Outcrossing in common bean

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ABSTRACT

Jalo Precoce, Pérola, Carioca and Michelite cultivars of Common Bean (*Phaseolus vulgaris* L.) were used to estimate natural hybridization rates. Two experiments were carried out, one in the "wet" season and the other in the 1997/1998 "fall-winter" season. Randomized complete blocks in a split plot scheme were used , with three replications. Four row spacings (0.20 m, 0.40 m, 0.60 m and 0.80 m) were randomized within the plots. Cultivars with a recessive genetic trait were planted in subplots, in alternate rows, with the IAPAR 44 cultivar , which has violet flowers and hypocotyl determined by dominant genes. In the "wet" season the Jalo Precoce, Pérola, Carioca and Michelite cultivars had average natural hybridization rates of 1.23, 1.34, 1.25 and 0.71%, respectively. The mean percentages were 0.82, 0.75, 0.79 and 0.76% for Jalo Precoce, Pérola, Carioca and Michelite cultivars in the "fall-winter" season, respectively. The estimates of natural hybridization had magnitudes which ranged from 0.71 to 1.66%.

KEY WORDS: *Phaseolus vulgaris* L., natural hybridization, cross-pollination.

INTRODUCTION

The flower structure of the common bean (*P. vulgaris* L.) characterizes it as a self pollinating plant since the reproductive organs are surrounded by petals, with self pollination taking place on or before the anthesis. However, cross pollination rate in self pollinating species in the field depends on cultivar type, distance among plants and environmental conditions, which, in turn, is affected insect population and activity (Vieira, 1978; Marques Júnior and Ramalho, 1995).

Knowledge of the outcrossing rate of a crop species in a specific environment is important when choosing a breeding procedure for crop improvement, germplasm maintenance, and commercial seed production.

Some plant breeders from México and Brazil have reported that the extent of outcrossing is probably negligeable with averages between 0.0 to 4.5% (Vieira, 1960; Pompeu, 1963; Pacova and Rocha, 1975; Pereira Filho and Cavariani, 1984; Crispin-Medina, 1960). Moderate levels of outcrossing (3.7% to 13%) were reported by breeders in Ethiopia, Sweden, and México (Stoetzer, 1984; Kristofferson, 1921; Miranda-Colin, 1974). Ibarra-Perez et al. (1997) reported that the mean outcrossing rate for the six white-seeded lines ranged from 4.41 to 10.16%. Junqueira Netto and Lasmar Filho (1971) found 1.02% of hybrid plants in the "wet" season in Lavras, Minas Gerais. Ortega (1974) also carried out outcrossing experiments during the "wet" and "dry" seasons in Venezuela.

Choice of germplasm and estimation method can influence levels of outcrossing (Wells et al., 1988). The above mentioned authors suggested that the genetic variation in the natural hybridization rates for the six cultivars studied in California was due to the great difference between the estimated mean for the FM 53 cultivar (66.8%) and the combined mean estimated for the other five cultivars (19.3%). An estimated natural hybridization mean of 18.8% was reported for the cultivars planted in May, while a considerable increase in hybridization rate was observed for the cultivars planted in July. The mean percentage of natural hybrids for the six cultivars was 27.3%. Nagata and Bassett (1985) tests on the F_3 progeny cultivated under field conditions showed natural cross pollination rates from 5.0 to 47% in 1980 and 56% in the F_4 progeny. This percentage decreased in 1982, remaining at 10%.

This contamination can act on superior genotypes, affecting genetic purity and seed production.

The efficiency and precision of the method used to determine the incidence of natural hybridization depends largely on the genetic marker used. An ideal marker should have the characteristics of dominance

and stablility, regardless of the environmental effects, and it should be easily distinguished. It should also appear during the first developmental stages of the plant. The hypocotyl color in beans fulfils all of these requirements (Alan and Moh, 1966).

Finally, depending on the rate of natural hybridization, intercrossing in a recurrent selection program may be advantageous. This study was conducted to determine the rate of natural hybridization in common bean cultivars, under field conditions, during the "wet" and "fall-winter" seasons in Maringá, State of Paraná.

MATERIAL AND METHODS

The present study was carried out at the Iguatemi Experimental Farm and the progenies were assessed in a greenhouse at the Nucleus for Research Applied to Agriculture (NUPAGRI), Laboratory for Seed Analysis and Irrigation Training Center at Maringá State University. Common bean seeds from Jalo Precoce and Michelite, Pérola, Carioca and IAPAR 44 cultivars were used.

Outcrossing experiments were carried out in August, 1997 and April, 1998, for the first and second planting, respectively. Pérola, Carioca, Michelite and IAPAR 44 cultivars have a longer cycle (95 days) than the Jalo Precoce cultivar (80 days). Jalo Precoce was planted 17 days after the other cultivars. A randomized complete block design with three replications and with split plot treatments was used for the two assays. The distance between rows was (0.20 m; 0.40 m; 0.60 m and 0.80 m) in the plots, while the cultivars were randomized in the sub-plots. Five rows of the IAPAR 44 cultivar were sown in the sub-plots. This cultivar has purple colored flowers and hypocotyls, which are controlled by a dominant gene used as a genetic marker to identify natural crosses. Other four rows were sown with one of the Michelite, Pérola, Carioca or Jalo Precoce cultivars, which have white flowers and green hypocotyls controlled by a recessive gene. Therefore, the odd and even numbered rows contained cultivars with the dominant and the recessive gene, respectively. The seeds were sown 0.10 m apart by hand, so that at flowering there was a sowing density of 15 plants per meter.

Natural hybridization estimates were obtained from the portion of offspring with violet hypocotyl derived from parents with green hypocotyl. Data was submitted to tests of normality and homogeneity of variance to check the basic analysis of variance. The pollinating insect population was identified in all the cultivars and spacings assumptions were met. Whenever the assumptions were not met, data were transformed by $\sqrt{y+0.5}$, where y is the rate of natural hybridization, according to Snedecor and Cochran (1967).

Row spacing, cultivars and the row spacing x cultivar interaction effects were considered fixed while the remaining effects were considered random. Estimates of the expected mean squares and combined residues followed the recommendations of Zonta (1978).

RESULTS AND DISCUSSION

The results of the joint analysis of variance showed significant effects ($P<0.05$) for the seasons x cultivars, row spacing x seasons and row spacing x cultivars x seasons interaction. However, the main effects of sowing seasons, cultivars and row spacing and the interaction row spacing x cultivar were not significant (Table 1). Ibarra-Perez et al. (1997) obtained similar results to those of this study when working with hybridization in the common bean.They reported that the year effects on the cross fertility rate were not significant. However, the year x location, sowing season and cultivars and location x sowing season x cultivars interactions were significant. Table 1 shows that the general reverse transformed mean of the natural hybridization rate of the cultivars was 0.45% for the four row spacings and two seasons. Results from this study indicated that the environment had a variable impact on the mean natural hybridization rate of the cultivars.This rate has already been estimated at almost twice this value, assuming that the natural hybridization rate within rows is the same as that among rows. The mean value obtained in this work is greater than several estimates obtained in studies carried out on common bean natural hybridization in Brazil and abroad (Mackie and Smith, 1935; Elgueta and Baillon, 1944; Ortega, 1974; Pacova and Rocha, 1975; Tucker and Harding, 1975; Park et al., 1996). The spacing x cultivar x season interaction in the analysis of variance was significant ($P<0.05$), allowing further statistical analyses.

Regression analyses of the natural hybridization data a function of the spacing for each season's cultivar were performed. The hybridization response to spacing up to a minimum value was obtained at approximately 0.60 m, for both the Jalo Precoce and

Pérola cultivars for the "wet" seasons with determination coefficient R^2=0.86 and R^2=0.99. The highest natural hybridization rate was recorded at 0.20 m row spacing. For instance, Alan and Moh (1966) reported the percentage of outcrossing detected was 0.20 and 0.19% at the 0.50 and 1 m distance, respectively. However, the percentage at the 0.25 m distance was 0.05%. According to the authors, it is possible that as the plants were so closely planted, it reduced the total plant area of the female parent exposed for insect visits.

The natural hybridization rates shown by the Carioca and Michelite cultivars for the "wet" seasons are not significantly different ($P<0.05$) among the row spacings. The studied cultivars in the "fall-winter" period of 1998 also were not significantly different due to spacing.

The common bean cultivars were assessed in each season and spacing in the next partition of the spacing x cultivar x season interaction. Table 2 shows the natural hybridization percentages of the Jalo Precoce, Pérola, Carioca and Michelite cultivars, respectively, depending on the different row spacings, which were obtained in the "wet" and "fall-winter" period of 1997/1998.

The Jalo Precoce, Pérola, Carioca and Michelite cultivars showed a similar tendency for the natural hybridization rate for each of the row spacings, both for the "wet" and the "fall-winter" seasons.

The highest natural hybridization rate was 1.66% for the Jalo Precoce and Pérola cultivar, while for the other cultivars there was a gradual reduction in the percentages, up to a value of 0.71. The estimates of natural hybridization were comparable to those reported by Vieira (1960), Pompeu (1963) and Pacova and Rocha (1975). In this study, during the "wet" 1997 season, the cultivar Michelite showed a lower cross-natural rate, when compared to other cultivars.

The Pérola cultivar showed a significant difference in the natural hybridization percentage in the 0.20 m between row spacing in the "wet" and "fall-winter" seasons, with 1.66% and 0.78%, respectively (Table 3). The Pérola cultivar has a flower morphology more suitable for insect penetration and permanence, suggesting the different behavior of the Pérola cultivar

Table 1. Combined analysis of variance of percent natural hybridization for four genotype, four spacings and two seasons, in 1997 and 1998, at Maringá, PR.

Source of variation	Degrees of freedom	Mean squares
		Natural hybridization (%)
Replication/seasons	4	0.0588
Seasons (S)	1	2.8722
Spacing (EP)	3	0.2070
EP x S	3	0.0943 [1/]
Error (a)	12	0.0058
Cultivars (C)	3	0.5282
EP x C	9	0.0323
S x C	3	0.4678 [1/]
EP x C x S	9	0.0267 [1/]
Error (b)	48	0.0107
C.V. (%) error (a)	7.93	
C.V. (%) error (b)	10.34	
Mean total (%)	0.96	Reverse transformed mean: 0.45

[1/] Mean square significant at P=0.05.

Table 2. Percentage of natural hybridization, obtained for the four cultivars in the wet and fall-winter planting seasons, in four spacings, in Maringá, PR [1/].

Planting season	Spacing (m)	Cultivar			
		Jalo Precoce	Pérola	Carioca	Michelite
		(%)			
"wet"	0.20	1.66 a	1.66 a	1.41 a	0.71 a
	0.40	1.10 a	1.26 a	1.17 a	0.71 a
	0.60	1.14 a	1.13 a	1.17 a	0.71 a
	0.80	1.01 a	1.31 a	1.25 a	0.71 a
"fall-winter"	0.20	0.89 a	0.78 a	0.86 a	0.79 a
	0.40	0.82 a	0.75 a	0.80 a	0.78 a
	0.60	0.80 a	0.74 a	0.77 a	0.75 a
	0.80	0.77 a	0.74 a	0.76 a	0.73 a

[1/] Means followed by the same letter in the line did not differ at the 5% level of probability by the F test.

compared to the other cultivars. The difference obtained in the 0.20 m row spacing was statistically significant at the level of 5% probability by the F test. In addition, Jalo Precoce, Carioca and Michelite cultivars in any of the four row spacings were not significantly different among the planting seasons. The Pérola cultivar (Table 3) at the 0.20 m between row spacing showed greater natural hybridization values in the "wet" season, similar to those found by Marques Júnior and Ramalho (1995). According to author (Vieira, 1960; Pompeu, 1963; Marques Júnior and Ramalho, 1995; Ibarra-Perez et al., 1997), the percentage of natural hybridization in the common bean crop varies from one season to another.

Eight types of insect were identified in the cultivar flowers: *Frankliniella occidentalis*, *Trigona* sp., *Apis mellifera*, Halictidae and *Xylocopa* sp. predominated among the others.

The changes in the natural hybridization rate among the different years may be associated with the variation in the number of insects. Although this was not assessed numerically, the insects were more frequent in the "wet" season than in the "fall-winter" season. The temperatures are lower in the "fall-winter" season and thus a lower insect frequency was found in this study.

The relative contribution by insects frequenting flowers to outcrossing among different cultivars showed average natural hybridization rates of 1.23, 1.34, 1.25 and 0.71% for Jalo Precoce, Pérola, Carioca and Michelite, respectively for "wet" seasons. In the "fall-winter" period the mean percentages were 0.82, 0.75, 0.79 and 0.76, respectively for the Jalo Precoce, Pérola, Carioca and Michelite cultivars. The natural hybridization estimates obtained in this study ranged from 0.71 to 1.66%. The Jalo Precoce and Pérola cultivars showed a quadratic response to the spacing for the "wet" season. Although the rate of outcrossing in Maringá was relatively low, it was sufficient to permit some crossing between individuals of the same population, increasing slightly the genetic variability. The isolated cultivars which were obtained from individuals in the fields and selected by the pedigree method may have appeared as result of such sporadic crossings. It also suggests that, in the seed multiplication plots, the cultivars should be planted in isolation or with borders to avoid possible contamination by natural crossings.

RESUMO

Cruzamento natural em feijoeiro comum

Os cultivares Jalo Precoce, Carioca, Pérola e Michelite foram utilizados para estimar a taxa de hibridação natural em feijoeiro *Phaseolus vulgaris* L., em

Table 3. Percentage of natural hybridization, obtained in the four cultivars in the wet and fall-winter planting seasons in four spacings, in Maringá, PR [1/].

Cultivar	Planting season	Spacing (m)			
		0.20	0.40	0.60	0.80
		(%)			
Jalo Precoce	‘‘wet’’	1.66 a	1.10 a	1.14 a	1.01 a
	"fall-winter"	0.89 a	0.82 a	0.80 a	0.77 a
Pérola	‘‘wet’’	1.66 a	1.26 a	1.13 a	1.31 a
	"fall-winter"	0.78 b	0.75 a	0.74 a	0.74 a
Carioca	‘‘wet’’	1.41 a	1.17 a	1.17 a	1.25 a
	"fall-winter"	0.86 a	0.80 a	0.77 a	0.76 a
Michelite	‘‘wet’’	0.71 a	0.71 a	0.71 a	0.71 a
	"fall-winter"	0.79 a	0.78 a	0.75 a	0.73 a

[1/] Means followed by the same letter in the column did not differ at the 5% level of probability by the F test.

Maringá, Paraná. Esses cultivares apresentam como fator genético recessivo, flores brancas e hipocótilo verde. Foram conduzidos dois ensaios, um no período das “águas” e o outro no período de “outono-inverno” nos anos agrícolas de 1997/1998. O delineamento experimental utilizado foi em blocos casualizados, em esquema de parcelas subdivididas, com três repetições. Na parcela casualizou-se os quatro espaçamentos entre fileiras (0,20 m, 0,40 m, 0,60 m e 0,80 m). Nas subparcelas foram semeados os cultivares possuidores do fator genético recessivo, em fileiras alternadas com o cultivar IAPAR 44 portador de flores e hipocótilo violeta, fator este, condicionado por gene dominante. No período das “águas” os cultivares Jalo Precoce, Pérola, Carioca e Michelite apresentaram taxa de hibridação natural de 1,23; 1,34, 1,25 e 0,71%, respectivamente. As percentagens médias obtidas no perído de “outono-inverno” foram 0,82, 0,75, 0,79 e 0,76%, para os cultivares Jalo Precoce, Pérola, Carioca e Michelite, respectivamente. As estimativas de hibridação natural apresentaram magnitudes que variaram de 0,71 a 1,66%.

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