

Concurrent selection for low coumarin and multi-stemmed crowns in annual sweetclover

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Introduction Annual sweetclover (*Melilotus alba* Desr) is a reseeding, drought tolerant forage legume that is well adapted to the alkaline and neutral soils of the US Southern Great Plains. Coumarin is a phytochemical found in sweetclover that is a precursor to dicoumarol, which causes a toxic bleeding disease in cattle. The inheritance of low coumarin in sweetclover is determined by a single gene (Goplen *et al.*, 1957), but current low coumarin cultivars are all biennial, northern types. The ability of annual sweetclover to develop a multi-stemmed crown, similar to alfalfa (*Medicago sativa* L.), is also under genetic control (Hartwig, 1942). The objective of this breeding program is to combine the traits of annual flowering, low coumarin and multi-stemmed crowns into improved sweetclover germplasm.

Materials and methods Hand pollinations and bee cage crosses were made between “Denta” (low coumarin biennial) and “Emerald” (high coumarin, multi-stemmed annual) sweetclover with the high coumarin allele used as a genetic marker to identify true hybrids. The Emerald parents were grouped by maturity with a 60 day range of flowering date. The Denta parents were grown under a 20 hr daylength in a growth chamber to force flowering. The low coumarin status of each Denta plant was confirmed before the crossing program began and all seed were harvested from the Denta parents. Hybrids were identified based on the presence of coumarin using a rapid assay (Gorz and Haskins, 1958). All hybrids were self-pollinated in the greenhouse and F₂ seed produced in the spring and summer of 2002. Fifteen hundred F₂ seedlings from each of seven parental maturity groups, for a total of 10,500 plants, were planted for evaluation and screening in the fall of 2002. Emerald and Denta seedlings were also planted for use as checks. A simultaneous seedling screen was initiated for low coumarin (*cu cu*) and the multi-stemmed trait.

Results From 338 hand crosses, 36 hybrids were identified. Forty-seven hybrids were identified from bee cage crosses. About 240,000 F₂ seed were produced from hybrids between Denta and seven different maturity groupings of the Emerald parent. Based on a preliminary study, we developed a screening technique to identify the multiple stem trait in young (6 to 8 weeks of age) sweetclover seedlings. The growth of secondary stems from the axillary bud of the unifoliate leaf and from the axillary buds of the cotyledons was used as a positive signal for the potential development of the multi stem trait. About 2700 (25.7%) sweetclover plants were identified with the potential to express the multiple stem trait. Each seedling was then evaluated for coumarin content and those testing positive were discarded. Four hundred and ninety-one (4.6%) sweetclover seedlings were identified with the trait combination of multiple stem crowns and low coumarin. Artificial lighting was used beginning in early November, 2002 to extend the daylength and force early flowering. By mid December 2002 about 25% of the F₂ selections were flowering. Evaluation, selection and seed production continued in the greenhouse on the low coumarin + multi-stemmed selections. This group of plants represents a selection intensity of about 4% on the original F₂ population of 10,500 plants. In February 2003 another 150 plants were discarded due to severe powdery mildew infection and/or general low vigor. Flowers on each plant were hand tripped to insure pollination. Seed was produced on 193 plants from late spring to mid summer with seed yields ranging from 0.5g to 5.0g per plant. Plants with annual (*AA* and *Aa*) growth habit were identified based on flowering date, relative to biennial (*aa*) plants. About 25 biennial plants were noted that did not flower until spring 2004. Progeny testing will be necessary to identify F₃ families that will breed true for the annual trait.

Conclusions A concurrent seedling screen was successful in the intermediate state of an annual sweetclover breeding program to develop F₃ families with both low coumarin and multi-stemmed crowns.

References

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