Unequal Growth Rate of Pollen Tubes from Normal and Stringless Pea Genotypes

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Abstract. There was no difference in percentage in vitro germination of pollen from stringless pea (*Pisum sativum* L.) cv. Sugar Daddy and stringy 'Oregon Sugarpod II' (OSP) and 'OSU 705' (705). However, pollen tubes of 'Sugar Daddy' grew more slowly in vitro than those of OSP or 705. Differences in pollen tube growth rate were demonstrated in vivo following time-course pollinations involving reciprocal crosses of 'Sugar Daddy' with OSP and 705, along with the selfed parents. After 8 hours, pollen tubes from stringless peas ("stringless" pollen) had entered 13% of the ovules compared with 51% for those from stringy peas ("stringy" pollen). Stringless pollen tubes entered 29% and stringy pollen tubes 66% of the ovules after 10 hours. The slower growth of stringless compared with stringy pollen tubes is a plausible explanation for previously observed deficiencies of stringless plants in segregating populations.

Development of edible pod peas with stringless pods has been of interest to breeders for many years, but especially so since "snap peas," those with thick pod walls conditioned by the gene *n* (Wellensiek, 1925), became commercially important after the introduction of 'Sugar Snap' by C. Lamborn of Gallatin Valley Seed Co. (Thorndyke, 1983). Snap peas with stringless pods have since been introduced by Lamborn, and stringless cultivars are being commercially processed.

Lamprecht (1938) described a stringless character controlled by a single recessive gene he called *sin*, but his material has apparently been lost. Wellensiek (1971) described a stringless mutation he obtained by treating the cultivar Dominant with ethyleneimine. His material, which is temperature sensitive in that stringlessness is not expressed below 15°C, has been used to develop the stringless edible pod pea (snow pea) 'Nofila' (Nunhem-Zaden Seed Co., Haelen, Holland) and several stringless snap peas, including 'Sugar Daddy' (Gallatin Valley Seed Co., Twin Falls, Idaho).

McGee and Baggett (1992) found that breeding stringless peas is complicated by apparent pleiotropic associations between stringlessness, smaller pod size, and lower productivity, and by recovery in the F₃ of fewer stringless segregates than expected for a single recessive gene or even duplicate re-

cessive genes. They concluded that a single recessive gene, designated *sin-2*, was involved. They found that the number of stringless segregates was adequate to fit a single recessive gene hypothesis (1 stringy : 1 stringless) when the backcross F₁ × stringless parent was tested. The reciprocal backcross, stringless parent × F₁, was deficient in stringless segregates, indicating that pollen carrying the stringless allele from the heterozygous F₁ was less competitive than pollen carrying the stringy allele.

The purpose of the present study was to compare the viability and growth rate of pollen from stringless-podded pea plants with pollen from stringy-podded plants to determine if differences in pollen tube growth rate could be responsible for the aberrant ratios of stringless : stringy plants observed in segregating generations.

Germination and pollen tube growth in vitro. The percent germination and growth rates of pollen from stringless and stringy sources were compared in vitro. Standard microscope slides were coated with a high-sucrose liquid medium (200 g sucrose, 15 g agar, 1 liter dH₂O, 20 ppm boric acid) and placed individually in 8.5-cm-diameter petri dishes. Flowers of stringy, edible pod 'Oregon Sugarpod II' (OSP, Baggett, 1982), snap pea 'OSU 705' (705), and stringless snap pea 'Sugar Daddy' were collected from vigorous greenhouse-grown plants. Greenhouse temperatures ranged from 21°C to ≈24°C day, and 15.5°C night. Fresh pollen, collected from the anthers of several plants before 10:00 AM, was dusted onto the cooled medium-coated slides. High humidity was maintained by sealing the lids onto the petri dishes with parafilm. The slides were placed in a well-lighted room at 30°C for 4 h, after which pollen germination was halted by placing the petri dishes in a freezer. This procedure was repeated on different days a total of four times

for pollen germination and three times for pollen tube growth. Germinating pollen was observed using standard bright field microscopy. The central portion of each slide was scanned and percent germination determined from the first 100 pollen grains observed. Pollen was considered germinated if the pollen tube was at least as long as the diameter of the pollen grain. Length of pollen tubes of the first 20 pollen grains encountered was measured using an ocular micrometer. Percent germination data were transformed by arcsin before analysis of variance.

The percent germination in vitro of pollen from all three cultivars was >90% (Table 1). There were no significant differences in percent germination due to pollen source. Pollen tubes of 'Sugar Daddy' were significantly shorter than those of either 705 or OSP, and pollen tubes of OSP were significantly shorter than those of 705.

Pollen tube growth in vivo. Reciprocal cross-pollinations were made in the greenhouse between 'Sugar Daddy' and 705 and between 'Sugar Daddy' and OSP. Each of these three parents was also manually self-pollinated. Flowers were emasculated before anthesis, after the filaments had fully elongated, and a liberal quantity of fresh pollen was dusted onto the receptive stigma. After 8, 10, and 24 h, pollinated flowers were excised and fixed in FAA [formaldehyde, 95% ethanol, and glacial acetic acid (1:8:1 by volume)] for 24 h and then transferred to 70% ethanol for storage. A total of 18 to 23 flowers was used for each cross or self-pollination within each time interval in a completely randomized design.

After removal from 70% ethanol, the flowers were rinsed in tap water and placed in 8 N NaOH for 8 h to soften and clear tissues. Flowers again were rinsed in tap water and transferred to distilled water for 1 to 2 h. Petals and sepals were removed, the pistil and ovary were dissected longitudinally, and the ovarian walls were removed. The remaining material, consisting of the pistil, the vascular strand from the lateral carpellary bundle, and the attached ovules, was placed in a small amount of stain (2% aniline blue in 20% K₃PO₄) on a microscope slide. Using ultraviolet light and aniline blue stain, callose, which lines and forms plugs in the pollen tubes, fluoresces bright bluish-white against a pale blue-gray background. Pollen

Table 1. Mean percent germination and tube length of pollen from stringless 'Sugar Daddy' and stringy (OSP and 705) podded peas when cultured in vitro for 4 h.

Cultivar	Germination* (%)	Pollen tube length* (mm)
Sugar Daddy	96.4	0.78 a
OSP	93.7	1.02 b
705	96.9	1.48 c
SE	2.8	0.02

*Nonsignificant ($F = 0.703$), determined by analysis of variance of arcsin-transformed data.

*Mean separation by Fisher's protected LSD at $P = 0.05$.

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Table 2. Mean squares for percent* fertilized ovules after varying time intervals following pollination with pollen from stringy or stringless-podded peas.

Source of variation	df	Mean squares		
		Hours after pollination		
		8	10	24
All crosses	6	4,464.30**	2,639.01**	684.36**
Stringless vs. stringy male, not selfed	1	15,008.64**	8,291.45**	2,137.42**
Stringless vs. stringy male, selfed	1	9,159.31**	6,962.32**	16.84
Residual	4	2,662.34**	903.24**	1,871.86**
Error	130, 72*	116.40	177.34	124.96

*Transformed by arcsin for analysis.

*Error df for 8 and 10 h.

*Error df for 24 h.

**Significant at $P = 0.01$.

Fig. 1. Percent of pollen tubes from stringless ('Sugar Daddy') and stringy (OSP) sources reaching ovules after controlled time course pollinations. (Letters a and b within each time denote mean separation by Fisher's protected LSD at $P = 0.05$.)

Fig. 2. Percent of pollen tubes from stringless ('Sugar Daddy') and stringy (705) sources reaching ovules after controlled time course pollinations. (Letters a, b, and c within each time denote mean separations by Fisher's protected LSD at $P = 0.05$.)

tubes growing down the style and into the ovules were observed with a Zeiss universal epi-fluorescence microscope (Model 47-34-15, Carl Zeiss, Oberkochen, Germany). The number and position of ovules with penetrating pollen tubes were determined and expressed as a percentage of the total number of ovules.

Following controlled time-course pollinations, there were significant differences in the percent of pollen from stringy vs. stringless sources in effecting fertilizations in vivo (Table 2). These differences were especially pronounced in the hours immediately following pollination but diminished as time progressed (Figs. 1 and 2).

Eight hours after pollination, pollen tubes

from 'Sugar Daddy' had entered <20% of the ovules of OSP, 705, or 'Sugar Daddy'. In contrast, pollen from 705 had entered 33% of the available ovules in self-pollinations, and >60% of the ovules of 'Sugar Daddy' flowers. OSP pollen tubes had entered ~55% of the ovules in both self-pollinations and in crosses with 'Sugar Daddy'. At 24 h, the only significant difference in the percentage of ovules fertilized by pollen from stringless and stringy sources was in a lower percentage of OSP and 705 ovules fertilized by 'Sugar Daddy' pollen.

Analysis of contrasts of means was used to differentiate between pollen performance per se and pollen performance as influenced by the genotype and environment of the style. The contrasts (Table 2) indicate that at each time interval, there are significant differences in the percentage of ovules fertilized, differences that can be attributed directly to the genotype of the pollen and are not merely reflections of the influence of the stylar environment.

The experiments described in this report were undertaken because segregation ratios observed in F_2 , F_3 , and backcross progenies involving stringless x stringy heterozygotes as the pollen source failed to produce the number of stringless-podded plants expected for a monogenic recessive character (McGee and Baggett, 1992). The assumption that stringlessness is controlled by a single recessive gene was based largely on its origin as a chemically induced mutation. The common factor shared by the F_2 and the backcross, stringless parent $\times F_1$, was that they resulted entirely from pollinations involving pollen from heterozygous plants. In the case of the F_2 families (derived from stringy F_2 plants), the segregating two-thirds of the families originated from selfed heterozygous plants, and the remaining one-third came from homozygous stringy plants. Because the reciprocal backcross, $F_1 \times$ stringless parent, was produced from stringless pollen and gave the expected number of stringless segregates, attention was focused on the germination and growth rate of pollen bearing the stringless gene.

Stringless pollen is functional, as indicated by normal pod set and fill usually observed in stringless cultivars. Since the percent

pollen germination of stringy and stringless pollen is equal, the slower growth rate of pollen tubes carrying the stringless gene *sin-2* supports a conclusion that stringless pollen may be less competitive than stringy pollen during germination and penetration of the style.

Time-course pollination studies (Figs. 1 and 2) provide more direct support for unequal pollen tube growth as an explanation for a deficiency of stringless individuals. Pollen tubes carrying the stringy allele grew down the style more quickly and reached the ovules before pollen tubes carrying the stringless allele. Ten hours after pollination, tubes from stringy pollen entered >60% of the ovules compared with ~30% for stringless pollen. The faster growth rate of stringy pollen is an effective mechanism for gametophytic selection and results in a disproportionate representation of stringy alleles in the next generation. Other examples have been documented in which genetic ratios or expressions were affected by unequal pollen tube growth in both selective (Searcy and Mulcahy, 1985; Zamir and Gadish, 1987; Zamir et al., 1981) and neutral (Barnes and Cleveland, 1963) environments.

Our results provide an example of overlapping effects of a gene that is expressed in both the gametophytic and sporophytic generations. When *sin-2* is expressed sporophytically, pods are stringless; however, there is a reduction in pod and plant size and plant productivity (McGee and Baggett, 1992). Gametophytic expression results in slow pollen tube growth. Although expression of *sin-2* apparently reduces the fitness of both gametes and sporophytes, it can result in unique and useful cultivars.

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