In Vitro Cell. Dev. Biol.—Plant 41:274–280, May–June 2005
DOI: 10.1079/IVP2005638

1054-5476/05 $18.00+0.00

TISSUE CULTURE AND PLANT REGENERATION OF THE SALT MARSH MONOCOTS *JUNCUS ROEMERIANUS* AND *JUNCUS GERARDI*

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(Received 5 January 2004; accepted 23 December 2004; editor P. Lakshmanan)

Summary

Tissue culture and plant regeneration protocols for the salt marsh plants *Juncus roemerianus* Scheele and *Juncus gerardi* Loisel. were developed. *J. roemerianus* callus was induced from mature seeds cultured on Murashige and Skoog (MS) medium supplemented with 2.22 μM 6-benzylaminopurine (BA), 5.37 μM α-naphthaleneacetic acid (NAA), 2.26 μM 2,4-dichlorophenoxyacetic acid (2,4-D), and 50 ml l^{-1} coconut water (callus induction medium). The callus was subcultured on MS medium containing 2.22 μM BA, 5.37 μM NAA, and 9.05 μM 2,4-D for callus maintenance. Shoot regeneration occurred 2 wk after transferring the callus onto shoot regeneration medium, which consisted of MS medium containing BA or thidiazuron. A high frequency of shoot regeneration was obtained when the medium contained 13.3 μM BA. Regenerated shoots were transferred to MS medium supplemented with 10.7 μM NAA for root production. Rooting did not occur in the shoots regenerated on the thidiazuron-containing media. The callus induction medium for *J. roemerianus* was also effective in inducing callus of *J. gerardi* from young inflorescences. The same medium was also used for callus maintenance. Shoot regeneration occurred 10 d after transferring the callus onto MS medium supplemented with 0.44 μM BA and 0.57 μM indole-3-acetic acid. Root regeneration occurred after transferring the shoots onto MS medium plus 0.44 μM BA and 14.8 μM indole-3-butyric acid. The regenerated plants of both *J. roemerianus* and *J. gerardi* grew vigorously in potting soil in the greenhouse. *J. roemerianus* regenerants also grew well in a saltwater-irrigated field plot. Tissue culture-produced plants of *J. roemerianus* and *J. gerardi* can be used for planting in created or restored wetlands.

Key words: black needle rush; blackgrass; organogenesis; restoration; salt marsh.

Introduction

Juncus roemerianus Scheele, black needle rush, is one of the dominant species in salt marshes along the south Atlantic and Gulf coasts of the USA (Eleuterius, 1984). *J. roemerianus* is also a component of salt marshes in Delaware, where it reaches its northward limit (Eleuterius, 1976a). In addition to occurring in patches among other marsh species, monospecific stands of this species often occur as large bands above the *Spartina alterniflora* Loisel. zone of the low marsh. *J. roemerianus* has wide environmental tolerances (Eleuterius, 1984) and adapts to a broad range of physical and chemical habitats (Woerner and Hackney, 1997). Shoot production occurs throughout the year and dead shoots remain standing for a long time, resulting in dense assemblages of shoots with a high biomass (Eleuterius and Caldwell, 1981; Christian et al., 1990). The aerial production of *J. roemerianus* in a Georgia salt marsh is estimated to be 2200 g m^{-2} yr^{-1} (Gallagher et al., 1980).

Juncus gerardi Loisel., blackgrass, is found in salt marshes along the North Atlantic coast in New England and Canada, and is common in the mid-Atlantic region (Reimold, 1977). *J. gerardi* mostly occurs in patches in high marshes along the terrestrial marsh borders (Nixon, 1982). It tolerates harsh physical stress and can ameliorate soil salinity, thereby facilitating the settlement of other plants (Bertness and Hacker, 1994). Its shoot production mostly occurs in spring and summer. The aerial production of *J. gerardi* is estimated to be 616 g m^{-2} yr^{-1} in a Maine salt marsh (Linthurst and Reimold, 1978). The ecological importance of *J. roemerianus* and *J. gerardi* is particularly manifested by their substantial primary production, their value as wildlife habitat (Stout, 1984; Nixon, 1982), and their contribution to shoreline stabilization.

Juncus roemerianus and *J. gerardi* propagate mainly through extensive rhizome growth (Eleuterius, 1976b; Bouzille et al., 1997). Seed germination and seedling establishment are vulnerable to environmental stress and are therefore very limited (Eleuterius, 1984; Shumway and Bertness, 1992). Large numbers of macrophytes are often required for wetland creation and restoration efforts. However, collecting plants from natural marshes may be ecologically damaging and is usually controlled by state regulations. *In vitro* tissue culture offers an alternative for efficiently propagating plants in an environmentally benign way. In addition, the application of somaclonal variation via an established tissue culture system makes it possible to produce wetland plants with characteristics not readily found in wild populations that may be useful for marsh creation and restoration as shown in the field studies by Seliskar (1998) and Seliskar and Gallagher (2000). Tissue culture techniques have been developed in other *Juncus*

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spp., *J. accuminatus* Michaux (Sarma and Rogers, 1998) and *J. effusus* L. (Sarma and Rogers, 2000), as well as other marsh species, including *Sporobolus virginicus* (L.) Kunth (Straub et al., 1992), *Distichlis spicata* (L.) Greene (Straub et al., 1989), *Typha latifolia* L. (Rogers et al., 1998), *Typha angustifolia* L. (Rogers, 2003), *Phragmites australis* (Cav.) Trin. ex. Steud. (Straub et al., 1988), *Carex lurida* Wahlenb. (Rogers, 2003), *Scirpus polyphyllus* Vahl (Rogers, 2003), *Scirpus robustus* Pursh (Wang et al., 2004), *Kosteletzkya virginica* (L.) Presl. (Cook et al., 1989), and *Spartina* spp., *S. patens* (Aiton) Muhl. (Li et al., 1995), *S. cynosuroides* (L.) Ruth (Li and Gallagher, 1996), and *S. alterniflora* Loisel. (Wang et al., 2003). Here we describe *in vitro* plant regeneration protocols for *J. roemerianus* and *J. gerardi*.

Materials and Methods

Mature inflorescences of *Juncus roemerianus*, collected from the marsh at Assawoman Wildlife Area in Delaware in October, were stored at 4°C in a refrigerator for 9 mo. Seeds were carefully picked after breaking the husk of the capsular fruits of each inflorescence. The seeds were pooled and then selected randomly for the tissue culture studies. These small oval seeds were about 0.7 mm long. They were disinfected by shaking in a solution of 20% commercial bleach (containing 5.25% sodium hypochlorite by weight) plus 10% ethanol for 20 min. The bleach–ethanol solution with seeds was then poured through a sterilized metal sieve, following which the seeds were rinsed five times with sterile water. Six to seven seeds were then placed in each Petri dish on gelled MS-based medium (Murashige and Skoog, 1962; GibcoBRL, UK) for callus induction. Four media formulations were tested; MS plus 2.22 μM 6-benzylaminopurine (BA), 5.37 μM α-naphthaleneacetic acid (NAA), and 2.26 μM 2,4-dichlorophenoxyacetic acid (2,4-D), with or without the addition of 50 ml l^{-1} coconut water (from mature coconuts purchased at a local grocery store), MS plus 9.05 μM 2,4-D, and MS plus 5.71 μM indole-3-acetic acid (IAA) and 2.26 μM 2,4-D. These media were modifications of callus induction media used in another salt marsh monocot, *Spartina patens* (Li et al., 1995). In addition, MS medium without growth regulators (MS0 medium) was used as a control. Seeds were placed on at least three Petri dishes of each medium. All media in this study, including those for the tissue culture of *J. gerardi*, contained 3% sucrose and were adjusted to pH 5.7 prior to autoclaving at 20 psi (1.4 kg cm^{-2}) for 20 min. Gel-Gro (ICN Biomedicals, Aurora, OH) at a concentration of 0.28% was used to solidify the media. Cultures were grown in 60 mm × 15 mm Petri dishes (15 ml medium per dish), under 30 μE m^{-2} s^{-1} light (provided by Philips cool white fluorescent tube lights) with a 12 h light period at a temperature of 25°C.

Five weeks after placing the seeds on callus induction media, calluses produced were maintained on (1) medium with the same formulation as that used for successful callus induction (MS medium supplemented with 2.22 μM BA, 5.37 μM NAA, 2.26 μM 2,4-D, and 50 ml l^{-1} coconut water), or (2) MS medium containing 9.05 μM 2,4-D, 5.37 μM NAA, and 2.22 μM BA. Globular callus, that was yellow and compact in appearance, was selected for subculture at 5-wk intervals. After three subcultures, the globular callus was transferred onto MS0 medium or MS with different concentrations of BA or thidiazuron (TDZ) for shoot regeneration (Table 1). Seven to nine calluses (about 0.5 cm in diameter) were cultured in each of at least three Petri dishes of each medium. Six weeks after transferring the callus onto shoot regeneration medium, data on the percentage of callus forming shoots and number of shoots per regenerating callus (callus that produced shoots) were collected. Subsequently, regenerated shoots (in the form of clusters of three to 12 entangled shoots approximately 1–3 cm in length) from the medium giving the most successful regeneration rate were transferred onto root regeneration media, the formulations of which are shown in Table 2. Four or five clusters of shoots were transferred onto each of at least three Petri dishes of each root regeneration medium. The percentage of shoot clusters with roots and the number of roots per shoot on each cluster with roots were scored 6 wk after transferring the shoot clusters onto root regeneration medium. From shoot regeneration to root regeneration, all experiments were repeated at least three times. Forty-two regenerated plants were then carefully separated and planted in pots (17 cm in diameter) containing potting soil (Promix®) in the tissue culture room for 1 mo. The plants were subsequently moved into the greenhouse for further growth. The natural light in the greenhouse was supplemented with light provided by metal halide lamps (Voigt Lighting, PA; 254 μE m^{-2} s^{-1}). Seven months later, the root and soil mats of five regenerated plants were cut into four even sections and planted into 17-cm diameter pots. After another 10 mo. in the greenhouse, the five plants, each with three replicates, were planted in a field plot in Lewes, DE, in late June 2003 (one pot was maintained as stock in the greenhouse). Wild plants of *J. roemerianus* were dug from the natural marsh where the inflorescences for this study were collected. These plants with associated soil clumps were planted in the same size pots used for planting regenerated plants. After 1 wk in the greenhouse, they were planted in triplicate in the field plot at the same time that the regenerated plants were planted. The plot was flood-irrigated with 10 ppt (parts per thousand, 10 g salt l^{-1}, measured by refractometer) salt water twice a week. Four months later, the above-ground shoots of the *J. roemerianus* plants were harvested. The maximum plant height and circumference of the shoot clump were measured after the planting in June and again just prior to the harvest in October. Plant aerial biomass and shoot density were quantified after the harvest. Lab tissue culture data and plant field growth data were analyzed by one-way ANOVA and Fisher's least significant difference test, using SPSS software. A paired sample *t*-test was used to compare field data collected in June and October.

For *Juncus gerardi* tissue culture, early-stage inflorescences (4–6 d after emergence) were used for callus induction. The inflorescences were collected from the marsh at Greenhill Light near Lewes, DE in May. The inflorescences were soaked for 20 min in the disinfection solution containing 20% bleach plus 10% ethanol, followed by five rinses with sterile water. The inflorescences were then placed on MS medium containing 2.22 μM BA, 5.37 μM NAA, 2.26 μM 2,4-D, and 50 ml l^{-1} coconut water, which was

TABLE 1

REGENERATION OF *JUNCUS ROEMERIANUS* SHOOTS FROM CALLUS GROWN FOR 2 wk ON MEDIA WITH BA OR TDZ

Medium	Percentage of calluses forming shoots	Mean number of shoots per regenerating callus
MS0	0 a	0 a
MS + 2.22 μM BA	29 b	3.2 b
MS + 13.3 μM BA	74 c	7.9 c
MS + 2.27 μM TDZ	36 b	7.2 c
MS + 13.6 μM TDZ	69 c	7.8 c

MS0, MS medium without growth regulator.

Means within *columns* followed by the same *letter* are not significantly different at the 5% level using one-way ANOVA and Fisher's least significant difference test.

TABLE 2

ROOT REGENERATION OF *JUNCUS ROEMERIANUS* FROM SHOOT CLUSTERS GROWN FOR 4 wk ON MEDIA WITH DIFFERENT AUXINS

Medium	Percentage of shoot clusters forming roots	Mean number of roots per shoot on the root-regenerating shoot cluster
MS0	0 a	0 a
MS + 11.4 μM IAA	53 b	0.5 b
MS + 9.84 μM IBA	57 b	0.6 b
MS + 10.7 μM NAA	96 c	2.9 c

MS0, MS medium without growth regulator.

Means within *columns* followed by the same *letter* are not significantly different at the 5% level using one-way ANOVA and Fisher's least significant difference test.

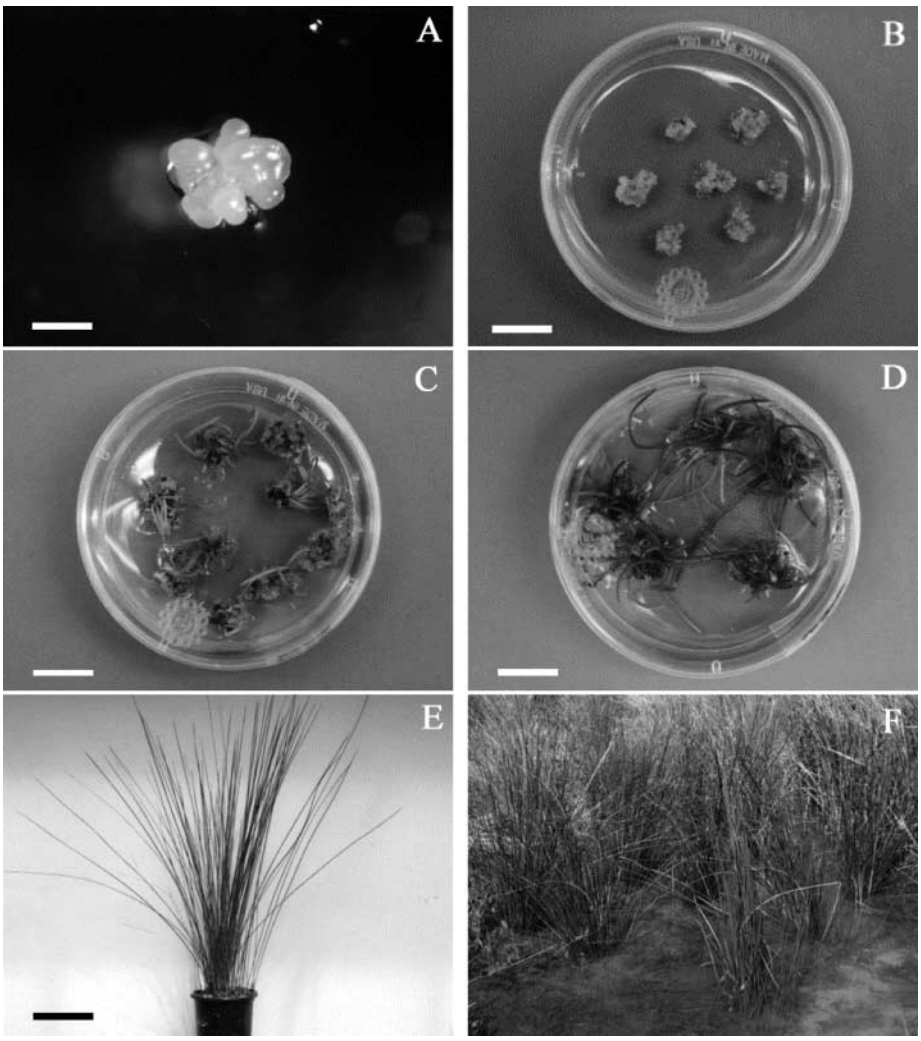

FIG. 1. *A*, Induced callus of *Juncus roemerianus* (2 wk after placing the seed on callus induction medium) (*bar* = 1.4 mm). *B*, Maintained callus (4 wk after the third transfer of callus onto maintenance medium) (*bar* = 11.3 mm). *C*, Regenerated shoots (4 wk after transferring the callus onto shoot regeneration medium) (*bar* = 11.5 mm). *D*, Regenerated whole plants (1 mo. after transferring the shoots onto root regeneration medium) (*bar* = 11.4 mm). *E*, Regenerated plants growing in soil in the greenhouse for 7 mo. (just before repotting) (*bar* = 173.9 mm). *F*, Regenerated plants growing in a field plot for 4 mo.

successfully used as callus induction medium for *J. roemerianus*. Four weeks later, the calluses produced were transferred onto the same medium for callus maintenance, and subcultured three times at 3-wk intervals. For shoot regeneration, the callus was transferred onto MS medium plus 13.3 μM BA and MS plus 0.44 μM BA and 0.57 μM IAA. After 4 wk, the regenerated shoots were transferred onto MS medium plus 10.7 μM NAA and MS plus 0.44 μM BA and 14.8 μM indole-3-butyric acid (IBA), for root regeneration. MS0 medium was used as the control. Four weeks later, the regenerated plants were planted in potting soil in the tissue culture room for 1 mo. and subsequently moved into the greenhouse for further growth. The protocol for successful regeneration of *J. gerardi* was repeated three times, but detailed growth data were not collected for this species.

RESULTS

About half of the *Juncus roemerianus* inflorescences produced seeds but none produced more than five seeds. On average, two seeds were collected per inflorescence. Pooled data of three experiments showed that 26% of seeds (one out of six, two out of six, and two out of seven seeds, respectively) produced callus after 1 wk of culturing on MS medium supplemented with 2.22 μM BA, 5.37 μM NAA, 2.26 μM 2,4-D, and 50 ml l^{-1} coconut water (callus induction medium). Calluses formed were yellow, globular, and compact (Fig. 1*A*), and one callus clump was obtained per seed. On the other media tested, no seed germination or callus induction was observed.

The induced callus grew slowly to about 8–10 mm in diameter after 5 wk of culture. The calluses were then separated into 2–3 mm pieces for subculture. They became desiccated when subcultured on callus induction medium, but grew slowly and retained the yellow, globular, and compact appearance on MS medium containing 2.22 μM BA, 5.37 μM NAA, and 9.05 μM 2,4-D (Fig. 1*B*). Shoot regeneration of *J. roemerianus* occurred 2 wk after transferring the callus onto shoot regeneration media containing BA

or TDZ (Table 1). MS medium with either 13.3 μM BA or 13.6 μM TDZ was effective in inducing shoot regeneration, with 69–74% of the calluses forming shoots. With these media the number of shoots per regenerating callus was greater than seven (Fig. 1*C*). Both shoot regeneration frequency and regenerated shoot number increased with the increase in BA concentration from 2.22 to 13.3 μM. An increased TDZ concentration from 2.27 to 13.6 μM resulted in increased shoot regeneration frequency although it did not significantly affect the number of shoots produced per regenerating callus (Table 1). The callus in our culture has maintained consistent regeneration ability for 3 yr.

The regenerated shoots of *J. roemerianus* were entangled in clusters on both the BA- and TDZ-supplemented media. One cluster of three to 12 shoots from each callus clump was commonly observed. The shoot clusters were carefully removed from the callus, and moved onto root regeneration medium (Table 2). Root production occurred in 1 wk on MS media containing either 11.4 μM of IAA, 9.84 μM IBA, or 10.7 μM NAA. These media were effective in stimulating root regeneration of shoots regenerated with BA, but not with TDZ. Significantly greater root regeneration frequency (96%), as well as the greatest number of roots per shoot (about three), were achieved on the MS medium containing 10.7 μM NAA (Fig. 1*D*). No roots were induced on the MS0 medium. One to three whole plants were recovered per shoot clump. The most effective protocol for tissue culture and plant regeneration of *J. roemerianus* is summarized in Table 3.

Forty-one regenerated plants (98%) transferred to potting soil survived in the tissue culture room and all of these grew vigorously in the greenhouse (Fig. 1*E*). In the field plot, all the regenerated plants survived and vigorous plant growth was observed 4 mo. after planting (Fig. 1*F*). This was demonstrated by the spread of the plants from an average circumference of 29.2 cm in June to 75.9 cm in October ($\alpha = 0.05$). Maximum plant height was 108.5 cm in June and 106.7 in October, exhibiting no significant difference ($\alpha = 0.05$). No significant difference was found among the five regenerants in circumference or maximum height in either June or October. In October, mean plant biomass and shoot density of the five regenerants were 2051.8 g dry weight m^{-2} and 4301 shoots m^{-2}, respectively, but no significant differences were found among them for either character ($\alpha = 0.05$). The wild plants showed few green shoots at the time they were initially dug from the natural marsh, during the week in the greenhouse, and during the 4-mo. period in the field plot.

Callus initiation in *J. gerardi* inflorescences occurred after 1 wk of culture on MS medium supplemented with 2.22 μM BA, 5.37 μM NAA, 2.26 μM 2,4-D, and 50 ml l^{-1} coconut water (Fig. 2*A*). The callus produced was yellow, globular, and compact, and maintained the same appearance during the subcultures on the same medium. Shoot regeneration occurred after 10 d of culturing the callus on MS medium supplemented with 0.44 μM BA and 0.57 μM IAA (Fig. 2*B*). The callus desiccated and blackened after shoot regeneration. Regenerated shoots were then transferred onto MS plus 0.44 μM BA and 14.8 μM IBA, and root regeneration occurred within 1 wk after the transfer (Fig. 2*C*). Interestingly, MS plus 13.3 μM BA and MS plus 10.7 μM NAA, the two media that resulted in the highest level of shoot and root regeneration, respectively, for *J. roemerianus*, were not effective in inducing shoot and root regeneration of *J. gerardi*. The regenerated plants grew vigorously in the greenhouse (Fig. 2*D*). The protocol for callus culture and plant regeneration of *J. gerardi* is also summarized in Table 3.

Discussion

The *J. roemerianus* in our collection produced very few seeds. On average, only two seeds were collected per inflorescence, which usually contains 50–100 capsular fruits. The husk of most of the capsular fruits was not broken at the time of inflorescence collection; therefore, the seed loss to the field was limited. *J. roemerianus* is a gynodioecious plant, and it is composed of two plant forms based upon corresponding flower morphology: the pistillate (unisexual) flower and the perfect (bisexual) flower (Eleuterius, 1975). Because only the fertilized pistillate and the perfect flowers produce seeds, the occurrence of few seeds in our collection suggests that pollen (only produced by perfect flowers) may be in short supply at our collection site. The *J. roemerianus* population at our collection site is apparently dominated by unisexual plants with very few bisexual plants. Eleuterius (1975) also described that both plant forms of *J. roemerianus* occur in salt marshes along the Atlantic and Gulf Coasts, with large tracts dominated by the unisexual form, which reproduces primarily through vegetative growth.

J. roemerianus callus was obtained from mature seeds. Mature seeds have been used for callus induction in other monocots, including the wetland species *Scirpus robustus* (Wang et al., 2004) and *Distichlis spicata* (Straub et al., 1989). The type of explant is a

TABLE 3

PROTOCOLS FOR CALLUS CULTURE AND PLANT REGENERATION OF *JUNCUS ROEMERIANUS* AND *J. GERARDI*

Protocol	*Juncus roemerianus*	*Juncus gerardi*
PM	Mature seeds	Young inflorescences (4–6 d after emergence)
CI	MS + 2.22 μM BA + 5.37 μM NAA + 2.26 μM 2,4-D + 50 ml l^{-1} coconut water	MS + 2.22 μM BA + 5.37 μM NAA + 2.26 μM 2,4-D + 50 ml l^{-1} coconut water
CM	MS + 2.22 μM BA + 5.37 μM NAA + 9.05 μM 2,4-D	MS + 2.22 μM BA + 5.37 μM NAA + 2.26 μM 2,4-D + 50 ml l^{-1} coconut water
SR	MS + 13.3 μM BA	MS + 0.44 μM BA + 0.57 μM IAA
RR	MS + 10.7 μM NAA	MS + 0.44 μM BA + 14.8 μM IBA

MS-based media were used. PM, plant material for callus induction; CI, callus induction medium; CM, callus maintenance medium; SR, shoot regeneration medium; RR, root regeneration medium.

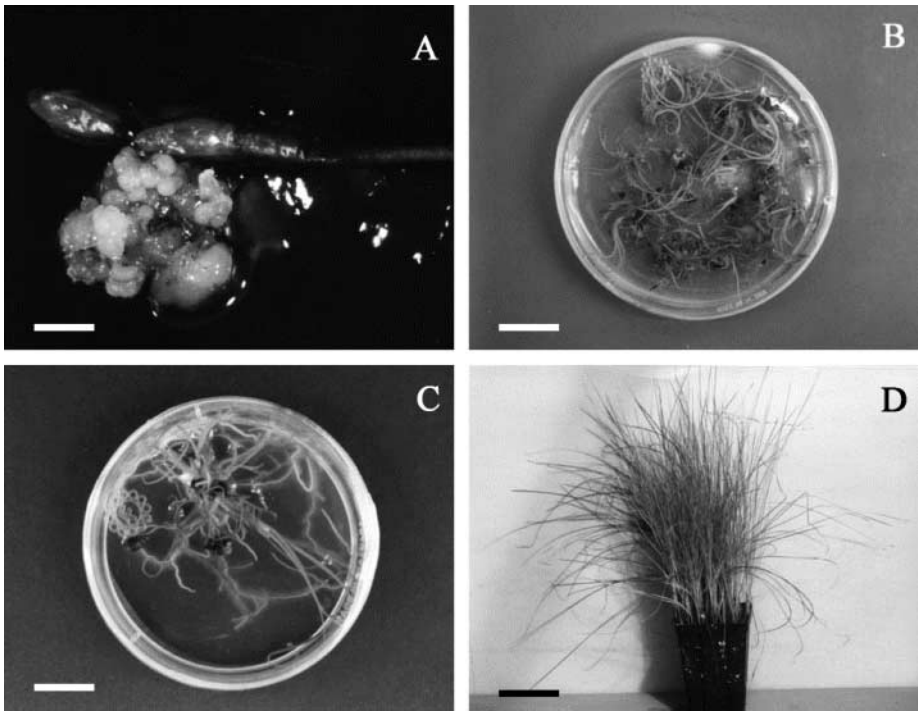

FIG. 2. *A*, Induced callus of *Juncus gerardi* 3 wk after placing the inflorescence (collected 6 d after emergence) onto callus induction medium (*bar* = 1.1 mm). *B*, Regenerated shoots (4 wk after transferring the callus onto shoot regeneration medium) (*bar* = 12.5 mm). *C*, Regenerated whole plants (4 wk after transferring the shoots onto root regeneration medium) (*bar* = 12.2 mm). *D*, Regenerated plants growing in soil in the greenhouse for 7 mo. (*bar* = 75 mm).

critical factor and can determine the capacity to induce regenerable callus. In addition to mature seed, various explants have been used for callus induction of other wetland monocots, such as immature inflorescences for *Sporobolus virginicus* (Straub et al., 1992) and *Phragmites australis* (Lauzer et al., 2000), and young seedlings for *Typha latifolia* (Rogers et al., 1998), *Spartina patens* (Li et al., 1995), *S. cynosuroides* (Li and Gallagher, 1996), and *S. alterniflora* (Wang et al., 2003), as well as *Juncus accuminatus* (Sarma and Rogers, 1998). In the tissue culture of another *Juncus* species, *J. effusus*, seedling explants were used as initial material and direct shoot regeneration was obtained without the callus stage (Sarma and Rogers, 2000).

The callus induction medium contained 50 ml l^{-1} coconut water. Coconut water contains cytokinins such as 9-β-D-ribofuranosylzeatin (Letham, 1974), zeatin, and zeatin ribosides (van Staden and Drewes, 1975). The biologically active compounds in coconut water have been proven to be effective in inducing callus production and plant regeneration of many plant species (Al-Khayri et al., 1992; Varshney et al., 1997). In the case of salt marsh plants, coconut water was used in the medium for the embryogenic callus induction of *Sporobolus virginicus* (Straub et al., 1992) and the callus maintenance of *Spartina patens* (Li et al., 1995). As with these salt marsh monocots, coconut water is required for the induction of morphogenic callus in *J. roemerianus* and *J. gerardi*.

Both BA and TDZ can induce shoot regeneration of *J. roemerianus*. However, only those shoots regenerated from BA-containing medium developed roots on the root induction medium, indicating that TDZ could be inhibitory to root regeneration in *J. roemerianus*. A similar result was reported for the tissue culture of *Spartina alterniflora* (Wang et al., 2003), but the exact reason for this observation is not clear. In a study of wheat and barley tissue culture, TDZ was found to be effective in inducing shoot regeneration directly from the induced callus after placing the immature embryos on the callus induction medium for 4 wk, and interestingly, it was not inhibitory to root regeneration (Shan et al., 2000). Seeds and segments of young seedlings were used to induce callus of *J. roemerianus* and *S. alterniflora*, respectively, and the calluses of both species were cultured for more than 15 wk before shoot induction. Differences in callus-initiating explants and callus culture time may have impacts on root regeneration from the shoots induced on TDZ-containing medium.

In this study, the tissue culture protocol of *J. roemerianus* was tested for *J. gerardi*. The callus induction medium for *J. roemerianus* was also effective for *J. gerardi*, although young inflorescences were used as the explant. However, different media were needed for *J. gerardi* callus maintenance, shoot regeneration, and root regeneration than for that of *J. roemerianus*, indicating species-specific requirements in tissue culture (Table 3). In the tissue culture of another *Juncus* spp., *J. accuminatus*, MS medium plus 20.7 μM picloram was used for callus induction and maintenance, MS plus 22.2 μM BA for shoot regeneration, and MS plus 0.54 μM NAA for root regeneration (Sarma and Rogers, 1998), indicating some similarities to *J. roemerianus* tissue culture, which also used BA and NAA for shoot and root regeneration. Plant regeneration in *J. gerardi* and *J. roemerianus* occurred via organogenesis, while inducing callus from seeds or inflorescences results in somatic embryogenesis in some other marsh species, such as *Scirpus robustus* (Wang et al., 2004) and *Distichlis spicata* (Straub et al.,

1989) (seeds), and *Sporobolus virginicus* (Straub et al., 1992) and *Phragmites australis* (Lauzer et al., 2000) (inflorescences).

The establishment of appropriate vegetation is a primary goal in wetland creation and restoration (Sullivan, 2001), which requires large amounts of planting material. Seeds, young plants grown from seed, and plugs containing roots and rhizomes, usually collected with sediment or mulch from natural populations, are the primary plant materials used for wetland restoration. However, collecting seeds and plugs can be ecologically damaging to natural wetlands and the introduction of undesirable species into restoration sites is a major concern. If a wetland creation or restoration project requires plants of *J. roemerianus* or *J. gerardi*, tissue culture-propagated plants could be an alternative source that is environmentally benign. This is especially important when plant material is limited or unavailable from other sources. For example, seed collection will be limited when seed production of *J. roemerianus* is extremely low, and collection of vegetative plants from natural marshes may be restricted by state regulations.

The regenerated plants of *J. roemerianus* grew vigorously in both the greenhouse and field plot. However, the wild plants appeared stressed in the natural marsh and showed very limited growth in our field experiment. *J. roemerianus* reaches its northern limit in Delaware (Eleuterius, 1976a) because of its cold intolerance. It is probable that the severe winter of 2002–2003 stressed the natural population of *J. roemerianus* from which we collected our plants and then resulted in the limited growth of the wild plants observed in the greenhouse and field plot.

The tissue culture process also provides a means of generating genetic variation in a plant (Larkin and Scowcroft, 1981) that may result in a selection with desirable attributes for solving a particular problem, e.g. marsh restoration in a disturbed site. The growth data we collected on the five regenerants in the field plot did not demonstrate significant differences among the five plant lines. However, testing a larger number of regenerants, conducting a longer-term study, and/or measuring more growth characters may result in evidence of somaclonal variation. Seliskar (1998) and Seliskar and Gallagher (2000) identified desirable somaclonal variation in wetland plants when they field-tested six to eight plant lines that were originally selected from a group of 50–100 regenerants potted in the greenhouse. The tissue culture process may produce genetic diversity, which may not be exhibited morphologically. Like most salt marsh monocots, both *J. roemerianus* and *J. gerardi* mainly propagate vegetatively and thus may have low genetic diversity. In the practice of wetland creation and restoration, regenerated plants with desirable genetic diversity may have a better ability to adapt in the field, e.g., perhaps to lower temperature. In addition, somaclonal variation makes it possible to produce plants with desirable characteristics not present in wild populations. In a field study of the salt marsh monocot *Sporobolus virginicus*, Seliskar (1998) identified a regenerant line that offered the best combination of characteristics for planting in a newly created marsh: high biomass, fairly high root plus rhizome biomass, and a tall canopy with a moderate stem density. In another field study, Seliskar and Gallagher (2000) identified a regenerant line of the salt marsh grass *Distichlis spicata* which had both a high biomass and a rapid decomposition rate, which may be desirable for use in locations where an increased input of detritus into the ecosystem is a priority. Such an attribute would be valuable for supporting the detritus food web in a newly created marsh where organic matter is scarce (Seliskar et al., 2002). In the cases of *J. roemerianus* and *J. gerardi*, the development of their tissue culture and plant regeneration protocols provides a potential to develop lines with desirable characteristics for solving specific problems in wetland creation and restoration.

Acknowledgements

Support for this research came from the University of Delaware Sea Grant College Program project R/ME-24 under Grant No. NA96RG0029 and the Estuary Enhancement Program of the Public Service Enterprise Group, Salem, New Jersey.

References

Al-Khayri, J. M.; Huang, F. H.; Morelock, T. E.; Busharar, T. A. Spinach tissue culture improved with coconut water. HortScience 27:357–358; 1992.

Bertness, M. D.; Hacker, S. D. Physical stress and positive associations among marsh plants. Am. Nat. 144:363–372; 1994.

Bouzille, J. B.; Bonis, A.; Clement, B.; Godeau, M. Growth pattterns of *Juncus gerardi* clonal populations in a coastal habitat. Plant Ecol. 132:39–48; 1997.

Christian, R. R.; Bryant, W. L., Jr.; Brinson, M. M. *Juncus roemerianus* production and decomposition along gradients of salinity and hydroperiod. Mar. Ecol. Prog. Ser. 68:137–145; 1990.

Cook, D. A.; Decker, D. M.; Gallagher, J. L. Regeneration of *Kosteletzkya virginica* (L.) Presl. seashore mallow from callus cultures. Plant Cell Tiss. Organ Cult. 17:111–119; 1989.

Eleuterius, L. N. The life history of the salt marsh rush, *Juncus roemerianus*. Bull. Torrey Bot. Club 102:135–140; 1975.

Eleuterius, L. N. The distribution of *Juncus roemerianus* in the salt marshes in North America. Chesapeake Sci. 17:289–292; 1976a.

Eleuterius, L. N. Vegetative morphology and anatomy of the salt marsh rush, *Juncus roemerianus*. Gulf Res. Rep. 5:1–10; 1976b.

Eleuterius, L. N. Autecology of the black needle rush *Juncus roemerianus*. Gulf Res. Rep. 7:339–350; 1984.

Eleuterius, L. N.; Caldwell, J. D. Growth kinetics and longevity of the salt marsh rush *Juncus roemerianus*. Gulf Res. Rep. 7:27–34; 1981.

Gallagher, J. L.; Reimold, R. J.; Linthurst, R. A.; Pfeiffer, W. J. Aerial production, mortality, and mineral accumulation–export dynamics on *Spartina alterniflora* and *Juncus roemerianus* plant stands in Georgia salt marsh. Ecology 61:303–312; 1980.

Larkin, P. J.; Scowcroft, W. R. Somaclonal variation – a novel source of variability from cell culture for plant improvement. Theor. Appl. Genet. 60:197–214; 1981.

Lauzer, D.; Dallaire, S.; Vincent, G. In vitro propagation of reed grass by somatic embryogenesis. Plant Cell Tiss. Organ Cult. 38:229–234; 2000.

Letham, D. S. Regulators of cell division in plant tissues. XX. The cytokinins of coconut milk. Physiol. Plant. 32:66–70; 1974.

Li, X.; Gallagher, J. L. Tissue culture and plant regeneration of big cordgrass, *Spartina cynosuroides*: implications for wetland restoration. Wetlands 16:410–415; 1996.

Li, X.; Seliskar, D. M.; Moga, J.; Gallagher, J. L. Plant regeneration from callus cultures of salt marsh hay, *Spartina patens*, and its cellular-based salt tolerance. Aquat. Bot. 51:103–113; 1995.

Linthurst, R. A.; Reimold, R. J. Estimated net aerial primary productivity for selected estuarine angiosperms in Maine, Delaware, and Georgia. Ecology 59:945–955; 1978.

Murashige, T.; Skoog, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473–497; 1962.

Nixon, S. W., The ecology of New England high salt marshes: a community profile. FWS/OBS-81/55 Washington, DC: US Fish Wildlife Service, Office of Biological Services; 1982.

Reimold, R. J. Mangals and salt marshes of eastern United States. In: Chapman, V. J., ed. Wet coastal ecosystems. Ecosystems of the world, vol. 1. Amsterdam: Elsevier Scientific Publishing Company; 1977:157–166.

Rogers, S. M. D. Tissue culture and wetland establishment of the freshwater moncots *Carex, Juncus, Scirpus*, and *Typha*. In Vitro Cell. Dev. Biol. Plant 39:1–5; 2003.

Rogers, S. D.; Beech, D. J.; Sarma, K. S. Shoot regeneration and plant acclimatization of the wetland monocot Cattail (*Typha latifolia*). Plant Cell Rep. 18:71–75; 1998.

Sarma, K. S.; Rogers, S. D. Plant regeneration and multiplication of the emergent wetland monocot *Juncus accuminatus*. Plant Cell Rep. 17:656–660; 1998.

Sarma, K. S.; Rogers, S. M. D. Plant regeneration from seedling explants of *Juncus effusus*. Aquat. Bot. 68:239–247; 2000.

Seliskar, D. M. Natural and tissue culture-generated variation in the salt marsh grass *Sporobolus virginicus*: potential selections for marsh creation and restoration. HortScience 33:622–625; 1998.

Seliskar, D. M.; Gallagher, J. L. Exploiting wild population diversity and somaclonal variation in the salt marsh grass *Distichlis spicata* (Poaceae) for marsh creation and restoration. Am. J. Bot. 87:141–146; 2000.

Seliskar, D. M.; Gallagher, J. L.; Burdick, D. M.; Mutz, L. M. The regulation of ecosystem functions by ecotypic variation in the dominant plant: a *Spartina alterniflora* salt marsh case study. J. Ecol. 90:1–11; 2002.

Shan, X.; Li, D.; Qu, R. Thidiazuron promotes *in vitro* regeneration of wheat and barley. In Vitro Cell. Dev. Biol. Plant 36:207–210; 2000.

Shumway, S. W.; Bertness, M. D. Salt stress limitation of seedling recruitment in a salt marsh plant community. Oecologia 92:490–497; 1992.

Stout, J. P. The ecology of irregularly flooded salt marshes of the northeastern Gulf of Mexico: a community profile. US Fish Wildl. Serv. Biol. Rep. 85(7.1):1–99; 1984.

Straub, P. F.; Decker, D. M.; Gallagher, J. L. Tissue culture and regeneration of *Distichlis spicata* (Gramineae). Am. J. Bot. 76:1448–1451; 1989.

Straub, P. F.; Decker, D. M.; Gallagher, J. L. Characterization of tissue culture initiation and plant regeneration in *Sporobolus virginicus* (Gramineae). Am. J. Bot. 79:1119–1125; 1992.

Straub, P. F.; Gallagher, J. L.; Decker, D. M. Tissue culture and long-term regeneration of *Phragmites australis* (Cav.) Trin. ex Steud. Plant Cell Tiss. Organ Cult. 15:73–78; 1988.

Sullivan, G. Established vegetation in restored and created coastal wetlands. In: Zedler, J. B., ed. Handbook for restoring tidal wetlands. Boca Raton, FL: CRC Press; 2001:119–155.

van Staden, J.; Drewes, S. E. Identification of zeatin and zeatinriboside in coconut milk. Physiol. Plant. 34:106–109; 1975.

Varshney, A.; Kant, T.; Kothari, S. L. Plant regeneration from coleoptile tissue of wheat (*Triticum aestivum* L.). Biol. Plant. 40:137–141; 1997.

Wang, J.; Seliskar, D. M.; Gallagher, J. L. Tissue culture and plant regeneration of *Spartina alterniflora*: implications for wetland restoration. Wetlands 23:386–393; 2003.

Wang, J.; Seliskar, D. M.; Gallagher, J. L. Plant regeneration via somatic embryogenesis in the brackish wetland monocot *Scirpus robustus*. Aquat. Bot. 79:163–174; 2004.

Woerner, L. S.; Hackney, C. T. Distribution of *Juncus roemerianus* in North Carolina tidal marshes: the importance of physical and biotic variables. Wetlands 17:284–291; 1997.