CELL BIOLOGY & MOLECULAR GENETICS

Infection of Somatic Embryos of Tall Fescue with *Acremonium coenophialum*

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ABSTRACT

The availability of a tall fescue (*Festuca arundinacea* Schreb.) clone whose members are infected with different strains of its endophytic fungus, *Acremonium coenophialum* Morgan-Jones & Gams, would help identify the individual contributions of the endophyte and tall fescue toward traits normally associated with endophyte-infected tall fescue plants. As embryos and very young seedlings are the only stages of the life cycle during which infection by the endophyte naturally occurs in planta, attempts were made to duplicate this phenomenon in vitro. Callus cultures of tall fescue were induced using leaf basal tissue as the explant source, and Murashige and Skoog (MS) basal medium supplemented with the synthetic auxins 2,4-D [(2,4-dichlorophenoxy)acetic acid], 2,4,5-T [(2,4,5-trichlorophenoxy)acetic acid], or picloram (4-amino-3,5,6-trichloropicolinic acid). Two tall fescue genotypes were exposed to four concentrations (15, 30, 45, or 60 μM) of each auxin. Calli were transferred to hormone-free basal medium to permit the development of somatic embryos. Elongating shoots were counted to determine the effectiveness of each treatment and transferred to fresh medium to permit their development into plants. The most effective and consistent auxin concentration to stimulate somatic embryogenesis was 2,4-D at 30 μM, producing almost 14 embryos per callus. Next, 60 explants of one fescue genotype were placed on MS medium supplemented with 30 μM 2,4-D. Two isolates of the endophytic fungus were used to infect the explants and developing calli by inoculating at weekly intervals. The highest infection rate was obtained by inoculating somatic embryos with the fungus at the time the callus was transferred to hormone-free medium, the stage at which somatic embryos begin to elongate and germinate. This stage corresponds with the stage at which infection is thought to occur in planta. This method will permit further studies of the interactions between tall fescue and its endophyte.

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TALL FESCUE is a pasture grass grown on 16 million ha in Canada and the southeastern USA (Bacon and Siegel, 1988). It is commonly infected with an endophytic fungus that produces ergopeptine alka-

Abbreviations: MS, Murashige and Skoog; 2,4-D, (2,4-dichlorophenoxy)acetic acid; 2,4,5-T, (2,4,5-trichlorophenoxy) acetic acid.

loids associated with the chronic health disorder in livestock known as fescue toxicosis (Hoveland et al., 1983). As infected plants are better able than noninfected plants to survive stress, eradication of the endophyte is not viable as a universal strategy to prevent fescue toxicosis (Hill et al., 1991). Infected tall fescue plants vary in levels of ergopeptine alkaloid production (Hill et al., 1990), suggesting a strategy whereby the beneficial qualities of the endophyte are maintained, but ergopeptine alkaloid content is decreased or eliminated through selection of low ergopeptine alkaloid-producing endophyte strains or plant–endophyte combinations.

An understanding of the factors controlling levels of ergopeptine alkaloid production would be facilitated if different plants of the same genotype infected with different endophyte strains were available. Normally, the endophyte is present at the growing points of the flowering stem and infects the ovaries prior to pollination. During germination of the seed, the hyphae grow from the maternal tissues in the seed and infect the embryo (Bacon and Siegel, 1988). Young seedlings have been artificially infected with the endophyte by inserting endophyte mycelium into the coleoptile of seedlings (Latch and Christensen, 1985), but this technique does not work on mature plants. Consequently, it has not been possible to introduce more than one strain of the endophyte into any given genotype of tall fescue. Callus cultures of tall fescue infected with the endophyte led to the recovery of regenerated plants, ≈17% of which were infected with the endophyte (Johnson et al., 1986).

Taken together, this information suggests that existing tissue culture techniques can be adapted to duplicate the natural endophyte infection process. Regeneration of grasses usually occurs through somatic embryogenesis (Vasil, 1988), whereby a cell from a piece of callus in culture becomes an embryo, which subsequently follows a developmental pathway nearly identical to that of an embryo developing inside a seed (Williams and Maheswaran, 1986). It follows that optimum efficiency of infection of tall fescue could be obtained if one first waits for somatic embryos to form on callus growing in culture, and then infects the embryos with endophyte at the time they begin to germinate. Using this method, an unlimited number of endophyte isolates could be introduced into a given plant genotype.

Initial reports on successful regeneration of tall fescue used various protocols based on the use of a synthetic auxin. While regeneration has occurred on tissues exposed to an auxin (Dale and Dalton, 1983), regeneration in most protocols (Kasperbauer et al., 1979; Kasperbauer et al., 1980; Lowe and Conger, 1979; Dalton, 1988) occurred upon lowering the auxin level in the medium following a prolonged callus phase. In red fescue (*Festuca rubra* L.), regeneration has occurred following complete removal of auxin from the medium (Zaghmout and Torello, 1989), an approach that, while widely used in several species, has not been commonly used for tall fescue (Eizenga and Dahleen, 1990). Recent reports of auxin-stimulated regeneration in red fescue and tall fescue have characterized regeneration as being via somatic embryogenesis (Dalton, 1988; Eizenga and Dahleen, 1990).

The objectives of this experiment were to (i) identify the most efficient regeneration system, and (ii) select the optimal stage of the regeneration process for infection by the endophyte.

MATERIALS AND METHODS

Sterilization of Explants

Two endophyte-free genotypes of 'Kentucky 31' tall fescue, designated FDN2 and FDN9, were selected because they differed in their level of alkaloid production when infected with endophyte. The procedure used to free these plants from their endophytes has been described (Hill et al., 1990). Leaf basal tissues were selected as the explant of choice because of their year-around availability. Explant tissues were collected in a manner similar to that of Kasperbauer et al. (1979). Two basic techniques and four variations of them were used to sterilize leaf basal tissues. The first technique consisted of surface sterilization in 70% ethanol for 5 s followed by 5 min in an 0.525% NaOCl solution (w/v) (i.e., 10% commercial bleach solution, v/v) and three sterile water washes (Latch and Christensen, 1985). Technique 2 consisted of surface sterilization in an 0.525% NaOCl solution for 2 min followed by flaming explants after dipping in 70% ethanol (v/v) (Tuite, 1969). Techniques 3 and 4 were variations of the first two. They consisted of 70% ethanol for 3 s, 0.525% NaOCl for 10 min, and three water rinses, with Technique 4 including a light flaming (with 70% ethanol) of the explant prior to the rinses. Finally, two techniques were variations of Technique 4; for Technique 5, the time in NaOCl was 15 min, while for Technique 6 it was 20 min.

Somatic Embryogenesis

The 2 cm explants consisting of leaf basal tissue from FDN2 and FDN9 were surface sterilized in 70% ethanol for 30 s, followed by 15 min in 0.525% NaOCl solution, and by three 2-min rinses in sterile water. The outer leaf tissue layers were peeled away, and the central 1 cm section of the yellow-green tissue was placed in a 100 by 20 mm petri dish with ≈20 mL of auxin-supplemented solid basal medium. Basal medium consisted of MS salts (Murashige and Skoog, 1962), B_5 vitamins (Gamborg et al., 1968), 3% sucrose (w/v), pH adjusted to 5.8, and 2 g of Gelrite (Collier, Nolan, Strickland Medical Supply, Norcross, GA) as a solidifying agent. Auxins consisted of picloram, 2,4-D, or 2,4,5-T added to the media in 15, 30, 45, or 60 μM concentrations. Media were autoclaved at 121 °C for 20 min. Auxin type, concentration, and fescue genotype were used as factors in a 3 × 4 × 2 factorial design with five replications. Cultures were maintained in a growth chamber at 25 ± 1 °C with fluorescent lamps providing ≈75 μmol m^{-2} s^{-1} of photons for 42 d, after which the calli were transferred to auxin-free medium to permit further development of the somatic embryos. After 28 d on the auxin-free medium, embryos had started germinating on the callus. The plantlets were counted and transferred to basal medium in Magenta boxes (Magenta Corp., Chicago, IL). The boxes were maintained in the growth chamber for 28 d, permitting the plantlets to grow sufficiently for transplantation into soil. The potting soil mixture consisted of equal parts by volume of soil and premixed potting soil (Hyponex, Atlanta, GA) in 5-cm pots placed in Dome Top trays (Gardener's Supply, Burlington, VT) to keep a high relative humidity. Plants were slowly hardened off by progressively removing the tray lids in the growth chamber after 1 wk. Plants were transferred to the greenhouse after an additional 1 wk.

Infection with Endophyte

Tall fescue Genotype FDN2 was chosen for the infection process because of its lower affinity for root production. Leaf basal tissue was collected from plants that were generated by somatic embryogenesis and grown aseptically in Magenta boxes to ensure a large supply of sterile explant tissue. After collection, all explants were placed on basal medium with 30 μM of 2,4-D, the auxin and concentration which had previously produced the most consistent results for both genotypes. A total of 60 explants were cultured. Two isolates of the endophyte, 2 and 11 (isolated from plants known to differ in alkaloid production), were used for inoculations. Endophyte 2 was isolated from an infected form of tall fescue Genotype FDN2, while Endophyte 11 was isolated from another infected tall fescue genotype, FDN11, not otherwise used in this experiment, by Dr. D.D. Pope, Department of Plant Pathology, University of Georgia, and maintained on basal medium.

Five explants were immediately inoculated with each endophyte (Week 0). Inoculation was effected by placing small bits of mycelium from the outer regions of the endophyte colony on cut ends of explants. Five additional explants were inoculated at weekly intervals (Days 7, 14, 21, 28, and 35) with each endophyte. Once callus growth had begun, inoculation was made by making a cut, a few millimeters in length, in the callus with a scalpel blade, and inserting small pieces of mycelium. After 28 d, calli were transferred to auxin-free medium to permit development of somatic embryos, whether or not they had been inoculated with the endophyte. Hence, the inoculation at 35 d was made on hormone-free medium. At Week 6 (Day 42), plantlets were removed from the calli and placed in Magenta boxes with auxin-free basal medium with a sucrose level lowered to 0.6%. Plantlets were maintained in the growth chamber and allowed to develop for 28 d, prior to their transfer to pots, hardened as described previously, and transferred to the greenhouse.

Plants were allowed to grow in the greenhouse for two months before they were screened for presence of the endophyte using the staining technique of Clark et al. (1983). Leaf sheath sections were collected from the plants; a razor was used to make a small cut across the inside surface at the base of the leaf sheath; and a section of the epidermis was peeled off and placed on a slide with the inner surface up and the cuticle down. A drop of aniline blue stain was added and blotted off after 30 s. A drop of water and a cover slip were then added. Intercellular hyphae running parallel to the cells were clearly visible whenever the reinfection process had been successful.

Statistical Analysis

Data were analyzed using PROC GLM of PC-SAS (SAS Institute, Cary, NC). Treatment means were separated using a protected LSD value generated from the mean squared error term (Steel and Torrie, 1980).

RESULTS

Sterilization Procedures

Sterilization of fescue explants from leaf basal tissues was complicated because of high numbers of microorganisms present near the crown of the plants. The initial sterilization technique (No. 1) resulted in a 60% contamination rate. This was only slightly better than that obtained with Technique 2, which had a 70% contamination rate. These contamination rates were still too high to proceed, so variations of the first two were performed. Exposure times to NaOCl were adjusted until a low contamination rate was found, along with low explant death caused by overexposure to NaOCl. Overall, those techniques that omitted flaming gave better results than those that did not. Technique 5 had an overall contamination and death rate of 44%. Increasing the exposure time in NaOCl (Technique 6) resulted in an explant death and contamination rate of 63%. Technique 5 was the choice for sterilization.

Somatic Embryogenesis

Data for callus induction and regeneration is presented in Table 1. Callus induction from explants in both genotypes of tall fescue was ≈50%. Contamination further reduced the number of calli for some of the treatments. Callus formation was visible as early as 1.5 wk. In most cases, the explant would curl during the callus formation stage. A yellow-green explant would sometimes turn brown and fall off from the

Table 1. Comparison of callus formation and regeneration in two genotypes of tall fescue following induction with three auxins at four concentrations.

		Fescue genotype						
		FDN2			FDN9			
Auxin	Conc.	Calli†	Embryos	Embryos/ callus	Calli†	Embryos	Embryos/ callus	Average embryos/callus‡
	μM	no.						
2,4-D	15	2	20	10.0	2	0	0.0	5.0
	30	2	40	20.0	5	55	11.0	13.6
	45	3	3	1.0	3	36	12.0	6.5
	60	4	10	2.5	2	25	12.5	5.8
2,4,5-T	15	–	–	–	4	18	4.5	3.0
	30	3	14	4.7	4	87	21.7	14.4
	45	1	0	0	2	25	12.5	6.0
	60	2	0	0.0	0	0	0.0	0.0
Picloram	15	–	–	–	1	0	0.0	0.0
	30	3	11	3.7	1	0	0.0	2.7
	45	2	0	0.0	3	0	3.0	0.0
	60	1	0	0.0	4	0	4.0	0.6
LSD (0.10)§								9.4

† Number of calli formed from five original explants.
‡ Average of two genotypes.
§ LSD value is for differences between number of embryos per callus for the genotype × auxin rate × auxin type interaction.

callus, and at other times it would be overgrown by the callus. Embryos could be seen during Weeks 3 and 4, but for the most part appeared during Weeks 5 to 7. Not all calli formed embryos. Some just produced roots, and others formed a combination of the two. Small embryos sometimes germinated and grew roots while still on the callus. Once removed from the callus, germinating embryos grew a root system within 2 or 3 d. The success rate was nearly 100% for converting embryos in Magenta boxes to potted plants in the greenhouse.

Genotypic and auxin effects were apparent in root formation from callus. Genotype FDN9 had a high affinity for root formation, as compared with genotype FDN2. The use of 2,4-D resulted in less root formation from callus than did the other auxins. The lower concentrations of 15 and 30 μM 2,4,5-T produced more roots than the 45 and 60 μM concentrations. Picloram produced the most roots of all the auxins evaluated, with the lower concentrations (15 μM and 30 μM) being the most rhizogenic.

The low number of calli that survived in each treatment prevented significant differences from being detected at the 0.05 probability level. At the 10% level, the two-way interactions between main effects (genotype × auxin type, genotype × auxin rate, and auxin type × auxin rate) were not significant as determined by the PROC GLM procedure. A three-way interaction existed, which suggested that different tall fescue genotypes are affected differently by the type and rate of auxin used for embryogenesis.

Infection with Endophyte

Data for inoculations with endophyte are presented in Table 2. Inoculations were made during Weeks 0 through 5, but only inoculations made during Weeks 3 to 5 were successful in obtaining regenerated plants with introduced endophytes (Fig. 1a). Mycelia overgrew the calli following the Weeks 0 to 1 inoculations. No inoculations were made after Week 5, as somatic embryos had germinated into small seedlings. Endophyte mycelia readily infect calli and quickly establish colonies on callus tissue (Fig. 1b). Mycelia were seen on the crown of germinating embryos (Fig. 1c), as were hyphae exiting from leaves of young seedlings growing in culture (Fig. 1d). Such hyphae have been observed previously in endophyte-infected plants growing in a culture vessel (Johnson et al., 1986).

Table 2. Recovery of plants following infection with *Acremonium coenophialum* at various times and number of infected plants recovered.

	Week of inoculation of callus tissues†						
	0	1	2	3	4	5	Total
	no.						
Plants	0	0	29	19	23	6	77
Infected plants	0	0	0	1	2	1	4

† Weeks 0 to 4 were on medium with 30 μM 2,4,D; Week 5 was on hormone-free medium.

DISCUSSION

Previous evaluations of the efficiency of various auxins in the induction of tall fescue callus have provided conflicting results. The auxin 2,4-D has been reported to provide better callus growth than 2,4,5-T (Conger et al., 1978; Eizenga and Dahleen, 1990) or pCPA (p-chlorophenoxyacetic acid) (Eizenga and Dahleen, 1990). On the other hand, both 2,4-5-T and pCPA have been considered superior to 2,4-D (Dahleen and Eizenga, 1990), as have dicamba (2-methoxy-3,6-dichlorobenzoic acid) and picloram (Conger et al., 1982). In the results presented here, 2,4-D produced the most consistent results for both genotypes of fescue tested. The conflicting results reported in the literature may be attributed to genotypic differences among tall fescue plants.

Following the callus phase, regeneration in grass species most commonly occurs via somatic embryogenesis following exposure to an auxin, most frequently 2,4-D (Vasil, 1985; Vasil, 1987). When various auxins have been evaluated for regeneration in tall fescue, 2,4-D has resulted in higher (Kasperbauer et al., 1979; Dahleen and Eizenga, 1990) or equal (Eizenga and Dahleen, 1990) regeneration frequencies. Similar results were obtained in this study. While both genotypes regenerated well with 2,4-D, only FDN9 regenerated well with 2,4,5-T, and neither genotype did well with picloram.

To study the effects of various endophytes on a given genotype of tall fescue, it is desirable that the culture system does not introduce any somaclonal variation into the plants, as such variation could be confounded with the influence of the endophyte. In monocots, plants regenerated through somatic embryogenesis usually are genetically stable, and their genotype should reflect that of the original plant. This phenomenon has been attributed both to the single-cell origin of somatic embryos (Vasil, 1987) and to the inability of developing embryos to tolerate many abnormalities (Ozias-Akins and Vasil, 1988). Plants of red fescue regenerated via somatic embryogenesis from 4-yr-old cultures were all phenotypically stable (Zaghmout and Torello, 1989).

Nevertheless, regenerated plants of tall fescue have frequently displayed somaclonal variation (Conger et al., 1980; Reed and Conger, 1985; Eizenga, 1987, 1989; Dahleen and Eizenga, 1990). In all these cases, regeneration was invariably obtained following callus phases lasting about 2 to 4 mo. In two reports of regeneration in tall fescue, somaclonal variation was restricted to plants regenerated from aged callus (Kasperbauer et al., 1979; Kasperbauer and Eizenga, 1985). Cytogenetic and isozyme analyses of tall fescue plants regenerated following a callus phase of ≈1 mo. failed to reveal any somaclonal variation (Eizenga and Dahleen, 1990). This study also limited the callus phase to 4 wk. Thus far, all regenerated plants from this study are indistinguishable morphologically from other plants of the same genotype.

The presence of the auxin induces the development of somatic embryos, while subsequent transfer to low-auxin or auxin-free medium permits germination of the embryos. The results presented here suggest that there is an optimal stage for infection of somatic embryos by the endophyte, corresponding to the week-long period beginning with embryo transfer to hor-

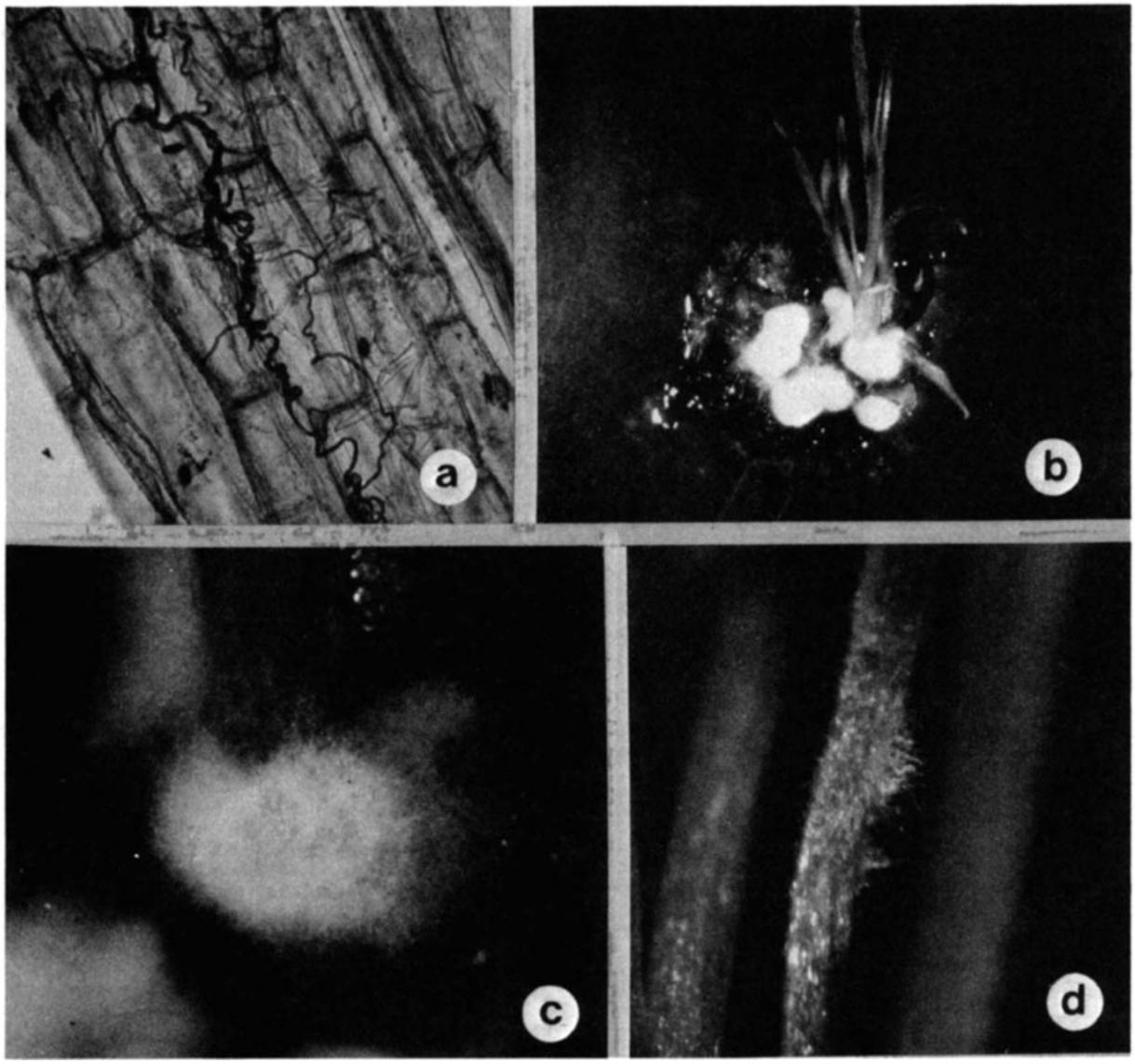

Fig. 1.(a) Hyphae of *Acremonium coenophialum* Isolate 11 inside a leaf of tall fescue genotype FDN2 regenerated from tissue culture (320×).(b) Colonies of *A. coenophialum* Isolate 11 growing on tall fescue FDN2 callus and a seedling germinated from a somatic embryo (6×).(c) A colony of *A. coenophialum* growing along the crown of a germinating somatic embryo (34×).(d) Hyphae of *A. coenophialum* protruding from a leaf of a seedling germinated from a somatic embryo and growing in a Magenta box (51×).

mone-free medium. The growth and germination experienced by somatic embryos at the time of auxin removal is probably analogous to that of germinating zygotic embryos at the time natural infection occurs. The frequency of infection obtained from inoculations during this period is similar to that reported in an earlier report of callus infection of tall fescue (Johnson et al., 1986).

Only 5% of the plants obtained in this study were infected with the endophyte. This frequency could have been higher if all the inoculations had been made during Weeks 4 and 5, when the frequency of infection approached that reported by Johnson et al. (1986). While this report does not improve on the frequency of infection, two important advances have been made. First, a 10- to 12-wk period for callus development was used in the previous report. Following inoculation with the endophyte, an additional 10-wk period was allowed to permit infection of the callus. Finally, an additional 4 wk were necessary on regeneration medium to obtain plants. With the procedure used for our study, it was possible to establish infected plants in the greenhouse 10 wk from the time uninfected explant tissues were initially placed in culture. Secondly, by limiting exposure to 2,4-D to 4 wk, the callus phase was limited to that time period, greatly limiting the time period during which somaclonal variation could occur and accumulate prior to regeneration.

It is now possible to introduce various endophytes into a common fescue genotype in a timely manner. Knowing that somatic embryos can be infected instead of callus, embryogenic suspensions (Zaghmout and Torello, 1989; Rajoelina et al., 1990) become viable as a strategy to generate large numbers of embryos for infection. Our continuing studies are designed to generate large populations of tall fescue plants infected

with various endophyte isolates. These populations should help identify factors that control alkaloid levels in endophyte-infected tall fescue plants, as well as other factors that confer stress resistance to infected plants. As such, this is the first step toward understanding interactions that occur between tall fescue and its endophyte, as well as developing agronomic protocols for exploiting this relationship.

REFERENCES

Bacon, C.W., and M.R. Siegel. 1988. Endophyte parasitism of tall fescue. J. Prod. Agric. 1:45–55.

Clark, E.M., J.F. White, and R.M. Patterson. 1983. Improved histochemical techniques for the detection of *Acremonium coenophialum* in tall fescue and methods of *in vitro* culture of the fungus. J. Microbiol. Methods 1:149–155.

Conger, B.V., J.V. Carabia, and K.W. Lowe. 1978. Comparison of 2,4-D and 2,4,5-T on callus induction and growth in three *Gramineae* species. Environ. Exp. Bot. 18:163–168.

Conger, B.V., K.W. Lowe, and J.V. Carabia. 1980. Relative DNA content of cells in *Festuca arundinacea* and *Dactylis glomerata* calli of different ages. Environ. Exp. Bot. 20:401–408.

Conger, B.V., L.L. Hilenski, K.W. Lowe, and J.V. Carabia. 1982. Influence of different auxins at varying concentrations on callus induction and growth from embryo and leaf tip explants in *Gramineae*. Environ. Exp. Bot. 22:39–48.

Dahleen, L.S., and G.C. Eizenga. 1990. Meiotic and isozymic characterization of plants regenerated from euploid and selfed monosomic tall fescue embryos. Theor. Appl. Genet. 79:39–44.

Dale, P.J., and S.J. Dalton. 1983. Immature inflorescence culture in *Lolium, Festuca, Phleum* and *Dactylis*. Z. Pflanzenphysiol. 111:39–45.

Dalton, S.J. 1988. Plant regeneration from cell suspension protoplasts of *Festuca arundinacea* Schreb. (tall fescue) and *Lolium perenne* L. (perennial ryegrass). J. Plant Physiol. 132:170–175.

Eizenga, G.C. 1987. Cytogenetic and isozymic characterization of anther-panicle culture derived tall fescue aneuploids. Euphytica 36:175–179.

Eizenga, G.C. 1989. Meiotic analysis of tall fescue somaclones. Genome 32:373–379.

Eizenga, G.C., and L.S. Dahleen. 1990. Callus production, regeneration and evaluation of plants from cultured inflorescences of tall fescue (*Festuca arundinacea* Schreb.). Plant Cell Tissue Organ Cult. 22:7–15.

Gamborg, O.L., R.A. Miller, and K. Ojima. 1968. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. 50:150–158.

Hill, N.S., W.C. Stringer. G.E. Rottinghaus, D.P. Belesky, W.A. Parrott, and D.D. Pope. 1990. Growth, morphological, and chemical component responses of tall fescue to *Acremonium coenophialum*. Crop Sci. 30:156–161.

Hill, N.S., D.P. Belesky, and W.C. Stringer. 1991. Competitiveness of tall fescue as influenced by *Acremonium coenophialum*. Crop Sci. 31:185–190.

Hoveland, C.S., S.P. Schmidt, C.C. King, Jr., E.M. Clark, J.A. McGuire, L.A. Smith et al. 1983. Steer performance and association of *Acremonium coenophialum* fungal endophyte on tall fescue pasture. Agron. J. 75:821–824.

Johnson, M.C., L.P. Bush, and M.R. Siegel. 1986. Infection of tall fescue with *Acremonium coenophialum* by means of callus culture. Plant Dis. 70:380–382.

Kasperbauer, M.J., R.C. Buckner, and L.P. Bush. 1979. Tissue culture of annual ryegrass × tall fescue F_1 hybrids: Callus establishment and plant regeneration. Crop Sci. 19:457–460.

Kasperbauer, M.J., R.C. Buckner, and W.D. Springer. 1980. Haploid plants by anther-panicle culture of tall fescue. Crop Sci. 20:103–107.

Kasperbauer, M.J., and G.C. Eizenga. 1985. Tall fescue doubled haploids via tissue culture and plant regeneration. Crop Sci. 25:1091–1095.

Latch, G.C.M., and M.J. Christensen. 1985. Artificial infection of grasses with endophytes. Ann. Appl. Biol. 107:17–24.

Lowe, K.W., and B.V. Conger. 1979. Root and shoot formation from callus cultures of tall fescue. Crop Sci. 19:397–400.

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

Ozias-Akins, P., and I.K. Vasil. 1988. In vitro regeneration and genetic manipulation of grasses. Physiol. Plant. 73:565–569.

Rajoelina, S.R., G. Alibert, and C. Planchon. 1990. Continuous plant regeneration from established embryogenic cell suspension cultures of Italian ryegrass and tall fescue. Plant Breed. 104:265–271.

Reed, J.N., and B.V. Conger. 1985. Meiotic analyses of tall fescue *Festuca arundinacea* plants regenerated from callus cultures. Environ. Exp. Bot. 25:277–284.

Steel, R.G.D., and J.H. Torrie. 1980. Principles and procedures of statistics. 2nd. ed. McGraw-Hill Book Co., New York.

Tuite, J. 1969. Plant pathological methods. Burgess Publ. Co., Minneapolis.

Vasil, I.K. 1985. Somatic embryogenesis and its consequences in the gramineae. p. 31–47. *In* R.R. Henke et al. (ed.) Tissue culture in agriculture and forestry. Vol. 31. Plenum Press, New York.

Vasil, I.K. 1987. Developing cell and tissue culture systems for the improvement of cereal and grass crops. J. Plant Phys. 128:193–218.

Vasil, I.K. 1988. Progress in the regeneration and genetic manipulation of cereal crops. Bio/Technology 6:397–402.

Williams, E.G., and G. Maheswaran. 1986. Somatic embryogenesis: Factors influencing coordinated behaviour of cells as an embryogenic group. Ann. Bot. 57:443–462.

Zaghmout, O.M.F., and W.A. Torello. 1989. Somatic embryogenesis and plant regeneration from suspension cultures of red fescue. Crop Sci. 29:815–817.