

## Seed yield components in F2 genotypes of interspecific hybrids of *Lotus uliginosus* and *Lotus japonicus*

ALVARO MESSA<sup>1</sup>, RODRIGO SALDÍAS<sup>1</sup>, ALICIA CASTILLO<sup>1</sup>, JORGE MONZA<sup>2</sup>, OMAR BORSANI<sup>2</sup>, [MÓNICA REBUFFO](#)<sup>1\*</sup>

<sup>1</sup>*Instituto Nacional de Investigación Agropecuaria, Ruta 50 km 11, Colonia, Uruguay*

<sup>2</sup>*Departamento de Biología Vegetal, Facultad de Agronomía, Montevideo, Uruguay.*

\* *Corresponding author*

[click here for Spanish version](#)

The compatibility among species of *Lotus* and the rhizobia that are able to nodulate them determine the efficiency of the biological fixation of nitrogen. The plants of *L. corniculatus* L. and *L. glaber* Mill. fix nitrogen with bacteria of the genus *Mesorhizobium*, while *L. subbiflorus* Lag and *L. uliginosus* (Schkuhr) form effective nodules with *Bradyrhizobium* and ineffective with *Mesorhizobium*. The genetic and metabolic mechanisms that are involved in the incompatibility of plant-bacteria nitrogen fixation are unknown, which could be studied by means of the hybridization of two species of different symbiotic group (*L. uliginosus* x *L. japonicus* Regel (Larsen) ecotipo Gifu). *L. uliginosus* is a perennial diploid alogamous species, with 6 to 15 flowers per umbel and vegetative multiplication by rhizomes, whereas *L. japonicus* is a diploid autogamous species with 1 to 3 flowers per umbel and without vegetative reproduction. Therefore, this hybrid will also allow to analyze the possibility to combine morphological characteristic of these species, such as the natural self-pollination and the presence of rhizomes. The hybridization was carried out without emasculation, previous verification of self-incompatibility of *L. uliginosus* maternal plants. In vitro multiplication of the F1 embryos was carried out 15-21 days post-pollination because the hybrid seed aborted before reaching maturity. 170 F1 embryos were able to develop on agar and reach to adult plant, of which 3 fertile plants were identified (6-4, 6-6 and 6-81). 1800 F2 individuals were achieved from the cross of these fertile plants through a new phase of embryo rescue. The fertility of this F2 population was evaluated by its seed production as autogamous (natural self-pollination), self-pollination (forced self-pollination) and cross-pollination (polycrosses between genotypes of the same genetic origin). Umbels labeling at the beginning of blooming allowed to identify at least 30 F2 genotypes that behave as strict autogamous in the absence of pollination insects (the characteristic of the father *L. japonicus*). These genotypes are in very low frequency in different crosses (6-4 x 6-6; 6-4 x 6-81; 6-6 x 6-81; 6-6 x 6-4; 6-81 x 6-6), and their fertility was disparate. The largest number of plants identified as autogamous, including some of the most fertile ones, came from the cross of 6-81 x 6-6. Paintbrush manual pollination with pollen of the same genotype (forced self-pollination) was carried out in all F2 genotypes. Most plants recovered fertility with crossed pollination (pollen of other genotypes from the same hybrid), while 428 plants produced seed with forced self-pollination. Within the latter group of plants, 107 flowers/plant have been pollinated on average, of which 21.6 pods have been obtained, that represented a low level of fertility (25.5%). The pods harvested had 147

viable seeds and 41 immature seeds, representing 25% aborted seeds. The number of pollinated flowers, as well as the components of seed yield presented a high variability inside each cross, even though average fertility were similar for cross 6-4 x 6-6 and reciprocal cross (6-6 x 6-4), 6-4 x 6-81 and 6-6 x 6-81. Crosses of F1 genotype 6-81 with pollen of genotypes 6-4 or 6-6 produced very scarce number of embryos and no fertile plant was obtained by embryo rescue. Cross 6-4 x 6-6 presented more abundant blossom, that allowed to reach 126 pollinated flowers /plant on average, although it was recorded high variability, with a range from 10 to 298 flowers/plant. Crosses between 6-6 x 6-4 and 6-6 x 6-81 presented more morphological similarities with *L. uliginosus*, with 64% of plants with presence of rhizomes. On the other hand, cross of 6-4 x 6-6 had 56% of plants with absence of rhizomes, and the proportion increases to 85% in the cross 6-4 x 6-81 whose descendant had larger similarities with *L. japonicus*. It will be necessary to evaluate the next generation fertility to determine the stability of reproductive parameters, especially in the descendant of different F2 genotypes. Research financed by the LOTASSA project ([www.lotassa.org](http://www.lotassa.org)).