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Determination of the outcrossing rate of *Phaseolus vulgaris* L. using seed protein markers

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Summary

The outcrossing rates of four varieties of *Phaseolus vulgaris* in Asturias (Northern Spain) were studied using seed protein polymorphisms as genetic markers. No evidence of outcrossing was obtained, and the outcrossing rate of this species at Asturias was estimated, with a confidence of 95%, as being less than 0.74%. The usefulness of seed proteins as genetic markers for obtaining estimates of outcrossing is also discussed.

Introduction

The outcrossing rate of a species is a very important aspect to be considered in order to establish strategies for plant breeding or germplasm management and conservation. The common bean (*Phaseolus vulgaris* L.) has been considered a highly self-pollinated species. However, the outcrossing rates reported so far range from less than 1% (Ortega, 1974; Tucker & Harding, 1975; Park et al., 1996) to as high as 66% (Wells et al., 1988). High outcrossing rates are often considered to be caused by pollination through insects, such as thrips or different species of bees (Antunes et al., 1973; Wells et al., 1988; Park et al., 1996).

The estimation of the outcrossing rate in these experiments was based on the expression of a monogenic dominant marker in the offsprings of at least two jointly grown genotypes differing for such marker. The color of either the hypocotyl, the flower or the seed, all of them determined by the *P/p* gene (Leakey, 1988), was the marker analyzed in most cases. The use of these morphological characters, as well as most molecular markers, such as isozymes or DNA polymorphisms, have the disadvantage of requiring the sowing of the offspring in order to evaluate their expression. In the case of seed color, the evaluation has to be done after the growing cycle is completed due to the maternal origin of the testa.

This paper reports the results of a study in which seed proteins have been used as markers in order to estimate the outcrossing rates in four *P. vulgaris* cultivars in Asturias (Northern Spain).

Materials and methods

Plant material

Cultivars Andecha (A), Bonafema (B), Garrafal Oro (G) and Zondra (Z) were used in this study. These four cultivars have indeterminate climbing growth habit. Andecha and Bonafena are closely related white dry bean cultivars proceeding from a selection of Asturian landraces. Garrafal Oro and Zondra are commercial varieties commonly used for green pod production in Asturias.

Experimental design

Sowing was carried out simultaneously at two different localities at Asturias (Villaviciosa and Llanera) in 1992. In each plot, 30 plants of each type (A, B, G and Z) were sown. Plants of the same cultivar were never direct neighbors: rows having the sequence -A-B-A-B-, alternated with rows having the sequence -G-Z-G-Z-. A 60 cm plant spacing and a distance

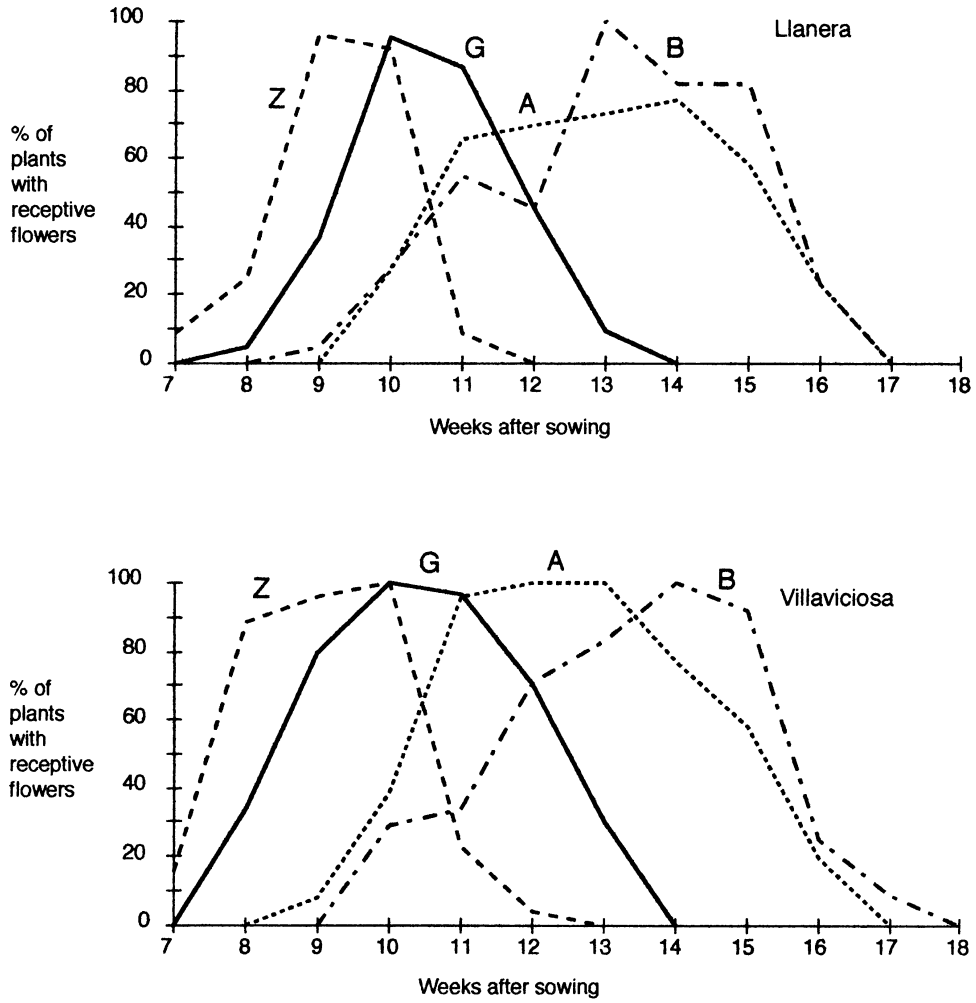

Figure 1. Flowering periods (determined as the percentage of plants with receptive flowers) of the cultivars Andecha (A), Bonafema (B), Garrafal Oro (G) and Zondra (Z) in the two localities.

between rows of 1 m were used in order to facilitate the collection of the seeds from each individual plant.

A honeybee hive was placed within the plot in Villaviciosa in order to ascertain the possible pollination effect caused by honey bees.

The flowering period (in weeks after the sowing date) was scored for each plant.

The plants were individually harvested and the protein pattern was analyzed in 800 seeds per cultivar (2 seeds per pod, 10 pods per plant, 20 plants per cultivar and locality).

Seed protein analysis

Seed protein patterns were determined by SDS-PAGE (Laemmli, 1970; Ma & Bliss, 1978) using 12% (w/v) polyacrylamide slab gels. Proteins were extracted from flour samples (0.01–0.02 g) taken from the raphe end of each seed, using a buffer (62 mM TRIS-C1H pH 8.8, 2% (w/v) SDS, 10% glycerol and 0.005% (w/v) bromphenol blue) during six hours at room temperature. The extracts were later reduced with one drop of 2-mercaptoethanol (2-ME). The mixture was heat-treated (100 °C) during 5 minutes, centrifuged and the supernatant was submitted to electrophoresis. The proteins were visualized using Coomassie Brilliant Blue R.

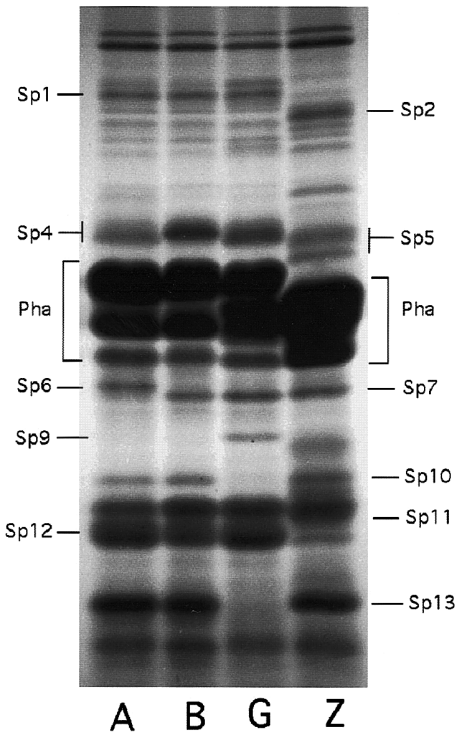

Figure 2. Polyacrylamide gel showing the seed protein patterns (SDS-PAGE) of cultivars Andecha (A), Bonafema (B), Garrafal Oro (G) and Zondra (Z). Bands corresponding to phaseolins (Pha) and other seed proteins are indicated (see text for explanations about allelic relationships).

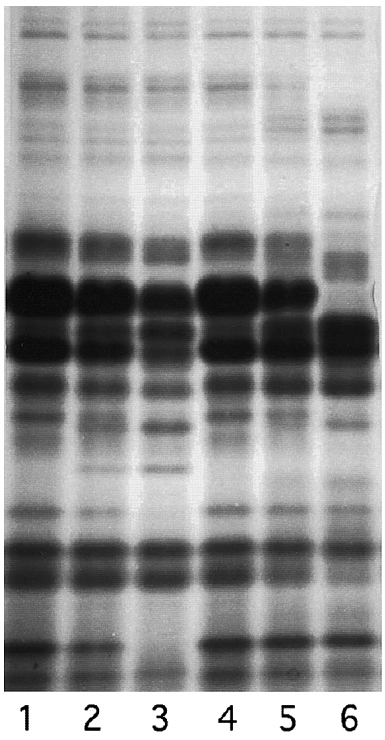

Figure 3. Polyacrylamide gel showing the protein patterns (SDS-PAGE) of F1 seeds obtained from artificial crosses between cultivars Andecha, Garrafal Oro and Zondra. lane 1: Andecha; lane 2: F1 Andecha × Garrafal Oro; lane 3: Garrafal Oro; lane 4: Andecha; lane 5: F1 Andecha × Zondra; lane 6: Zondra.

Apparent molecular weights of seed proteins were determined from the mobilities of the following proteins included in the MV-SDS-200 kit (Sigma): myosin (205,000), b-galactosidase (116,000), phosphorilase B (97,400) bovine albumin (66,000), egg albumin (45,000) and carbonic anhydrase (29,000).

Results and discussion

Synchrony in flowering periods should be taken into account in the determination of the outcrossing rate between two given varieties. Figure 1 represents the flowering periods of the four cultivars in the two localities. A relatively high degree of coincidence appeared, on one hand, between cultivars G and Z, which were characterized by an earlier and shorter flowering period, and on the other hand, between the dry bean cultivars A and B, having a later and longer one. The maximum synchrony in flowering between the four cultivars was produced from the 10th to the 11th week after sowing.

The seed protein electrophoretic patterns (SDS-PAGE) of the four cultivars A, B, G and Z are shown in Figure 2. Within each cultivar, all seeds had the same protein pattern. According to the phaseolin classification of Brown et al. (1981), cultivars A and B showed a phaseolin pattern type T, whereas cultivars G and Z showed phaseolin patterns types C and S, respectively. Among the other protein bands appearing in Figure 2, the following codominant allelic pairs were previously established from segregation studies (Ferreira, 1996): band clusters indicated as Sp1 and Sp2; band clusters indicated as Sp4 and Sp5, bands indicated as Sp6 and Sp7; bands indicated as Sp9 and Sp10; and band clusters indicated as Sp11 and Sp12 (probably corresponding to lectins). Band indicated as Sp13 segregated as presence-absence.

These different protein patterns allow the identification of all hybrid seeds proceeding from any outcross between the four cultivars. Figure 3 shows some examples of hybrid seeds obtained from artificial crosses between cultivars A, G and Z.

The protein pattern of all seeds scored corresponded to that of the female parent from which they proceeded, i.e., no evidence of outcrossing was observed.

The value for the outcrossing rate (α) of a given cultivar can be calculated as follows (Tucker & Harding, 1975). Let T be the observed frequency of outcrosses in a progeny. The probability (p) of observing at least one seed showing evidence of outcrossing among 800 seeds proceeding from a given cultivar is:

$$p = 1–(1–T)^{800}$$

Since no evidence of outcrosses was observed, p can be considered as the confidence for a T value. Setting a confidence of 95% (p =0.95) a value of T = 0.0037 results.

As it is not possible to detect seeds derived from outcrosses between plants of the same cultivar, T is smaller than the true outcrossing rate, α. Since, in the experiment carried out in this work there is the same number of plants of the four cultivars, considering a 100% coincidence in their flowering periods, the outcrossing rate between plants of the same cultivar would be $\alpha/4$. Then, the observed frequency of outcrosses between plants of different cultivars, T, would be $3\alpha/4$ (α = 4/3T). From the results shown in Figure 1, a minimum value of flowering period coincidence of 50% can be deduced (outcrossing rate between plants of the same cultivar = $\alpha/2$), and therefore, a maximum value of α would be estimated as 2T.

For these above reasons, it can be concluded that the true outcrossing rate of the four cultivars analyzed should not be higher than 0.74%.

This low outcrossing rate agrees with the results obtained in the majority of previous studies on outcrossing in common bean (Mackie & Smith, 1935; Barrons, 1939; Elgueta & Baillon, 1944; Vieira, 1960; Crispin-Medina, 1960; Pompeu, 1963; Alan & Moh, 1966; Antunes et al., 1973; Ortega, 1974; Miranda Colin, 1974; Tucker & Harding, 1975; Vieira Pacova & Rocha, 1975; Pereira & Cavariani, 1984; Stoetzer, 1984; Martin & Adams, 1985; Brunner & Beaver, 1989; Triana et al., 1994; Park et al., 1996). Only four of these cases reported outcrossing rates higher than 5% for some varieties.

Wells et al. (1988) reported exceptionally high levels of outcrossing (up to 85%) that were later explained by Ibarra-Perez et al. (1997) through the existence of a high heterogeneity in outcrossing rates among plants (ranging from 0.0 to 78%) within the same variety. In the present work, the individualized offsprings of 160 plants (40 plants per variety; 20 seeds per plant) were analyzed, but no such a variation was found.

The results obtained indicate that in the environments in which this work was carried out, even with a high density of honeybees, no special precautions for avoiding outcrossing have to be taken to obtain selfed seed. This work also indicates the usefulness of seed proteins as genetic markers in the estimation of outcrossing rates in common bean. Seed proteins provide a relatively high number of codominant markers, so allowing to identify all possible hybrids between different cultivars. Therefore, a unique experiment involving the simultaneous growth of several varieties can be performed in order to estimate their specific outcrossing rates.

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References

Alan, J.J. & C.C. Moh, 1966. Determinación del porcentaje de cruzamiento natural en el frijol común (*Phaseolus vulgaris* L.) en Alajuela, Costa Rica. Turrialba 16: 156–158.

Antunes, F.I., J.G.C. da Costa & E.A. Oliveira, 1973. Natural hybridization in *Phaseolus vulgaris* L. in Pelotas (Brasil). Anu Rep Bean Improv Coop 16: 61–62.

Barrons, K.C., 1939. Natural crossing in beans al. different degrees of isolation. Proc Amerc Soc Hortc Sci 36: 637–640.

Brown, J.W.S., Y. Ma, F.A. Bliss & T.C. Hall, 1981. Genetic Variation in the subunits of Globulin-1 storage protein of french bean. Theor Appl Genet 59: 83–88.

Brunner, B.R. & J.S. Beaver, 1989. Estimation of outcrossing of the common bean in Puerto Rico. Hortic Sci 24: 669–671.

Crispin-Medina, A., 1960. Cruzamiento natural en frijol. Agricultura Tecnica en Mexico 11: 38–39.

Elgueta, M. & L. Baillon, 1944. Ensayo de fecundación ajena en frejoles. Agricultura técnica 4: 38–40.

Ferreira, J.J., 1996. Caracterización y mapas genéticos en Phaseolus vulgaris L para la mejora genética de la variedad granja asturiana. Ph.D. Thesis, University of Oviedo.

Ibarra-Perez, F.J., B. Ehdaie & J.G. Waines, 1997. Estimation of outcrossing rate in common bean. Crop Sci 37: 60–65.

Laemmli, V.K., 1970. Cleavage of structure proteins during assembly of the head of bacteriophage T4. Nature 22: 680–685.

Leakey, C.L.A., 1988. Genotypic and phenotypic markers in common bean. In: P. Gepts (Ed.), Genetic Resources of Phaseolus Beans, pp. 245–327. Kluwer Academic Publishers.

Ma, Y., & F.A. Bliss, 1978. Seed proteins of common bean. Crop Sci 18: 431–437.

Mackie, W.W. & F.L. Smith, 1935. Evidence of field hybridization in beans. J Amer Soc Agron 27: 903–909.

Martin, G.B. & M.W. Adams, 1985. The role of outcrossing in the generation of variability in Malawian bean landraces. Annu Rep Bean Improv Coop 28: 49–50.

Miranda-Colin, S., 1974. Cruzamiento natural en frijol. Campo 49: 34–40.

Ortega, S.V., 1974. Polinización cruzada natural de la caraota (*P. vulgaris* L.) en Venezuela. Agronom. Tropical 24: 27–32.

Park, S.J., T.E. Michaels, J.R. Myers, D.W.A. Hunt & K. Stewart-Williams, 1996. Outcrossing rates of common beans grow in Ontario and Idaho. Annu Rept bean Improv Coop 39: 90–91.

Pereira Filho, I.A. & C. Cavariani, 1984. Taxa de hibridaçao natural do feijoeiro comun en Patos de Minas, Minas Gerais. Pesquisa Agropecuaria Brasileiro 19: 1181–1183.

Pompeu, A.S., 1963. Polinizaçao cruzada natural no freijoeiro. Bragantia 22: 53–57.

Stoetzer, H.A.I., 1984. Natural cross-pollination in bean in Ethiopia. Annu Rep Bean Improv Coop 27: 99–100.

Triana, B., M. Iwanaga, M. Rubiano & M. Andrade, 1994. A study of allogamy in wild *Phaseolus vulgaris* L. In: W.M. Roca, J.E. Mayer, M.A. Pastor-Corrales & J. Tohme (Eds.), Phaseolus Beans Advanced Biotechnology Research Network. pp. 97–103. CIAT, Cali, Colombia.

Tucker, C.L. & J. Harding, 1975. Outcrossing in common bean *Phaseolus vulgaris* L. J Amer Soc Hort Sci 100: 283–285.

Vieira, C., 1960. Sobre a hibridaçao natural em *Phaseolus vulgaris* L. Revista Ceres 11: 103–107.

Vieira-Pacova, B.E. & A.C.M. Rocha, 1975. Hibridaçao natural no feijoeiro (*Phaseolus vulgaris* L.), em Linhares, Espirito Santo. R Ceres 22: 157–158.

Wells, W.C., W.H. Isom & J.G. Waines, 1988. Outcrossing rates of six common bean lines. Crop Sci 28: 177–178.