5-1973

Genetic Studies of Induced Mutants in *Melilotus alba* III. Folded Leaflet, Elongatyed Stem, and Short-Petiole Dwarf

R. R. Ronnenkamp
United States Department of Agriculture

Francis A. Haskins
University of Nebraska-Lincoln, fhaskins@neb.rr.com

Herman J. Gorz
United States Department of Agriculture

Genetic Studies of Induced Mutants in *Melilotus alba* III. Folded Leaflet, Elongated Stem, and Short-Petiole Dwarf[1]

R. R. Ronnenkamp, F. A. Haskins, and H. J. Gorz[2]

ABSTRACT

Observations of the F_1, F_2, and F_3 progenies of crosses between normal annual *Melilotus alba* Desr. and three mutants, folded leaflet, elongated stem, and short-petiole dwarf, indicate that each of the mutant characters is controlled by a single recessive gene. Proposed symbols for the three mutant genes are *f*, *el*, and d_{sp}, respectively. In limited tests for linkage, *el* appeared to be linked (12% recombination) to a gene for multifoliolate leaves (*Mf*). No linkage was detected between *f* and *Mf*, *cu* (low *o*-hydroxycinnamic acid), or *b* (low β-glucosidase activity).

Additional index words: **Sweetclover, Ethyl methanesulfonate.**

SWEETCLOVER (*Melilotus* spp.) has been used for forage and soil improvement for many years. Early genetic studies of this crop dealt primarily with improving forage type and yield, but some attention was given to genetic analysis of mutant characters, particularly in the species *Melilotus alba* Desr. Inheritance studies in *M. alba* up to 1965 were reviewed by Smith and Gorz (10). Only a few added traits have been analyzed since 1965 (1, 2, 3, 4).

A successful attempt to induce mutants in an annual strain of *M. alba* by treatment of seeds with ethyl methanesulfonate was reported by Kleinhofs, Gorz, and Haskins (7). This paper deals with the inheritance of three of the mutants isolated in that study.

MATERIALS AND METHODS

The three mutant characters selected for study are folded leaflet, elongated stem, and short-petiole dwarf. Following their isolation by Kleinhofs et al. (7) in the M_2 generation after seed treatment, each of the three mutant lines was carried through at least three generations of selfing before being crossed with the normal progenitor line. As described by Gengenbach et al. (2), plants of this progenitor line, which originated as a plant introduction (P.I. 165,554), are relatively small, annual, and autogamous, and they mature rapidly. Brief descriptions of the mutant lines follow:

Folded leaflet. Typical mutant leaflets were folded on the adaxial side from the tip to about half the length of the leaflet (Fig. 1,A). Variation in expression within some individual plants ranged from an extreme mutant phenotype (leaves with leaflets folded from the tips to about three-fourths of the length, and tips stuck together) to an apparently normal phenotype. The degree of mutant expression tended to be most extreme in leaves formed early during the growth of the plants. Mutant plants were slightly shorter than normal.

Elongated stem. Mutant plants were characterized by elongated internodes and by a marked reduction in branching and leaf production. Classification was done most readily on chamber-grown plants about 1 week after planting. At this stage of growth, cotyledons of mutant plants were about 2 cm above the soil surface, and cotyledons of normal plants were barely above the surface (Fig. 1,B). The stem color on all elongated-stem plants observed in these experiments was light green, in contrast to the reddish color of normal stems.

Short-petiole dwarf. Early growth of mutant plants was extremely slow, and branching did not occur. Leaves were much smaller than normal and were attached to the stem by very short petioles (Fig. 1,C). Plants were usually classified at an age of 2 to 3 weeks, at which time a several-fold difference in height existed between mutant and normal plants.

Other mutant traits used in linkage tests include *Mf*, multifoliolate leaves (2); *cu*, low *o*-hydroxycinnamic acid content (5); and *b*, low β-glucosidase activity (5, 9).

Plants were grown in growth chambers or in the greenhouse in milk cartons or wood flats containing a mixture of 4 parts soil, 1 part sand, and 1 part peat. All seeds were hand-scarified before planting. Planted seeds were covered with a 5-mm layer of fine sand containing Orthocide[3] (0.11 g/liter of sand) to reduce damping off. Continuous light (cool white fluorescent lamps, ca 10,000 lux) and constant temperature (ca 23C) were provided in the growth chambers. To bring greenhouse-grown plants into

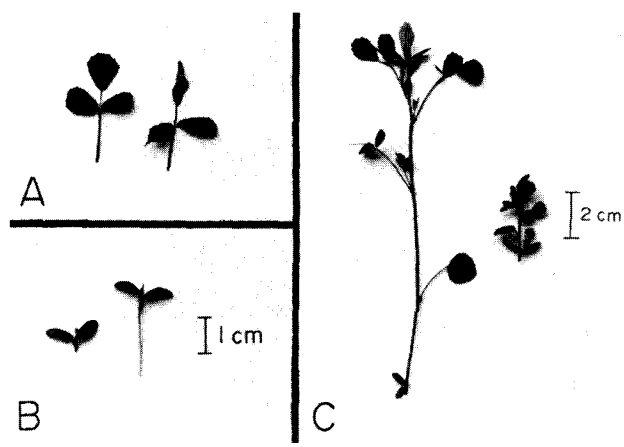

Fig. 1. Normal (left) and mutant (right) phenotypes. A. Folded leaflet. B. Elongated stem (seedlings excised at soil surface 7 days after planting). C. Short-petiole dwarf (plants excised at soil surface 25 days after planting).

[1] Contribution from the Agricultural Research Service, USDA and the Nebraska Agricultural Experiment Station, Lincoln. Published as Paper No. 3482, Journal Series, Nebraska Agr. Exp. Sta. The work reported was conducted under project 12-50, Nebraska Agr. Exp. Sta., and constituted part of a thesis submitted by the senior author to the University of Nebraska in partial fulfillment of the requirements for the M.S. degree. Received Nov. 20, 1972.

[2] Formerly Graduate Assistant in Agronomy, University of Nebraska (presently Graduate Fellow in Agronomy, Purdue University); Bert Rodgers Professor of Agronomy, University of Nebraska, Lincoln 68503; and Research Geneticist, ARS, USDA, Lincoln, Nebr., respectively.

[3] Mention of a specific product is for identification only and does not imply endorsement by the U. S. Department of Agriculture.

Table 1. Pooled data for F_2 segregations of normal and mutant phenotypes, with X^2 values for goodness of fit to a 3:1 ratio.

	Mutant character		
	Folded leaflet	Elongated stem	Short-petiole dwarf
Number of families	8	8	4
Segregation, Normal:Mutant	615:193	593:173	385:121
Sum of family X^2 values	4.68	5.64	4.36
X^2, pooled data	0.54	2.38	0.32
P lies between	0.25-0.50	0.10-0.25	0.50-0.75
X^2, heterogeneity	4.14	3.26	4.04
P lies between	0.75-0.90	0.75-0.90	0.25-0.50

Table 2. Distributions of segregating and nonsegregating F_3 families from normal F_2 plants, with X^2 values for goodness of fit to a 2:1 ratio.

	F_3 families observed			
Mutant	Segregating	Nonsegregating	X^2 value	P lies between
Folded leaflet	13	7	0.03	0.75-0.90
Elongated stem	15	5	0.63	0.25-0.50
Short-petiole dwarf	14	6	0.10	0.50-0.75

flower during fall and winter months, natural light was supplemented throughout the night with incandescent light.

Reciprocal crosses were made between each of the three mutants and the normal line when flowers were in the bud stage as previously described (2). Seeds obtained from the crosses were planted and the phenotypes of the resulting F_1 plants were observed. On the average, about 100 selfed seeds were harvested from each F_1 plant. These seeds were planted to produce the F_2 generation of plants, which were classified as normal or mutant. For each of the three mutant characters, about 25 seeds from each of 20 phenotypically normal F_2 plants were planted, as were 25 seeds, when available, from each of four or more mutant plants. The resulting F_3 families were classified as true-breeding, for the normal or mutant phenotype, or segregating. For F_2 and segregating F_3 families, uncorrected chi-square (X^2) values were calculated for goodness of fit to a 3:1 ratio. X^2 values for pooled families and for heterogeneity also were calculated. In addition, X^2 values were used to test goodness of fit, to a 2:1 ratio, of segregating vs non-segregating F_3 families from normal F_2 plants.

In a very limited investigation of possible linkage relationships, the following crosses were made, using plants homozygous for the indicated characters: folded leaflet $\times$ *MfMf*; folded leaflet $\times$ *cucubb*; and elongated stem $\times$ *MfMf*. Crosses were advanced to the F_2 generation, and results were analyzed in 2-gene combinations to detect possible linkage. The X^2 test was used to detect significant departure from the 9:3:3:1 ratio expected in independent inheritance. Where linkage was indicated, the product method described by Kramer and Burnham (8) with the tables of Immer and Henderson (6) were used to estimate map distance and standard error.

RESULTS AND DISCUSSION

Reciprocal crosses between the normal line and each of the three mutants produced phenotypically normal F_1 plants. F_2 ratios did not differ significantly from the 3:1 expected in a single-gene mode of inheritance (Table 1). Among F_3 families from normal F_2 plants, satisfactory fits to the expected ratio of 2 segregating:1 nonsegregating were observed (Table 2). In addition, the ratio of normal to mutant plants in segregating F_3 families was approximately 3:1 for each mutant (Table 3). In F_3 families from mutant F_2 plants, only mutant individuals were observed.

All available evidence supports the conclusion that each of the three mutant characters is controlled by a single recessive gene. The following gene symbols are proposed: folded leaflet, *f*; elongated stem, *el*; and short-petiole dwarf, d_{sp}.

Table 3. Pooled data from segregating F_3 families from original crosses of normal and mutant, with X^2 values for goodness of fit to a 3:1 ratio.

	Mutant character		
	Folded leaflet	Elongated stem	Short-petiole dwarf
Number of families	13	15	14
Segregation, Normal:Mutant	241:81	256:68	253:92
Sum of family X^2 values	10.94	19.16	14.50
X^2, pooled data	0.004	2.78	0.51
P lies between	>0.90	0.05-0.10	0.25-0.50
X^2, heterogeneity	10.94	16.38	13.99
P lies between	0.50-0.75	0.25-0.50	0.25-0.50

Table 4. Classification of F_2 plants from crosses between the multifoliolate-leaf mutant and the elongated-stem mutant. Data from three F_2 families are pooled.

Phenotype	Genotype	Observed	Expected for 9:3:3:1	X^2
Multifoliolate	Mf_El_	298	253.69	7.74
Multifoliolate, elongated	Mf_elel	29	84.56	36.51
Normal	mfmfEl_	24	84.56	43.37
Elongated	mfmfelel	100	28.19	182.93
		451	451.00	270.55
				P<0.005

Analysis of F_2 results from crosses made for possible linkage detection indicated that good fits to a 9:3:3:1 ratio were obtained when the following two-gene combinations were considered: *f* and *Mf*; *f* and *cu*; and *f* and *b*. Thus, *f* appears not to be closely linked to *Mf*, *cu*, or *b*. The cross of *elel* $\times$ *MfMf* yielded an F_2 generation that departed significantly from a 9:3:3:1 ratio (Table 4). Based on these data, the map distance between *el* and *Mf* was calculated as 11.9 $\pm$ 1.6 units.

Each of the three mutant lines reported in this paper is readily distinguished from the normal, and each grows with good vigor and produces adequate seed. With these attributes, the mutants should be useful in further studies of the genetics of *M. alba*.

REFERENCES

1. Gengenbach, B. G., H. J. Gorz, and F. A. Haskins. 1970. Genetic studies of induced mutants in *Melilotus alba*. II. Inheritance and complementation of chlorophyll-deficient mutants. Crop Sci. 10:154-156.
2. ———, F. A. Haskins, and H. J. Gorz. 1969. Genetic studies of induced mutants in *Melilotus alba*. I. Short-internode dwarf, curled leaf, multifoliolate leaf, and cotyledonary branching. Crop Sci. 9:607-610.
3. Goplen, B. P. 1967. A recessive pleiotropic gene conditioning unifoliolate leaves and cauliflower inflorescences in sweetclover. Can. J. Genet. Cytol. 9:136-140.
4. ———. 1969. Inheritance of pale cotyledon (*pc*) in sweetclover. Can. J. Genet. Cytol. 11:30-33.
5. ———, J. E. R. Greenshields, and H. Baenziger. 1957. The inheritance of coumarin in sweet clover. Can. J. Bot. 35: 583-593.
6. Immer, F. R., and M. T. Henderson. 1943. Linkage studies in barley. Genetics 28:419-440.
7. Kleinhofs, A., H. J. Gorz, and F. A. Haskins. 1968. Mutation induction in *Melilotus alba annua* by chemical mutagens. Crop Sci. 8:631-632.
8. Kramer, H. H., and C. R. Burnham. 1947. Methods of combining linkage intensity values from backcross, F_2 and F_3 genetic data. Genetics 32:379-390.
9. Schaeffer, G. W., F. A. Haskins, and H. J. Gorz. 1960. Genetic control of coumarin biosynthesis and β-glucosidase activity in *Melilotus alba*. Biochem. Biophys. Res. Commun. 3:268-271.
10. Smith, W. K., and H. J. Gorz. 1965. Sweetclover improvement. Advan. Agron. 17:163-231.