Plant Cell Tiss Organ Cult (2007) 90:71–78
DOI 10.1007/s11240-007-9255-y

ORIGINAL PAPER

Comparison of callus induction and plant regeneration from different explants in triploid and tetraploid turf-type bermudagrasses

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Received: 1 December 2006 / Accepted: 29 May 2007 / Published online: 4 July 2007

Abstract The turf-type bermudagrasses are genetically variable and do not respond uniformly to tissue culture and plant regeneration protocols. We evaluated the callus induction response of two explant types, young inflorescences and nodes, from multiple genotypes including triploid TifSport, TifEagle, and Tift97-4 and tetraploid Tift93-132, Tift93-135, Tift93-156 and Tift93-157 on MS medium supplemented with 1–1.5 mg l^{-1} 2,4-D + 0.01 mg l^{-1} BA + 1.16 g l^{-1} proline. Four types of callus were observed. Type I was fluffy, soft, and white non-embryogenic callus, common to all cultures. Type II was globular, transparent, and hard, but sticky callus, which was pre-embryogenic and could be selected for subculture. Type III callus was transparent, compact, and embryogenic. Type IV callus was opaque white and compact. Both Type III and Type IV calluses were embryogenic and regenerative. A combination of gelling agents in the medium (2 g l^{-1} Gelrite and 5 g l^{-1} agar) improved callus quality and increased the rate of compact callus formation during subculture. Embryogenesis from compact callus was observed in TifEagle, TifSport and Tift93-132, and shoots were regenerated on MS medium with 0.1 mg l^{-1} 2,4-D + 0.5–4.0 mg l^{-1} BA. Low intensity light treatment (30 μmol $m^{-}2$ s^{-1} of cool white fluorescence) to callus before regeneration greatly enhanced regeneration frequency from 6.7% to 40% in recalcitrant TifSport.

Keywords Bermudagrass · Callus induction and subculture · Embryogenic callus · Regeneration

Abbreviations

2,4-D	2,4-Dichlorophenoxyacetic acid
BA	6-Benzyladenine
MS	Murashige and Skoog
NAA	α-Naphthaleneacetic acid
TDZ	Thidiazuron
GA_3	Gibberellic acid
ABA	Abscisic acid

Introduction

Bermudagrass, a warm season turfgrass, is indigenous to Africa but now is distributed throughout the world in tropical to warm temperate climates between 45° north and 45° south latitudes. Bermudagrass is grown extensively on lawns, golf courses, sports fields and arenas, and in parks, coastal areas, and pastures. Common bermudagrass [*Cynodon dactylon*

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(L.) Pers.] is tetraploid ($2n = 4x = 36$) and African bermudagrass [*C. transvaalensis* Burtt Davy] is diploid ($2n = 2x = 18$). Persistent stolons and rhizomes of common bermudagrass give this grass cold and wear tolerance, while African bermudagrass is characterized by its fine texture. Bermudagrass spreads quickly by seeds and vegetative reproduction. Triploid hybrid bermudagrass (*C. dactylon* × *C. transvaalensis*) is the interspecific hybrid between common bermuda and African bermuda. Hybrid bermudagrass, which is pollen and seed sterile, is an ideal recipient for foreign genes because there is no risk of gene flow due to complete sterility.

In order to maintain turf quality and uniformity, soil fumigants and herbicides are applied to control weeds. Herbicide-resistant turf bermudagrass would allow not only control of common weeds but also selective elimination of herbicide-sensitive bermudagrass genotypes. Biotechnology provides an ideal way to introduce foreign genes such as those for herbicide resistance, drought and salt tolerance into turfgrasses (Chai and Sticklen 1998; Inokuma et al. 1998; Xiao and Ha 1997; Wang et al. 2003). Although there are some reports on bermudagrass tissue culture and transformation (Goldman et al. 2004a; Li and Qu 2004; Li et al. 2005), bermudagrass is still recalcitrant to embryogenic callus induction and shoot regeneration, when compared with other turfgrasses (Bai and Qu 2000; Chai et al. 2000; Cho et al. 2000; Dalton et al. 1999; Asano et al. 1998). The various ploidy levels and broad genetic diversity in bermudagrass add to the complexity of regeneration and transformation. Artunduaga et al. (1988, 1989) first reported using young inflorescences as explants to induce callus in bermudagrass but shoot regeneration was at low frequency. Chaudhury and Qu (2000) reported somatic embryogenesis in common bermudagrass. Li and Qu (2004) succeeded in biolistic transformation of a common bermudagrass line by adding BA to callus induction medium. Although Goldman et al. (2004b) obtained plant regeneration in triploid TifSport and TifEagle, the regeneration rates were low, especially in TifSport. Since transformation efficiency is dependent on regeneration efficiency, our objective was to evaluate the callus types and improve plant regeneration systems in bermudagrass using different explants and genotypes.

Materials and methods

Plant materials

Three triploid bermudagrass cultivars (TifEagle, TifSport, Tift97-4) and four tetraploid genotypes (Tift93-132, Tift93-135, Tift93-156, Tift93-157) were used in this study. TifSport and TifEagle are elite triploid, sterile hybrid bermudagrass cultivars (*C. dactylon* × *C. transvaalensis*, $2n = 3x = 27$) (Hanna et al. 1997; Hanna and Elsner 1999). Tift97-4 is an experimental dwarf, sterile triploid hybrid. The Tift93-series are common, seeded bermudagrass genotypes (*C. dactylon* $2n = 4x = 36$).

Young inflorescences, whose flag leaves were just about to emerge from the leaf sheath, were harvested from the field during the flowering season of 2004. The leaf-encased inflorescences were immersed in 70% ethanol for 1 min and then sterilized with 1.2% sodium hypochlorite for 20 min on a shaker (150 rpm), followed by 4 washes in sterile H_2O. The surface-sterilized inflorescences were dissected from the leaf tissues, cut into 5 mm segments, and inoculated on callus initiation medium. Nodes were sterilized and cultured according to Goldman et al. (2004a).

Culture medium and conditions

The inflorescences or node segments were first cultured on MS medium (Murashige and Skoog, 1962) supplemented with 1, 1.5 or 2 mg l^{-1} 2,4-D, 0, 0.01 or 0.02 mg l^{-1} BA, 1.16 g l^{-1} proline, 30 g l^{-1} sucrose and 3 g l^{-1} Gelrite (Sigma cat # 1910-250G) (callus initiation medium) for 30 days in dark. After 1 month, embryogenic calluses were selected and subcultured onto fresh MS medium supplemented with 1.0–1.5 mg l^{-1} 2,4-D and 0.02–0.5 mg l^{-1} BA (callus subculture medium) every 30 days according to Li and Qu (2004). Different concentrations of gelling agents [2 or 3 g l^{-1} Gelrite; 5, 8, or 16 g l^{-1} agar (Sigma cat # A1296)] were tested to improve compact callus frequency in preliminary experiments for callus subculture. Each treatment for the gelling agent experiment consisted of two replications with two culture dishes per replication. Water availability of media was measured and analyzed according to Klimaszewska et al. (2000). A disc of autoclaved filter paper (Whatman #1, 7.0 cm) was placed on the

surface of two-day-old medium (25 ml) in 9 cm diameter Petri dishes. Each Petri dish served as one replicate with nine replicates for each treatment. The Petri dishes were sealed with Parafilm and incubated for one day under the same conditions as the callus cultures after which the filter paper was weighed. Gelrite (2 g l^{-1}) plus agar (5 g l^{-1}) was chosen for routine subculture.

Compact embryogenic calluses were transferred to MS medium containing 0.1 mg l^{-1} 2,4-D and 0.5–4.0 mg l^{-1} BA (regeneration medium) for shoot regeneration. The regenerated shoots were transferred to MS or 1/2 MS medium without any growth regulators (rooting medium) for rooting.

All media were autoclaved at 121°C, 105 kPa for 20 min. Callus induction and subculture were conducted at 25°C in the dark. Shoot regeneration and rooting were conducted at 25°C under light (100 µmol m^{-2} s^{-1} from cool white fluorescent lamps with 16/8 h (day/night photoperiod). Pretreatment of TifSport callus prior to shoot regeneration was carried out under low intensity light (30 µmol m^{-2} s^{-1}) with a 16/8 h (day/night photoperiod).

Experimental design and statistical analysis

Twenty explants were placed in each Petri dish as a single replication for the callus induction test. Plates from inflorescence and node cultures were replicated four and three times, respectively, in each treatment. In the shoot regeneration test, twenty callus clusters in a Petri dish were used as one replication and there were three replications in each treatment. Data were collected 30 days after inoculation, arcsine transformed, and subjected to ANOVA using SAS (SAS Institute, Cary, NC). Mean separation was conducted by Fisher's least significant difference (LSD) ($P = 0.05$).

Results

Callus induction from inflorescences and nodes in triploid bermudagrass

ANOVA showed that the inclusion of low concentrations of BA in the medium had the greatest overall effect on each genotype in inflorescence culture (TifSport—F value = 17.97, $P < 0.0001$; TifEagle—F value = 9.92, $P < 0.001$; Tift97-4—F value = 18.67, $P < 0.0001$). Neither 2,4-D concentration nor the interaction between 2,4-D and BA concentrations showed significant effects for either explant or genotype. For node cultures, the effect of BA concentration was significant for TifEagle (F value = 4.04, $P < 0.05$) but not for TifSport.

The highest percentage of callus induction from cultured inflorescences, 100% for TifEagle, 72.5% for TifSport and 50.0% for Tift97-4, occurred on MS medium with 1.5 mg l^{-1} 2,4-D and 0.01 mg l^{-1} BA, although these responses were not significantly different from those on several other media (Table 1). Callus could be induced from TifEagle inflorescences on every medium tested and the frequency reached 100% on four of the media. In node culture, high callus induction rates were obtained on medium with 1 mg l^{-1} 2,4-D and 0.01 mg l^{-1} BA, i.e., 68.3% for TifSport and 78.3% for TifEagle, but these were not significantly different from any of the other media. The Tift97-4 nodes turned brown on all media and no vigorous callus was observed.

Calluses were initiated from florets of inflorescences as a fluffy, white, soft and non-embryogenic type (Type I), (Fig. 1A). After 1–2 subcultures, some globular semi-transparent calluses (Type II) were observed among the fluffy calluses (Fig. 1B). These globular transparent calluses were selected under a dissecting microscope for subculture. Different types of calluses were observed during subculture. Type I calluses decreased in frequency as globular, sticky Type II calluses increased through selection. Sometimes the Type II calluses were wrapped in a "membrane" of collapsed cells (Fig. 1C). Compact embryogenic calluses, which were desirable for multiplication and regeneration, were first found among Type I callus (Fig. 1D). By selection and multiplication, yellowish transparent compact calluses (Type III) (Fig. 1E) and white opaque compact calluses (Type IV) (Fig. 1F) were obtained in TifSport and TifEagle after several subcultures. In Tift97-4, mostly Type I and Type II calluses were observed and the compact Type III or Type IV calluses were rarely seen.

Inflorescence culture in tetraploid bermudagrasses

Four tetraploid genotypes showed high callus induction frequencies on MS medium with 1.5 mg l^{-1}

Table 1 Effects of different growth regulator combinations on callus induction from inflorescences and nodes

Growth Regulators (mg l^{-1})		Callus induction frequency (%)*					
		Inflorescence			Node		
2,4-D	BA	TifSport	TifEagle	Tift97-4	TifSport	TifEagle	Tif97-4
1	0	33.8 ± 16.0 d†	92.5 ± 6.5 bc	23.8 ± 9.5 bc	53.3 ± 22.5 a	65.0 ± 13.2 a	0
1.5	0	28.8 ± 8.5 d	91.2 ± 10.3 c	21.8 ± 7.5 bc	51.7 ± 10.4 a	61.7 ± 16.1 a	0
2	0	35.0 ± 12.2 cd	90.0 ± 7.1 c	12.5 ± 5.0 c	55.0 ± 8.7 a	71.7 ± 5.8 a	0
1	0.01	61.25 ± 6.2 ab	100 a	46.3 ± 16.5 ab	68.3 ± 12.5 a	78.3 ± 7.6 a	0
1.5	0.01	72.5 ± 11.9 a	100 a	50.0 ± 14.7 a	63.3 ± 2.9 a	71.7 ± 16.1 a	0
2	0.01	52.5 ± 20.2 bc	97.8 ± 4.8 ab	37.5 ± 10.4 ab	63.3 ± 12.6 a	71.7 ± 5.8 a	0
1	0.02	40.5 ± 11.9 cb	100 a	26.3 ± 8.5 bc	61.7 ± 15.3 a	65.0 ± 15.0 a	0
1.5	0.02	45.0 ± 5.8 bcd	100 a	21.3 ± 8.5 bc	53.3 ± 5.8 a	71.7 ± 7.6 a	0
2	0.02	41.3 ± 8.5 cd	98.8 ± 7.1 ab	32.5 ± 8.7 ab	50.0 ± 13.2 a	65.0 ± 15.0 a	0

Each replicate dish contained 20 explants and each treatment consisted of three (node) or four (inflorescence) replicates. Basal medium was MS + 1.16 g l^{-1} proline + 30 g l^{-1} sucrose + 3 g l^{-1} Gelrite. Data were collected after 30 days of culture

* Callus induction frequency included all types of callus

† Data are means ± standard deviation. Different letters within columns showed a significant difference at $P = 0.05$ as determined by Fisher's LSD

2,4-D and 0.01 mg l^{-1} BA, ranging from a high of 93% for Tift93-132, 87% for Tift93-157, 64% for Tift93-156, to 54% for Tift93-135. Most calluses originated from florets. Transparent compact (Type III) and opaque compact calluses (Type IV) formed after several subcultures by carefully selecting the globular calluses for multiplication. Type I calluses were always accompanied by compact calluses. Both Type III and Type IV calluses were highly regenerative, particularly Type III callus which was highly embryogenic. Embryo germination was observed during callus subculture (Fig. 1G).

Modified gelling agent during subculture increased callus quality

Different gel compositions were tested for their effect on compact callus formation because the calluses from inflorescences and nodes were frequently watery and sticky in the initiation phase. There was a significant effect of gelling agent/concentration on compact callus formation (F value = 24.88, $P < 0.0001$). The water availabilities of medium with 3 g l^{-1} Gelrite or 8 g l^{-1} agar alone were not significantly different from one another, but these two media had a significantly higher water availability than the combination of 2 g l^{-1} Gelrite and 5 g l^{-1} agar or 16 g l^{-1} agar alone (Table 2). The medium with a combination of gelling agents and lower water availability supported the development of a higher number of Type III and Type IV calluses from TifSport, TifEagle and Tift93-132 (Table 2). Medium with 16 g l^{-1} agar had significantly lower water availability than all other media tested and also supported the formation of significantly fewer compact calluses from two of the three genotypes. Furthermore, calluses kept on medium with 16 g l^{-1} of agar for more than 60 days differentiated roots on the surface and these calluses lost their regeneration ability. This rooted callus could not revert into Type III or Type IV compact callus.

Shoot regeneration from embryogenic calluses

Different BA concentrations in MS + 0.1 mg l^{-1} 2,4-D were tested for shoot regeneration. Clusters (~5 mm in diameter) of Type III and Type IV calluses from all genotypes developed green spots on the surface; however, only TifEagle, TifSport and Tift93-132 developed shoots on almost all media containing BA (Table 3). Although the calluses were

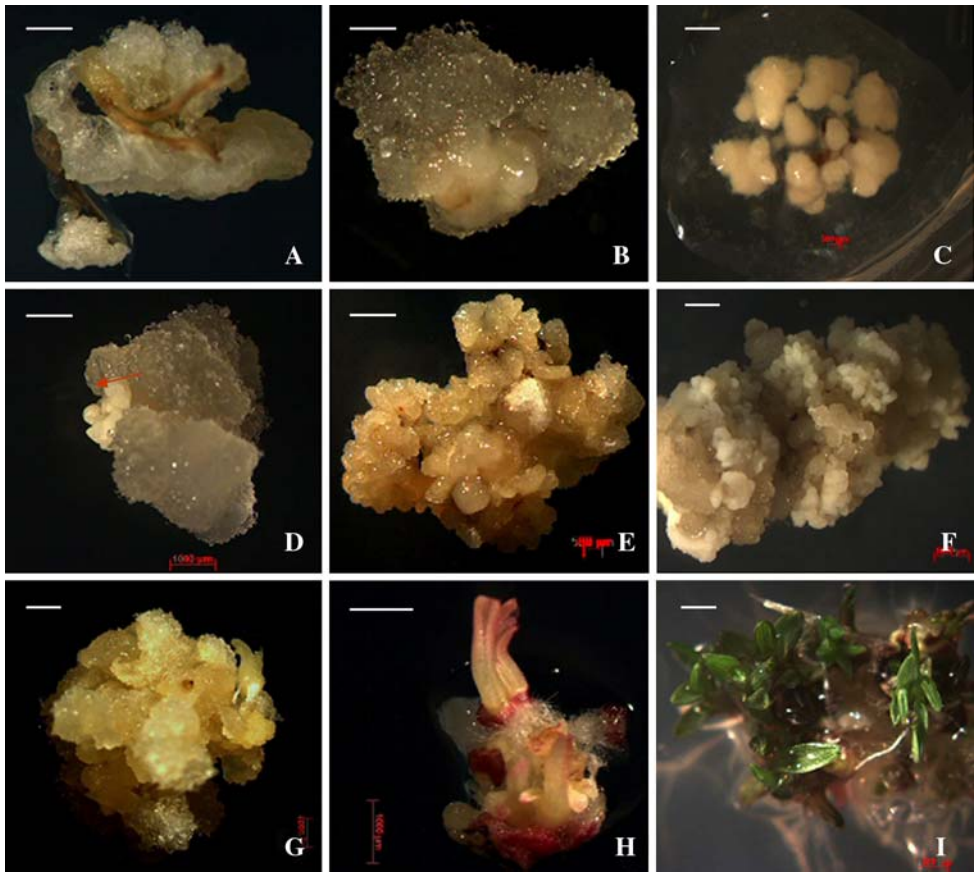

Fig. 1 Callus induction and shoot regeneration. **(A)** fluffy, white and soft callus (Type I); **(B)** globular sticky and transparent pre-embryogenic callus (Type II) embedded in fluffy callus; **(C)** globular sticky callus embedded in a watery membrane; **(D)** compact callus (red arrow) formed from the initial transparent watery callus; **(E)** transparent compact callus (Type III); **(F)** white compact callus (Type IV); **(G)** embryo germination during callus subculture in Tift93-132; **(H)** shoots with pigment without light treatment; **(I)** green shoot regeneration with light treatment. White bars represent 1 mm

Table 2 Effect of different gelling agent combinations on compact callus (Type III and Type IV together) frequency (%) after the second callus subculture

Medium	Water availability (mg)	Compact callus frequency (%) (No. compact calluses/total)			
		TifSport	TifEagle	Tift97-4	Tift93-132
Gelrite 3 g l^{-1}	653.1 ± 7.1 a*	2.5 ± 0.6 b (5/203)	9.6 ± 2.1 b (19/197)	0 (0/185)	8.3 ± 0.6 bc (13/156)
Gelrite 2 g l^{-1} + agar 5 g l^{-1}	629.1 ± 7.1 b	9.2 ± 1.4 a (20/218)	15.3 ± 3.1 a (31/203)	0 (0/155)	15.8 ± 3.5 a (19/120)
Agar 8 g l^{-1}	647.3 ± 9.4 a	2.5 ± 0.4 b (6/244)	6.7 ± 1.1 b (11/165)	0 (0/120)	7.2 ± 0.2 c (10/138)
Agar 16 g l^{-1}	597.6 ± 3.1 c	4.0 ± 1.5 b (8/198)	8.1 ± 1.4 b (17/211)	0 (0/145)	13.1 ± 2.3 ab (19/145)

The medium consisted of MS + 1.5 mg l^{-1} 2,4-D + 0.03 mg l^{-1} BA + 1.16 g l^{-1} proline + 30 g l^{-1} sucrose

* Data are means ± standard deviation. Values within columns with different letters were significantly different at $P = 0.05$

globular and compact in Tift93-156, roots instead of shoots grew in all treatments. Once the roots differentiated, no shoots were induced. Shoot regeneration from Tift97-4 was unsuccessful regardless of medium tested. Type III or Type IV calluses were not found in Tift97-4 cultures, and although there were some

shoot apices in the sticky callus of Tift97-4, they failed to regenerate into shoots.

The concentration of BA had a significant effect on green spot formation in TifSport (F value = 4.55, $P = 0.02$) and TifEagle (F value = 12.08, $P = 0.0006$) but not in Tift93-132 where 100% of the calluses on all media formed green spots. For shoot formation, the concentration of BA had low significance for TifSport (F value = 3.02, $P = 0.06$) but was highly significant for TifEagle (F value = 31.17, $P < 0.0001$) and Tift93-132 (F value = 12.34, $P = 0.0002$). The combination of 0.1 mg l^{-1} 2,4-D and 0.5 mg l^{-1} BA showed the highest percentage of shoot regeneration for Tift93-132, whereas for TifEagle, 0.1 mg l^{-1} 2,4-D + 2–4 mg l^{-1} BA gave the best shoot regeneration (Table 3). Extensive media modifications did not improve shoot regeneration frequency in TifSport (see below).

TifEagle showed a higher capacity for embryogenesis than TifSport among the triploid bermudagrasses, whereas a third triploid Tift97-4 showed no embryogenesis or shoot regeneration. Among the tetraploids, Tift93-132 and Tift93-156 showed high embryogenic callus induction, but only Tift93-132 showed shoot regeneration. Calluses from Tift93-156 readily differentiated only roots during subcultures. The roots probably inhibited shoot differentiation.

Stimulating shoot regeneration in the recalcitrant TifSport

In TifSport, there were three responses to regeneration medium, green spots on the callus surface, pink shoots (Fig. 1H) and green shoots (Fig. 1I). Green spots were easy to see on the callus surface but failed to develop into green shoots. The pink shoot apices grew into albino shoots in most cases. Almost all TifSport calluses turned brown under light and turned the medium brownish. In order to increase shoot regeneration frequency, various media modifications were tested, including using 0–1 mg l^{-1} NAA instead of 2,4-D and 0.5–4 mg l^{-1} TDZ instead of BA, as well as adding 0.25–1.0 mg l^{-1} ABA, 0.5 mg l^{-1} GA_3 or casein hydrolysate. However, no significant effects were observed from these modifications (data not shown). In order to reduce browning, the antioxidant polyvinylpolypyrrolidone (PVPP), the ethylene inhibitor $AgNO_3$ and activated charcoal were tested but were ineffective for TifSport (data not shown).

The only treatment that had a visible effect was light. The brownish necrotic response was greatly reduced in the Type III and Type IV calluses pretreated under low light intensity (30 μmol m^{-2} s^{-1}) for 10 days before transfer to regeneration medium. The results showed that there were significant increases in shoot regeneration in the light-treated callus compared with non-light-treated callus (Fig. 2). There also was a significant interaction between environment and BA (F value = 19.45, $P < 0.0001$). The shoot regeneration frequency for TifSport reached the highest point of 40% in the light-treated callus on medium with 0.1 mg l^{-1} 2,4-D and 2 mg l^{-1} BA. Essentially 100% of the regenerated shoots rooted well on rooting medium.

Table 3 Shoot regeneration of TifSport, TifEagle, Tift93-132

BA (mg l^{-1})	Callus with green spots (%)			Shoot regeneration frequency (%)		
	TifSport	TifEagle	Tift93-132	TifSport	TifEagle	Tift93-132
0.5	25.0 ± 10.0 a*	46.7 ± 15.3 cd	100 a	1.7 ± 2.9 ab	8.3 ± 5.8 bc	73.3 ± 12.6 a
1	21.7 ± 15.2 ab	63.3 ± 15.3 bc	100 a	0 b	21.7 ± 11.5 b	55.0 ± 13.2 b
2	5.0 ± 5.0 c	80.0 ± 8.7 ab	100 a	5.0 ± 5.0 ab	43.3 ± 17.6 a	33.3 ± 7.6 cd
3	3.3 ± 5.8 c	80.0 ± 8.7 ab	100 a	6.7 ± 2.9 a	56.7 ± 2.9 a	28.3 ± 5.8 de
4	0 c	81.7 ± 2.9 a	100 a	3.3 ± 2.9 ab	55.0 ± 13.2 a	11.7 ± 7.6 e
CK (MS0)	8.3 ± 7.6 bc	30.0 ± 5.0 d	100 a	0 b	0 c	50.0 ± 10.0 bc

The medium consisted of MS + 0.1 mg l^{-1} 2,4-D + 0.5–4 mg l^{-1} BA + 1.16 g l^{-1} proline + 30 g l^{-1} sucrose + 3 g l^{-1} Gelrite. The experimental unit consisted of a Petri dish containing 20 calluses, replicated three times. Data were collected after 30 days of culture

* Data are means ± standard deviation. Values within columns with different letters were significantly different at $P = 0.05$

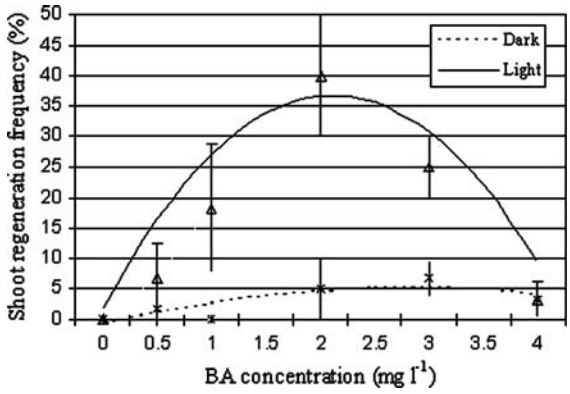

Fig. 2 Effects of light and BA on shoot regeneration in TifSport (Quadratic relationships between shoot regeneration frequency and BA were: Dark: $y = -1.1585 + 4.0595x—0.7037x^2$; Light: $y = -1.0798 + 32.8083x—7.73x^2$

Discussion

Explants for bermudagrass tissue culture

Young inflorescences are good explants to use for establishing tissue cultures in bermudagrass (Li and Qu 2004; Goldman et al. 2004ab). Embryogenic callus was induced from inflorescences of both triploid and tetraploid bermudagrass genotypes in this research. Due to the limited flowering season and young inflorescence availability in triploids, nodes are a possible alternative for inflorescences. However, only TifEagle showed good regeneration ability from node-derived embryogenic callus in this experiment and in other reports (Goldman et al. 2004ab; Hu et al. 2005). No regeneration was observed on node-derived callus in TifSport and no vigorous callus was induced from Tift97-4 nodes. Additional research is needed to increase the node-derived callus and the regeneration ability in these bermudagrass genotypes.

Effect of light on regeneration

Light is very important in plant growth. Plants use light not only as an energy source for photosynthesis but also as an important environmental signal (Liu et al. 2006). During plant callus induction and subculture, a dark environment normally is applied. In the present study, light appears to play an important role in the regulation of morphogenic potential in bermudagrass callus. In contrast to the typical effect of light, to induce the production of phenolic compounds (Weisshaar and Jenkins 1998), the light-treated TifSport callus produced less brown necrotic callus. The reason why light could reduce necrosis and increase shoot regeneration in TifSport is unknown.

Bermudagrass callus lost embryogenesis and regeneration ability after several months in culture. Selecting good quality callus, as Li et al. (2005) described, is one way to maintain embryogenesis and regeneration ability. In this study, a short period (~30 days) of exposure under light renewed the embryogenic potential of TifSport callus (data not shown).

Albinism is common in tissue cultures of some monocotyledonous plants, such as barley (Ziauddin and Kasha 1990; Bregitzer and Campbell 2001; Sharma et al. 2005), tall fescue (Bai and Qu 2000) and dune and swamp reed (Cui et al. 2002). Albinism may be a predictor for loss of embryogenic potential after long periods of culture and high levels of somaclonal variation (Ziauddin and Kasha 1990). Albinism appears to be genotype-dependent in bermudagrass. Albino shoot regeneration was a major problem in TifSport but was not encountered in TifEagle or Tift93-132. Bregitzer and Campbell (2001) reported genetic markers (QTLs) associated with green and albino plant regeneration in barley. His predictive model suggested that environmental factors played a relatively greater role in the regeneration of green plants than did genetic factors. In the present report, light treatment to callus increased green shoot regeneration in TifSport, but albino shoots always accompanied green shoots, grew faster, and to some degree, inhibited green shoot elongation. Additional studies are needed on ways to decrease albinism in TifSport.

Conclusions

Four types of calluses were observed and classified in our bermudagrass tissue culture research. Type I callus was the common response to in vitro culture of inflorescence and nodes in both triploid and tetraploid bermudagrass. By carefully selecting Type III and Type IV calluses, embryogenic compact callus lines were established. The combination of 2 g l^{-1} Gelrite and 5 g l^{-1} agar in the callus subculture medium

helped to induce more compact callus. If subculture conditions were not optimum, the compact embryogenic calluses reverted to non-embryogenic Type I and Type II calluses.

Light was first reported here to have an important role in regeneration of the recalcitrant TifSport. Treatment of the callus with low intensity light for 10 days before transfer to regeneration medium increased the plant regeneration frequency by 6–8-fold.

Acknowledgements We thank Evelyn Morgan and Judith Anne Bell for laboratory help; Larry Baldree, Amanda Webb and Jesse Harrison for greenhouse and field assistance; and Benjamin G. Mullinix for statistical analysis.

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