [20] reported near 100% callus induction on Bermudagrass seed explants in light/dark. In root explant, their naturally growing in dark condition might be the cause that the best callus induction happened in dark condition.

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