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Pollen morphology and in vitro germination characteristics of nodulating and nonnodulating soybean (*Glycine max* L.) genotypes

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Abstract Artificial hybridization in highly self-pollinated crop species such as soybean (*Glycine max* L.) is important for both generating genetic variability and segregation for selection. In higher plants, pollen is an agent for transmission of genetic information over generations. The objective of this study was to measure and compare both morphological (length, width) and in vitro germination (germination percentage tube length) characteristics of pollen from the nodulating soybean cultivar, Bragg, and a nonnodulating Bragg mutant line, Nod 139, obtained following ethyl methanesulphonate treatment. Highly significant ($P = 0.007$) differences in pollen length were observed between these two genotypes. Similarly, in vitro germination percent (G%) indicated highly significant ($P = 0.0001$) differences between these genotypes, suggesting that the nodulation trait produces variation in in vitro germination capacity of the pollen. It appears the nonnodulation trait in soybean alters pollen grain length and G%.

Keywords In vitro germination · Nodulating · Nonnodulating · Pollen-tube length

Introduction

Legumes symbiotically associate with rhizobia to form nodules that fix N_2 , thereby dispensing with the application of nitrogenous fertilizers. Consequently, nodulation is desirable from this perspective. However, nonnodulating mutants have been reported in a wide variety of leguminous species including soybean (William and Lynch 1954; Mathews et al. 1989), peanut (Gorbet and Burton 1979), peas (Kneen and LaRue 1984), sweetclover (Miller

et al. 1991), chickpea (Singh et al. 1992) and common bean (Park and Buttery 1994).

Inheritance studies of the nonnodulation trait in various legumes have been conducted (Peterson and Barnes 1981; Nigam et al. 1982; Davis et al. 1986; Miller et al. 1991; Singh et al. 1992; Gallo-Meagher et al. 2001). In soybean, a single locus containing the rj_1 allele was found to control nonnodulation in a spontaneous mutant (Williams and Lynch 1954). Mathews et al. (1989) observed another allele (*nod139*) at a different locus that conditioned nonnodulation in two ethyl methanesulphonate (EMS) derived soybean mutants.

There is a shortage of information on the potential pleiotropic effects of nonnodulation on other plant processes such as flowering, pollen production and germination. In higher plants such as soybean, pollen is the paternal agent for the transmission of genetic information over generations. Because soybean is at least 98% self-pollinated, cultivar development programs for this species rely on hybridization for generating genetic variability and the segregation necessary for selection. The fertilization ability of pollen grains is a function of both pollen germination as well as pollen-tube growth. In soybean, fertilization occurs approximately within 3 h after pollination. The objectives of our study were to (1) measure and compare the morphology and dimensions of pollen from a nodulating wild-type soybean cultivar, Bragg, and its nonnodulating mutant line, Nod 139 and (2) evaluate the in vitro pollen germination capacity and the pollen-tube growth from each genotype 3 h after inoculation on the medium.

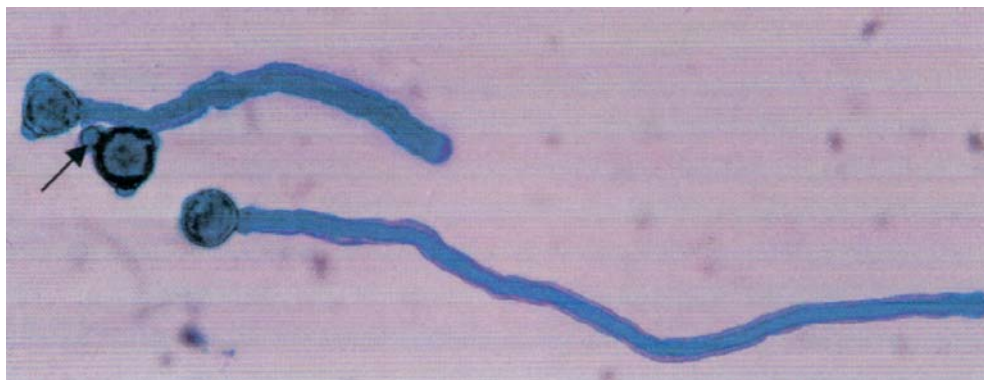
Materials and methods

Seeds of soybean cv. Bragg, homozygous for the nodulation trait, and the Bragg EMS-derived mutant line Nod 139 (Mathews et al. 1989) were grown in the greenhouse at day/night temperatures of approximately 28/20 °C and under a 14/10-h (day/night) photoperiod of natural light. Standard management and fertilization recommendations (including nitrogen) were followed at weekly intervals.

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Fig. 1 In vitro germination of soybean pollen grains: two germinated (*upper* and *lower*) grains showing pollen tubes of different lengths and grain (*arrow*) showing three pores



Mature flowers were randomly selected from each genotype between 9:00 and 10:00 A.M. on each of four consecutive days for pollen analysis. The length and width (in micrometers) of 100 dry pollen grains from each genotype were measured using a microprojector at 400× magnification. Pollen from each genotype was air-dried for 2 h and then inoculated onto an in vitro germination medium [5.0 g/l sucrose ($C_{12}H_{22}O_{11}$), 0.03% calcium nitrate ($Ca(NO_3)_2 \cdot 4H_2O$), 0.01% boric acid (H_3BO_3) and 0.5% agar] in petri dishes at room temperature (25 °C). Three h after inoculation, the petri dishes were flooded with a killing and fixing solution [5 parts formaldehyde ($HCHO$), 72 parts water (H_2O), 3 parts glacial acetic acid (CH_3OOH), 20 parts glycerin ($C_3H_5(OH)_3$)] to stop all activity.

The in vitro germination percent (G%) data were obtained by classifying the germination of 100 samples (ranging from 35 to 60 grains per sample) using a microprojector at 400× magnification. A grain was classified as germinated if at least one recognizable pollen tube was present. Pollen-tube length (PTL) data were obtained by measuring 184 randomly selected relatively straight pollen tubes that arose from grains producing only one pollen tube. The accuracy of the measurement increased if straight pollen were measured.

The experiment was a completely randomized design. An analysis of variance was performed on pollen grain dimensions (length and width) and in vitro germination characteristics (G% and PTL) using SAS procedures (SAS Institute 1989).

Results

Pollen morphology and dimensions

Dry pollen grains were rod-shaped. Differences in pollen length were highly significant ($P = 0.007$) with 30.8 μm and 29.5 μm for the nodulating and nonnodulating genotypes, respectively (Table 1). The differences in pollen width were not significant.

In vitro pollen germination

At the initiation of in vitro germination, pollen grains expanded to full volume by hydration, attaining a near-spheroidal shape with three distinct pores (Fig. 1). In some samples from both genotypes that had clumped pollen grains, the pollen tubes were entangled and appeared to grow faster than those from single grains.

The nodulating genotype significantly ($P = 0.0001$) exceeded the nonnodulating genotype by approximately

Table 1 Pollen grain length and width obtained in the nodulating genotype (Bragg) and nonnodulating mutant line (Nod 139) derived from Bragg (SE standard error)

Characteristic	Genotype	Mean ^a ± SE
Length ^b (μm)	Nodulating	30.8 ± 0.3
	Nonnodulating	29.5 ± 0.3
Width ^c (μm)	Nodulating	21.9 ± 0.2
	Nonnodulating	21.4 ± 0.3

^a $n = 100$

^b Probability (P) of t value = 0.0070

^c Probability (P) of t value = 0.1154

Table 2 In vitro pollen germination percentage (G%) and pollen-tube length (PTL) obtained in soybean genotypes Bragg (nodulating type) and Nod 139 (nonnodulating type)

Characteristic	Genotype	Mean ^a ± SE
G% ^b	Nodulating	76.3 ± 1.9
	Nonnodulating	64.1 ± 2.2
PTL ^c (μm)	Nodulating	314.0 ± 6.2
	Nonnodulating	295.3 ± 6.3

^a n : Germination percentage = 100 samples; tube length = 184 μm

^b Probability (P) of t value = 0.0001

^c Probability (P) of t value = 0.1190

20.0% in terms of pollen G% (Table 2). However, PTL measurements indicated the lengths of the two genotypes were not significantly different.

Discussion

We report here a relatively simple medium and reproducible procedure for in vitro germination of soybean pollen. In vitro germination of pollen from other crop species (Pfahler 1971; Pereira 1997) suggests that medium requirements such as optimal sucrose concentrations may vary widely within a species. Pfahler (1981) suggested that differences in in vitro pollen germination could be the result of complex interactions between the morphology and physiology of the pollen grain and the medium components. The structure and physiology of

the pollen grain is under genetic control and the medium composition is critical for both germination and tube growth.

The morphological traits of soybean pollen grains observed in this study, particularly the width, agree with those reported by Abel (1970). However, Vanhof and Harder (1995) found variations in pollen diameter within 17 species of papilionaceous legumes and attributed the variation to a trade-off between grain diameter and the number of pollen grains produced per flower. Quantitative variation in pollen grain dimension and volume has also been reported in other crop species (Pfahler et al. 1980, 1996, 1998). The differences in pollen dimensions would translate into considerable differences in the volume of pollen grains between the genotypes. If the pollen grains of each genotype are identical in shape, the smaller volume of the Nod 139 (about 90% of Bragg) grains might suggest that less energy would be available for germination and tube growth at both the in vitro and in vivo level. The differences between the two genotypes in in vitro germination characters may further indicate that pollen genotypes containing the non-nodulating allele influenced pollen transmission which, in turn, would result in aberrant Mendelian ratios. Aberrant ratios in any species make inheritance studies involving qualitative characters extremely difficult to interpret.

Differences in pollen morphology and in vitro germination characteristics may probably explain why, from an evolutionary standpoint, the nonnodulating trait is not fit, there is a lack of nitrogen fixation on the whole plant level, but there also may be a reduction in pollen transmission, especially in hybrids where competition with pollen containing the nodulation allele is present.

It appears the nonnodulating trait in soybean alters pollen germination capacity. However, we could not make unequivocal conclusions because of the limited number of genotypes used in this study. Linkage studies between the pollen traits and other nonnodulation loci in soybean could be interesting.

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