

## **Theoretical bases for the elaboration of a breeding program. *Lotus glaber* as an example.**

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Before starting a breeding program, problems or aspects that need to be improved in the species of interest have to be identified and listed. Among others, the following are apparently of concern in *Lotus glaber*: slow seed germination, seedling weakness, lack of persistence, susceptibility to stresses (abiotic: soil salinity and flooding, and biotic: insects) and winter and early spring forage production. Next, it is necessary to consider: which problems can be solved by cultural and/or chemical practices, what is the effectiveness of these practices and their cost in relation to the expected results, which could be solved only by breeding and, if the solution to a problem can be approached in both ways, which of them is the most cost-effective. If the breeding approach seems convenient from the economic or environmental point of view, it is necessary, before making a decision, to know (or determine if not known) the type of genetic control of the trait(s) of interest: (a) nuclear (mono- or oligogenic with none or little environmental influences on the expression, or oligo- or polygenic, with the expression highly influenced by the environment) or (b) cytoplasmic. Nuclear genes have biparental inheritance and follow Mendel's laws, thus predictions can be made regarding expected genotypes/phenotypes and proportions in controlled crosses, in contrast with cytoplasmic genes that can have either uni- or biparental inheritance and do not follow Mendel's laws. Also, it has to be considered that a given trait can be under control of nuclear or cytoplasmic genes, or under control of cytoplasmic genes that interact with nuclear genes (i.e. chlorophyll content or male sterility) and that an enzyme can be composed of various polypeptides, some of them codified by nuclear genes and the others by cytoplasmic genes (i.e. the enzyme RUBISCO).

If breeding is deemed appropriate, it has to be decided what type of cultivar will be developed (improved population, synthetic variety, hybrid, etc.). For doing this, it has to be considered: (a) that *Lotus glaber* is a species herbaceous, perennial, diploid and self-incompatible, that reproduces sexually and has related diploid and polyploid species that can be manipulated in vitro and are amenable to transgenesis, and (b) the complexity of the process, the minimum required time, the likelihood of maintenance of the genetic identity of the cultivar (given the feasibility of natural hybridizations and re-seeding), the available infrastructure and equipments, and costs in relation to benefits. The first step to start a breeding program is to gather a collection of germplasm (commercial cultivars, advanced selections, natural populations), and to study the heritable variation within and between genetic materials. If no heritable variation is found for the trait(s) of interest, the

breeder can recur to: (a) interspecific hybridization (a collection of species and genotypes has to be gathered and controlled crosses performed; if hybridization barriers are detected, strategies have to be applied or developed to circumvent them, i.e., ploidy level manipulations), (b) *in vitro* manipulations to select pre-existing somaclonal variation or generate it, (c) induced mutagenesis with chemical or physical agents, (d) transgenesis for monogenic traits. It is important to analyze the advantages and disadvantages of each approach taking into account effectiveness and costs. For *in vitro* manipulations, the correlation with the *in vivo* behavior has to be established. Breeding has been considered an art and a science. But modern breeding has to be carried out on solid scientific bases, making use of sophisticated tools only if the cost-benefit relations warrant it.