9-1971

Cross-Fertilization in *Melilotus alba*

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Published in Crop Science (September-October 1971) v. 11, no. 5

CROSS-FERTILIZATION IN *MELILOTUS ALBA*[1]

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ABSTRACT

Two simply inherited characters, *o*-hydroxycinnamic acid (*o*-HCA) content and β-glucosidase activity, were used as genetic markers in studying the frequency of cross-fertilization in *Melilotus alba* Desr. Based on the *o*-HCA marker, the effective cross-fertilization averaged 58% for two harvest years in a biennial population and 67% in a 1-year study with an annual population. Use of the β-glucosidase marker provided a cross-pollination estimate of 72% in the biennial population. The extent of natural crossing in sweetclover appears to be sufficient to restore normal vigor in the production of varieties synthesized from selected inbred lines.

Additional index words: **Sweetclover, *o*-hydroxycinnamic acid, β-glucosidase, Genetic markers.**

POLLINATION of sweetclover (*Melilotus alba* Desr.) flowers is effected principally by honey bees, although a variety of other insects also is known to pollinate flowers of this species (1, 2). Flowers do not set seed unless tripped (2); tripped flowers of cultivated forms of *M. alba* may be self- or cross-pollinated. This combination of self-fertility and pollination by insects results in a seed crop that is produced by a mixture of self- and cross-fertilization. It would be expected that the relative extent of selfing and crossing would be influenced by many interacting factors, including weather and soil conditions, number and kind of pollinating insects, variety of sweetclover, and plant spacing.

Literature reports dealing with the estimation of cross-fertilization in *M. alba* are rare. In 1927 Smith (11) reported the use of natural hybrids in a study of the inheritance of annual versus biennial growth habit. He observed that among 227 seeds produced by natural pollination, 126 gave rise to hybrid plants. From these results 56% cross-fertilization may be inferred. Smith did not describe the planting arrangement of the two types used in this study. Later, Kirk (9) observed a crossing percentage of 57% in producing natural hybrids in an inheritance study of the dwarf branching habit of growth. Individual plants having the dwarf habit, which is inherited as a simple recessive, were surrounded for several meters by plants having the normal habit of growth. Progeny of 13 plants of the dwarf branching type were classified for habit of growth.

Neither of the studies just cited was designed to yield an accurate estimate of the percentage of cross-fertilization in *M. alba*. The objective of the present study was to obtain such an estimate, utilizing the *Cu/cu* and *B/b* allelic pairs as genetic markers. The *Cu/cu* alleles influence the content of *o*-hydroxycinnamic acid (*o-HCA*) glucosides and are independent of the *B/b* alleles that determine the presence or absence of β-glucosidase activity. Plants or the *CuCu* genotype are high in content of *o-HCA* glucosides, and preparations of the *BB* genotype possess β-glucosidase activity (6, 10). The *Cu* and *B* genes are both lacking in dominance (3, 7). These alleles, derived from *M. dentata* (Waldst. & Kit.) Pers. by interspecific hybridization, have not been used previously in cross-fertilization studies.

[1]Contribution from the Plant Science Research Division, Agricultural Research Service, U. S. Department of Agriculture, and the Nebraska Agricultural Experiment Station, Lincoln, Nebr. 68503. Supported in part by the National Science Foundation (Grant Nos. GB-1148 and GB-8280). Published with the approval of the Director as Paper No. 3068, Journal Series, Nebraska Agr. Exp. Sta. Research reported was conducted under Project No. 27. Received March 1, 1971.

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Materials and Methods

'Denta' sweetclover, a variety low in *o*-HCA, was used as the seed parent in this study. Approximately 50% of Denta plants have the *cucubb* genotype; the remaining 50% are *cucuB-* (4). Both types were used in this experiment, the genotype of individual plants being determined by means of a rapid, qualitative test (8). The variety 'Evergreen' (*CuCuBB* genotype) was used as the pollen parent. Plantings were established in drilled rows. Rows of Evergreen were 0.9m apart and single Denta plants were 3m apart in the Evergreen rows. In odd-numbered rows, the first Denta plant was 1.5m from the end of the row; in even-numbered rows this distance was 3m. The total area of the experiment was approximately 350 m^2. The maturity of the variety Evergreen is similar to that of Denta. In these experiments, Evergreen began flowering several days earlier than Denta, and both varieties continued flowering for approximately the same period of time. Identical plantings of Denta and Evergreen were made in 1963 and 1965; seed production occurred in 1964 and 1966.

Cross-fertilization in annual *M. alba* was measured in 1965 using a low *o*-HCA line having the *cucuBB* genotype. The high *o*-HCA variety, Floranna, served as the pollen parent. The total area and arrangement of plants were similar to those used in the Denta × Evergreen experiments described in the preceding paragraph.

Progenies of individual Denta plants in the Denta × Evergreen experiments were grown in rows approximately 5 cm apart in 35- × 50-cm greenhouse flats containing a 3:1 mixture of composted soil and sand. The number of plants per progeny varied from 10 to 400 because of wide differences in seed production among the Denta plants. Progenies were grown for approximately 2 to 3 months under natural illumination supplemented with incandescent light to provide an 18-hour photoperiod.

When most plants in the progenies were at an early bud stage, a portion of a leaflet from each plant was tested qualitatively for *o*-hydroxycinnamic acid as described previously (8). Progenies of *cucubb* plants also were tested for β-glucosidase activity by a similar rapid procedure (8).

Results and Discussion

Progenies from Denta plants of the *cucubb* genotype contained an average of 61% high-*o*-HCA seedlings compared to 56% for plants of the *cucuB-* genotype (Table 1). These differences in percentage cross-

Table 1. Frequency of *Cu* plants in progenies of Denta plants of the *cucubb* and *cucuB-* genotypes interplanted with the variety, Evergreen (*CuCuBB* genotype).

Genotype of Denta parent	Harvest year	Number of progenies	No. of plants tested	Avg % Cu per progeny*
cucubb	1964	13	895	62
	1966	29	2,095	60
	Both	42	2,990	61
cucuB-	1964	16	1,517	55
	1966	22	1,435	57
	Both	38	2,952	56
Mean (both genotypes and both years)				58

* Cu plants represent those resulting from fertilization of the Denta parent with pollen from the Evergreen parent. The frequency of such plants was calculated for each progeny. Values shown are the averages of these progeny values, without regard to differences in the number of plants in the various progenies.

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ing between the *cucubb* and *cucuB-* genotypes were nonsignificant within years and for both years. Results for the two harvest years were very similar, with an average of 58% cross-fertilization for the 2-year period. This value is probably somewhat underestimated, since experience with the qualitative test for *o-HCA* (8) suggests that the tendency to classify *Cucu* plants as low-*o*-HCA is greater than the tendency to classify *cucu* plants as high-*o*-HCA. Difficulties in correctly classifying *Cucu* plants are intensified if growth conditions are suboptimal.

A value of 72% cross-fertilization was obtained for the 2-year period by measuring the frequency, in progenies from *cucubb* plants, of plants having a high level of β-glucosidase activity. The *B/b* alleles, which control β-glucosidase activity, are probably better markers than the *Cu/cu* alleles, because the high activity of this enzyme permits excellent discrimination between *bb* and *Bb* genotypes in the qualitative test, even though the *B* allele is lacking in dominance. An additional advantage of using the *B/b* alleles as markers is the relatively simple procedure for the extraction of the substrate used in the test for the *B/b* alleles compared to the preparation of the β-glucosidase solution used in the detection of the *Cu/cu* alleles (8).

Cross-fertilization in annual *M. alba,* measured only in 1 season, averaged 67%. A total of 723 seedlings from 20 parent plants were tested. Only the *Cu/cu* alleles were used as genetic markers.

Inbreeding is used widely in sweetclover breeding programs and is known to result in a loss in vigor (5). The relatively high percentage of outcrossing observed in *M. alba* indicates that vigor would be recovered rapidly when inbred lines are used in producing a variety.

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