Ergopeptine Alkaloid Production by Endophytes in a Common Tall Fescue Genotype

N. S. Hill,* W. A. Parrott, and D. D. Pope

ABSTRACT

The fungal endophyte, *Acremonium coenophialum* Morgan-Jones & Gams, is present in many tall fescue (*Festuca arundinacea* Shreb.) pastures. It imparts fitness-related traits to the tall fescue plant but produces ergopeptine alkaloids, which may be the cause of fescue toxicosis in grazing livestock. Inasmuch as the endophyte provides the plant with added vigor, development of endophyte-infected tall fescue populations that are incapable of producing ergopeptine alkaloids may be a solution to providing a sustainable nontoxic grass base for livestock producers. Our objective was to study changes in ergopeptide alkaloid production in a common tall fescue genotype that was infected by two different endophyte isolates that express different levels of alkaloids when in their host plants. Endophyte-free tall fescue Genotype PDN2 explants were cultured in vitro to obtain 28-d-old calli. Endophytes EDN2 and EDN11 isolated from infected PDN2 and another plant genotype PDN11 were cultured and inserted into germinating somatic embryos by placing pieces of mycelium into slits at the base of the noninfected embryonic PDN2 explant. Both endophyte-infected and -free plants were regenerated from callus culture. Regenerated plants with each reinfected endophyte were grown with their endophyte-infected naturally occurring forms to compare endophyte behavior within a common plant genotype under greenhouse conditions. The PDN11 plant with its naturally occurring endophyte was a high alkaloid producer while PDN2 was a low alkaloid producer. When the endophytes EDN11 and EDN2 were reinfected into noninfected individuals of PDN2, the resulting plants were low alkaloid producers. These data suggest that the plant plays a major role in controlling the expression of the genetic potential of endophytes for ergopeptine alkaloid production.

THE FUNGAL ENDOPHYTE, *Acremonium coenophialum*, which is present in many tall fescue pastures, imparts fitness-related traits to tall fescue plants (Hill et al., 1990; Hill et al., 1991; Bacon and de Battista, 1990); however, the endophyte also produces ergopeptine alkaloids, which are responsible for vasoconstriction (Solomons et al., 1989) and reduced prolaction production (Strickland et al., 1989) by livestock consuming tall fescue herbage. Recent investigations in which ergopeptine alkaloids were infused into the vascular system of cattle (*Bos taurus*) have reproduced fescue toxicosis symptoms (G. Garner, 1990, personal communication). Livestock producers who depend on tall fescue as their grass base for forage systems therefore, face a biological dilemma, because pastures without the endophyte may not be as persistent as those with it, while those pastures containing endophyte produce livestock disorders. One solution to the dilemma might be to produce endophyte-infected tall fescue without the capacity to produce ergopeptine alkaloids. Considerable variability exists for alkaloid content within endophyte-infected tall fescue plant genotypes (Hill et al., 1990), but before breeding programs can be designed to reduce alkaloid content, a fundamental understanding of the interaction between plants and their endophytes is necessary. By using tissue culture and regeneration techniques, endophytes can be introduced into a common tall fescue genotype to study such interactions (Kearney et al., 1991). Our objective was to study ergopeptine alkaloid production in a common genotype of tall fescue infected by two different endophyte isolates that express different levels of alkaloids when in their host plants.

N.S. Hill and W.A. Parrott, Dep. of Agronomy, and D.D. Pope, Plant Pathology Dep., Univ. of Georgia, Athens, GA. Research supported by Southeast Regional USDA-IPM Grant no. 8900938. Received 13 Nov. 1990. *Corresponding author.

Published in Crop Sci. 31:1545–1547 (1991).

MATERIALS AND METHODS

Tissue Culture of Tall Fescue

Endophyte-infected tall fescue genotypes, PDN2 and PDN11, were selected from a pasture of 'Kentucky-31' tall fescue growing at the USDA-ARS Southern Piedmont Soil and Water Conservation Lab in Watkinsville, GA. Endophyte-free forms of the plants were generated from the infected forms as previously described (Hill et al., 1990). Following ≈12 generations of vegetative propagation, leaf basal tissues were selected as explant sources for callus culture. Explants were sterilized in a 10% (v/v) dilution of commercial bleach (0.525% NaOCl [w/v]) for 15 min, dipped in 70% ethanol, and flamed, and rinsed three times with distilled water (Kearney et al., 1991). Sections of explant were trimmed to 2-cm lengths and placed on a basal medium consisting of MS salts (Murashige and Skoog, 1962), B_5 vitamins (Gamborg et al., 1968), 30 g sucrose, and 2 g gelrite solidifying agent per liter of water. The final pH was 5.8. The basal medium was supplemented with 30 μM 2,4-D (2-4-dichlorophenoxyacetic acid). Explants were cultured in 2,4-D-supplemented basal medium for 28 d after which calli were transferred to hormone-free basal medium to permit development of somatic embryos.

Isolation and Culture of Endophytes

Fungal isolates from tall fescue genotypes PDN2 and PDN11 were obtained using the procedure described by Bacon and Siegel (1988). The endophyte isolates, EDN2 and EDN11, were maintained on strips of sterile MWCO 12 000 to 14 000 dialysis tubing (Union Carbide, New York) overlaying double-strength potato dextrose agar (Pope and Hill, 1991). Fungal isolates were identified as the endophyte by a positive reaction to an ELISA test (Johnson et al., 1982; performed by Dr. K. Gwinn Plant Pathology Dep., University of Tennessee, Knoxville) and by the presence of characteristic conidia and conidiophores.

Reinfection with Endophytes

After transfer of calli to hormone-free media, 12-wk-old mycelial cultures of endophytes EDN2 and EDN11 were used to inoculate germinating somatic embryos. These endophytes were selected because, when in association with plants PDN2 and PDN11, they had low and high alkaloid production, respectively. Inoculation was conducted by making a small slit on the somatic embryos and placing pieces of mycelia into the slit. Plantlets developed from the embryos were maintained in the growth chamber for 28 d prior to their transfer to pots, hardening, and transfer to the

Abbreviations: ELISA, enzyme-linked immunosorbent assay.

greenhouse. Plants were allowed to grow in the greenhouse for 6 months before being screened for presence of the endophyte using the aniline blue staining technique (Clark et al., 1983).

Greenhouse Growing Conditions

Endophyte-infected and noninfected individuals of tall fescue genotypes PDN11 and PDN2, and one each of the regenerated individuals of PDN2 with introduced endophytes, were vegetatively propagated from individual tillers and grown in a greenhouse with day/night temperatures of ≈29/21 °C. Explants from tall fescue genotype PDN-11 did not callus and therefore could not be reinfected with endophytes. Tillers were stratified by fresh weight and presence of tiller primordia to minimize variation of the resulting plants due to variation in propagules. Plants were arranged on the bench in a completely random assignment and fertilized weekly with 800 mg pot^{-1} of a commercial greenhouse liquid fertilizer containing 25, 87, and 166 g kg^{-1} of N, P, and K, respectively. Three replicates of each plant were grown. After ≈4 mo., herbage was hand clipped, freeze-dried, and stored at −70 °C until analyzed for alkaloids. The plants were harvested a second time after permitting the plants to regrow for ≈2 mo at which time herbage was harvested and dried as described.

Somaclonal Variation of Regenerated Plants

Twelve regenerated PDN2 plants that did not test positive for endophyte content were cultured in the greenhouse as described. Seven tillers were selected for uniformity and used to vegetatively propagate each of the regenerated plants in 15-cm pots. Vegetative propagules were used as replications within a completely random design whereby plants were permitted to grow in the greenhouse until flowering. Plants were harvested to a stubble height of 5 cm at flowering and oven-dried at 65 °C. Weight of the dried samples were determined and analyzed among regenerants and within regenerants to estimate somaclonal variability.

Ergopeptine Alkaloid Analysis

After each experiment, the dried herbage samples were ground to pass a 1-mm screen using a Wiley mill. Ergopeptine alkaloids were extracted and purified from 1-g subsamples of herbage as described by Hill et al. (1990). Purified alkaloid extracts were analyzed using a Shimadzu Model LC-600 high performance liquid chromatograph (Columbia, MD) equipped with a RF535 fluorescence detector. A mobile phase of 380 mL L^{-1} of acetonitrile in 0.001 M ammonium carbonate in distilled water was used to separate contents in an Alltech Associates (Deerfield, IL) adsorbosphere HS 3 micron C18 column. Column length and width were 100 by 4.6 mm and the injection volume was 20 μL. Excitation and emmision wavelengths were 250 and 420 nm, respectively. Total ergovaline content was calculated from the areas of the chromatograph corresponding to the ergovaline and ergovalinine peaks using ergotamine tartrate as an internal standard.

Table 1. Ergovaline content of tall fescue genotypes with endemic or introduced endophytic fungi on two sampling dates.

Plant genotype	Endophyte		Ergovaline	
	Endemic	Introduced	4 months†	6 months
			μg kg^{-1}	
PDN11	EDN11		3222	4696
PDN2	EDN2		517	1389
PDN2		EDN2	574	1324
PDN2		EDN11	704	876
PDN2	no endophyte		0	0
LSD (0.05) Plant/endophyte × age			407	

† Plant age after propagation.

RESULTS AND DISCUSSION

Somaclonal Variation among Noninfected Regenerated Plants

Endophyte-free regenerated plants from 28-d-old calli of PDN2, which did not become infected during endophyte reinoculation, were similar in appearance to each other. In addition, they had similar biomass production and flowering dates in the greenhouse. Biomass production averaged 6.28 g for all regenerated noninfected plants. The standard deviation of the mean biomass production among the regenerated plants was 0.35 g while the mean standard deviation within somaclones (i.e., effect of replication) was 0.64 g. The protocol used for regeneration was essentially the same as that used by Eizenga and Dahleen (1990), with the exception that they used panicle pieces to derive calli. In their study, they found no differences between parents and regenerated plants for chromosome number, isozyme analysis, and pollen stainability. Given that noninfected plant clones were similar in appearance, biomass production, and flowering date, and that other researchers found no somaclonal variation using similar regeneration procedures (Eizenga and Dahleen, 1990), it is unlikely that regenerated endophyte-infected tall fescue plants were somaclonal variants.

Alkaloid Content among Infected Regenerated Plants

Tall fescue genotype PDN11 was a high-alkaloid producer when in association with its endemic endophyte EDN11 (Table 1). Genotype PDN2 was a low alkaloid producer when infected with its endophyte EDN2. These data were consistent with preliminary studies conducted to design this series of experiments. When endophyte EDN2 was introduced into the noninfected calli of PDN2, the resulting plant behaved similarly to the PDN2 plant from which its endophyte was never removed. When endophyte EDN11 was introduced into calli derived from the noninfected form of PDN2, alkaloid content of the resulting infected plant was lower than that of the infected PDN11 plant from which its endophyte was never removed.

Ergopeptine alkaloid content increased numerically in all endophyte-infected plants with time, from 4- to 6-month-old plants (Table 1). This increase was presumed to be a consequence of larger and more vigorous plants that provided more substrate for the endophytes to utilize for alkaloid production. However, the increase was not significant when plant Genotype PDN2 was infected with the endophyte EDN11, suggesting that EDN11 and EDN2 behaved differently within a common tall fescue genotype. It is important to note that the ergopeptine alkaloid levels were the same in both PDN2 plants containing endophyte EDN2. This is despite the fact that the endophyte had never been removed from the plant in one case while, in the other, the endophyte had been

removed and later reinserted. Not only does this corroborate the lack of somaclonal variation during the regeneration process, it also indicates genetic stability of the endophyte during the culture and reinfection process. Likewise, it is important to note that ergopeptine alkaloid content of plants infected with endophyte EDN11 were different depending upon the plant genotype in which it resided.

The limited sample numbers with which this experiment was conducted do not permit far-reaching conclusions. Tall fescue genotype PDN11 does not appear amenable to callus induction using existing techniques, and consequently the reciprocal endophyte reintroduction has not been performed. However, these data suggest that the plant plays a major role in the expression of alkaloid production potential by endophytes, and although an endophyte may have a high capacity for ergopeptine alkaloid production, the genotype of the plant may mask the expression of that potential. This is a significant finding, as screening for endophyte-infected tall fescue plants low in ergopeptine alkaloid content does not assure that the endophyte remains a low alkaloid producer in subsequent seed generations. Therefore, when low-alkaloid-producing tall fescue–endophyte associations have been found, subsequent screening of their progeny will be essential to verify the true genetic potential of the endophyte.

REFERENCES

Bacon, C.W. and J. de Battista. 1990. Endophytic fungi of grasses. p. 231-256. *In* D.K. Arora (ed.) Advances in applied mycology. Marcel Dekker, New York.

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.

Eizenga, G.C., and L.S. Dahleen. 1990. Callus production, regeneration, and evaluation of plants from cultured inflorescences of tall fescue (*Festuca arundinacea* Shreb.). Plant Cell. 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., D.P. Belesky, and W.C. Stringer. 1991. Competitiveness of tall fescue as influenced by *Acremonium coenophialum*. Crop Sci. 31:185–190.

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.

Johnson, M.C., T.P. Pirane, M.R. Siegel, and D.R. Varney. 1982. Detection of *Epichloe typhina* in tall fescue by means of enzyme-linked immunosorbant assay. Phytopathology 72:647–650.

Kearney, J.F., W.A. Parrott, and N.S. Hill. 1991. Infection of somatic embryos of tall fescue with *Acremonium coenophialum*. Crop Sci. 31:979–984.

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

Pope, D.D., and N.S. Hill. 1991. Effects of various culture, media, antibiotics, and carbon sources on growth parameters of *Acremonium coenophialum*, the fungal endophyte of tall fescue. Mycologia 83:110–115.

Solomons, R.N., J.W. Oliver, and R.D. Linnabary. 1989. Reactivity of dorsal pedal vein of cattle to selected alkaloids associated with *Acremonium coenophialum*-infected fescue grass. Am. J. Vet. Res. 50:235–238.

Strickland, J.R., D.L. Cross, T.C. Jenkins, and M.A. Shoop. 1989. Bioassay of toxins of tall fescue using an isolated rat pituitary tissue perfusion system. p. 84–86. *In* H.A. Fribourg (ed.) Proc. of the Tall Fescue Toxicosis Workshop, Atlanta, GA. 13–14 Nov. Univ. of Tennessee Press, Knoxville.