

Exploring sweetclover potential

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White sweetclover (*Melilotus alba* Desr.) is a high-quality annual or biannual forage species that is adapted to various environmental conditions. It tolerates water deficit, moderate flooding, soils with medium to low productive potential, both heavy and sandy soils -and even being able to thrive in saline, alkaline-sodium soils-, but it cannot stand shade. The species has been widely used for multiple purposes, such as forage, honey production, green manure, and cover crop. Belonging to the legume family, sweetclover plants are erect, more or less branched and can reach 1.5 m in height. Another interesting characteristic is the exploration of the soil profile that the roots are able to do, which, added to the fixation of N due to the symbiosis with *Sinorhizobium meliloti*, leads to consider sweetclover as soil remedial and therefore to be utilized as service crop.

Sweetclover owes its name to the presence of a secondary compound with an aromatic ring called coumarin, which gives it a bitter taste and the characteristic sweetish smell of these species, reducing the palatability of *melilotus* plants. In poorly preserved sweetclover silage or hay, coumarin can change to dicumarol, which is a potent anticoagulant that causes the so called "sweetclover disease". The development of varieties with low coumarin content has been one of the primary objectives in breeding programs since the beginning of the last century worldwide. Obtaining these cultivars has been made possible by the transfer of the low coumarin gene present in *M. dentata*, a poorly productive wild species, into *M. alba* through interspecific crosses. The low coumarin content is determined by a single recessive gene, which makes necessary to thoroughly control the selection and seed increase processes.

Since 2012, INTA conducts a program to improve sweetclover by selecting for higher forage productivity, greater number of stems and late flowering, as well as reducing coumarin content.

The program used as a breeding initial population 13 accessions -two commercial cultivars and 11 ecotypes collected in various regions of the country- that were evaluated in a comparative yield trial with three replications carried out in four locations. Five populations were selected for higher biomass and seed production. Of those, 15 half-sib families (HSF) were obtained from each location that made up a trial with 60 HSF (10 plants per family; total: 600 plants). From these 60 HSF, the best 15 individuals were chosen, and seed was harvested from each one in only two locations. The next step was to plant this seed in the initial four locations to initiate the third selection cycle. Selection criteria were plant vigor, dry matter production (g plant^{-1}), height (cm), growth habit (erect, semi-erect or creeping), leafiness (visual scale), leaf/stem ratio, basal ramifications (visual scale), late flowering (days to flower), and flowering percentage. From this trial, five plants were selected in each location and the seed from those selected plants was collected in bulk (one bulk per location). These 4 bulks, plus a general bulk (GB) conformed by seed of the four bulks altogether, were evaluated in yield trials in two locations using commercial cultivars as checks. Results pointed out GB as the best experimental population. The registration process of GB as a new cultivar was initiated in 2019 at the National Institute of Seeds (INASE).

Regarding the selection for lower coumarin content, two populations with low coumarin content from the USA (Texas) were sown in the greenhouse and then coumarin content was evaluated using a fluorometric method.

Individual plants with the lowest content of coumarin (LCu) were selected and used as the donor parent to transfer the low coumarin gene into a local ecotype with good agronomical characteristics (F1LCu). The collected seed from those crosses was sown and the resulting plants were selfed, originating a new generation (F2LCu) which underwent a second cycle of selection for low coumarin content using a fluorometric method. The selected individuals were self-fertilized to originate F3LCu. Part of this was sown in 20 big bags in the greenhouse and polycrossed using honeybees as pollinators. Part of the remaining seed was sown, and the resulting plants were self-fertilized to fix the low coumarin character and the seed was harvested individually, originating 11 S1 families (S1LCu-1 to 11). Between five and 12 individuals from each family were evaluated in the greenhouse for leafiness (visual score 1 to 5), foliar area (visual score 1 to 5), diameter of the main stem base (mm), plant structure (visual), presence of multifoliolate leaves, height (cm), days to flowering, inflorescence length, when defoliated in vegetative state and earliness of flowering and fruit set. In the advanced flowering stage, a sample of leaflets was taken for determining coumarin content by HPLC. Thirty-five individuals with the desired traits and five individuals with poor agronomic performance were selected for contrasting purposes (checks). Among the selected plants, some showed an extremely low content of coumarin (0.01 – 0.38 mg g⁻¹). It is interesting to mention that the five individuals with normal coumarin content exhibited lower levels of coumarin (0.6 to 1.9 mg g⁻¹) when grown in the greenhouse in comparison to the same population grown in the field (mean = 7.92 mg g⁻¹). The selected 35 plants (desirable traits) were selfed to obtain S2LCu seed. The seed was sown in pots (3 - 5 from each selected plant) in May 2021 and are currently being grown in the greenhouse to obtain S3LCu seed. Next step will be to repeat the process for generating the S4LCu seed. Both F6LCu and S4LCu generations will be collected on an individual-plant basis to ensure genealogical traceability all the way down from the pure line (S4LCu) to the 11 plants of the F3LCu generation. Next step will be to increase these pure lines (S4LCu), harvesting each one individually. The obtained seed will be used for two purposes: a) to carry out field evaluation tests; and b) to make crosses with contrasting lines for traits of agronomic interest to generate mapping populations of those traits.