Title: Natural Outcrossing in Grasspea

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Abstract

The outcrossing rate of a species is important in designing experiments for inheritance and linkage studies and selection of appropriate breeding methods for crop improvement. Though predominantly self pollinated, frequent heterozygosity was found in isozyme studies grasspea (Lathyrus *sativus* L.). We established a study to estimate the rate of outcrossing in grasspea. Three planting methods, each with different frequency of the recessive parent, were repeated in three locations. Recessive white flower colour was used as a marker to detect outcrossing. Differences in the frequency of recessive alleles in the different planting methods were accounted in the calculation of outcrossing frequency. The estimates of outcrossing were homogeneous among eight families. The average outcrossing rate was 2.2%. We suggest that seed increases of grasspea be grown under isolation to maintain the genetic integrity of individual grasspea lines.

Introduction

The outcrossing rate of a species must be considered when making a decision on the most appropriate breeding method for improving the species. Knowledge of the outcrossing rate is also useful in designing experiments for genetic and linkage studies, and for maintaining genetic purity. Several studies in barley (Hordeum *vulgare* L.), lima bean *(Phaseolus lunatus* L.), wild oat *(Avena fatua* L.), and rose clover *(Trifolium hirtum* All.), all assumed predominantly self-pollinating species, have shown that low outcrossing rates (between 1 and 10%) had a significant effect on the genetic structure of the populations (Harding and Tucker 1964, Jain 1976). The observed heterozygosity in natural populations of grasspea *(Lathyrus sativus* L.) (Yunus *et al. 1991,* Chowdhury 1992) indicated the presence of outcrossing in this predominantly self-pollinated crop. Much bee activity was observed in *L. sativus* plots (Rahman *et al.* 1995) which indictes that bees are the predominant pollinators in grasspe. We conducted our experiment to determine the outcrossing rate in grasspea under field conditions in Saskatchewan.

Materials and methods

Selfed progenies of two grasspea lines, PI 426886b and PI 206891a, were selected for this study. The lines PI 426886 and PI 206891, obtained from the USDA, Regional Plant Introduction Station, Pullman, WA., were reselected for the uniformity of seed coat color which resulted the subacessions PI 426886b and PI 206891a. Line PI 426886b was homozygous dominant for blue flower color and PI 20689 1 a was homozygous recessive for white flower color. Previous studies had shown that blue vs. white flower color is conditioned by a single gene and blue is dominant over white. In F_2 population out of 100 individuals 27 and 73 were the white-flowered and blue-flowered

respectively and showed a good fit to 3: 1 ratio. All of the F, plants were blue-flowered (unpublished result). Three different experimental methods were used to estimate outcrossing rates.

Method 1:

The lines PI 426886b and PI 206891a were planted in alternate rows in a 4-row block with four replications. The rows were 3 m long with 1 m between rows and plants were spaced 15cm apart within each row. The white-flowered and blue-flowered plants were seeded in a 1:1 ratio. Five plants were randomly selected from each white-flowered row. Twenty seeds were sampled from each selected plant. Seed samples from the eight white-flowered rows were harvested, bulked into a family and progeny tested. The progeny of this family was checked for the frequency of blue-flowered heterozygotes. The outcrossing rate and its were estimated according to the procedure proposed by Jain (1979).

Method 2

Ten white-flowered plants were arranged in a staggered configuration (McKellar *et al*. 1991) and were surrounded by blue-flower plants, all with a 50 cm spacing between plants. The white-flowered and blue-flowered plants were seeded in a 1:2 ratio. One hundred seeds were sampled from each white-flowered plant. Seeds sampled from all 10 recessive plants were bulked into a family. Seeds of this family were sown in rows for progeny testing. The frequency of blue-flowered heterozygotes was determined. The outcrossing rate and its variance were calculated, as proposed by Jain (1979).

Method 3

This method is a modification of the design used by Wells *et al.* (1988). Thirty plants of dominant blue-flowered line PI 426886b were grown in each of 10 rows (300 plants), except that two plants of the recessive white-flowered line PI 20689 1 a were substituted into odd-numbered rows (10 plants). Thus, the white-flowered and the blue-flowered plants were grown in a ratio of 10:290 or 1:29. The inter- and intra-row spacing was 40 cm and 10 cm, respectively. This method was chosen to minimize the chance of homogeneous crossing. About 50 seeds were sampled from each of the recessive white-flowered plants (line PI 206891 a). Seed samples from the 10 white-flowered plants were bulked into a family, progeny tested, and the frequency of blue-flowered heterozygotes determined. The outcrossing rate and its variance were estimated, as proposed by Jain (1979).

Locations

Assuming the bee populations are different in number and kinds at different locations, the three methods were repeated at three locations near Saskatoon: 1) Preston Avenue plots, 2) the Kemen Farm, and 3) the Goodale Farm, in order to estimate the effect of different bee populations at the different locations on variation in outcrossing rate. Three families, one from each planting design, were grown at each location for a total of nine families. The outcrossing rate was estimated separately for each of the nine families. A χ^2 test for homogeneity was performed for homogeneity of the outcrossing rates for the nine families (Jain 1979).

Statistical Analyses

The outcrossing rate (t) was estimated as follows (Jain 1979):

$t = a/\{(a+b)*p\}$ ..... (1)

$\sigma^2_t = [t/\{(a+b)*p\}]*q/p$ ..... (2)

where, a and b are numbers of Aa (blue-flowered plants) and aa (white-flowered plants) among progeny-tested aa individuals; p and q are the frequencies of the A and a allele in the population in which outcrossing occurred.

The χ^2 for heterogeneity was calculated for the outcrossing rates among families as follows:

$\chi^2 = \Sigma\{(t_i - \bar{t})^2/s^2_i\}$; where

$\bar{t} = \Sigma(t_i/s^2_i)/\Sigma(1/s^2_i)$

where, t is the mean outcrossing rate, t, is the estimated outcrossing rate of different families and s_i is the standard error of the respective outcrossing estimates.

Results and Discussion

In some families, the number of progenies tested was less than planned due to either germination failure or because the parent plants did not produce enough seed. At the Goodale Farm, an early frost damaged the experiments which is reflected in a low number of observed progenies in method 1 and method 2 and loss of data for method 3.

The outcrossing rate ranged from 1.65 to 2.7% among the eight families (Table 1). The χ^2 test of homogeneity (χ^2 =1 SO, NS) shows that the outcrossing rate was uniform among the families and locations. The different planting methods had different gene frequencies for the recessive allele, but these differences were adjusted for in the estimate of outcrossing. Thus, the average outcrossing rate over the eight families was 2.16%.

The present outcrossing rate (2.16%) helps to explain the presence of heterozygosity in natural populations of grasspea. The grasspea lines are usually maintained by harvesting seed from lines grown in adjacent rows in the field. However, the outcrossing rate in this study (2.16%) suggest that individual lines of grasspea should be increased in isolation in order to maintain their genetic integrity. Further study should be conducted to determine the isolation distance required to minimize outcrossing in grasspea.

In our experiment the outcrossing rate was measured with only one genotype (PI 20689 1 a). However, inter-genotypic differences in outcrossing rate may also occur in grasspea. Rahman *et al.* (1995) observed significant variation in outcrossing rate among grasspea genotypes with different flower color. genotypes with red and white flower had the highest and lowest outcrossing rate (27.8% and 9.8%, respectively). It was assumed that these differences were due to the red-colored flowers attracting more bees than the white-colored flowers. The lower outcrossing rate in Saskatchewan may be explained by low bee activity due to fewer bees (no data collected) and the prevalence of low temperatures and strong winds in Saskatchewan during the grasspea growing season.

Metz *et al.* (1993) tested 180 faba bean *(Vicia faba* L.) genotypes and obtained outcrossing rates ranging from 1% to 55%. Similar large-scale trials should be conducted to provide a more generalize estimate of outcrossing rate in grasspea in Saskatchewan conditions. Moreover, since differential outcrossing rate might be locus dependent, as reported by Yunus *et al.* (1991) in grasspea, additional marker loci should be included.

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Table 1. Estimates of outcrossing rates (t) among eight different families of grasspea

Family[a]	(a+b)=N	a	b	q	p	t±SE
Ml-P	786	9	777	0.500	0.500	0.0229±0.0076
Ml-K	801	8	793	0.500	0.500	0.0199±0.0070
Ml-G	362	3	359	0.500	0.500	0.0165±0.0095
M2-P	1011	16	995	0.333	0.666	0.0237±0.0042
M2-K	958	17	941	0.333	0.666	0.0266±0.0046
M2-G	265	4	261	0.333	0.666	0.0226±0.0081
M3-P	520	10	510	0.033	0.9667	0.0199±0.0012
M3-K	503	10	493	0.033	0.9667	0.0206±0.0012
M3-G	-[b]					
Mean						0.0216

[a]M1-P= planting method 1 at the Preston Plots, Ml-K= planting method 1 at the Kemen Farm, M 1 -G= planting method 1 at the Goodale Farm, etc.

[b] = Missing.