Medicago lupulina petite line is useful in making crosses without emasculation

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Medicago lupulina is a predominantly self-pollinated annual species that has one seed per pod. *M. lupulina* has very small flowers that are difficult to pollinate by hand, and even more difficult to emasculate. Petite-1 *M. lupulina* serves as a marker for identification of self progeny (petite) versus hybrids (normal vigor). Moreover, it is partially self sterile thus enhancing the efficiency of hybrid seed production after hand crosses without emasculation. This has permitted us to make crosses and begin answering questions about the genetic biology of *M. lupulina*.

Petite-1 was discovered growing as a weed in the greenhouse on the University of Wisconsin Campus in May, 2002. We noticed that only about half of the flowers on the plant were setting seed, and realized that it had a potential for crossing without emasculation. We cross pollinated petite-1 with pollen collected from a normal *M. lupulina* growing in a different greenhouse section with a collection of *Medicago* species. When collecting or applying *M. lupulina* pollen, we wear magnifying goggles (2.5X). The point of a round toothpick was used to collect pollen by tripping about 10 normal flowers onto the point of the toothpick. Next, the toothpick with pollen was used to trip about 10 petite flowers on a raceme, and the remainder of the flowers on the raceme were picked off the minimize self seed production. The cross pollinated raceme was marked with a white thread. We found it interesting that tripped flowers are not obvious in *M. lupulina*, as they are in other *Medicago* species.

The labeled raceme was harvested about a month later, and retained until the fall greenhouse growing period. Eight seeds from the crossed raceme were planted in the fall and half were hybrids easily identifiable by normal growth and fertility (Figure 1) F2 seed was mature in about 5 weeks and was saved for later growouts. Petite was regrown from seed again in December of 2002 in the greenhouse, and crossed again in the same way in January 2003. At this time the greenhouse temperature does not exceed 75'F, even with full sun. Petite was almost completely self sterile at this time, and we did not pick off the untripped flowers on cross-pollinated racemes. A half dozen seeds from the January crosses were used in the next planting, and they were all hybrids. Thus, crossing was very efficient during the winter in our greenhouse.

Figure 1. Adult plants of Petite, F1 (Petite *x* Wt), and Wild type (Wt).

The F2s from the first round of crosses in 2002 were grown in the greenhouse along with the F1s from the second round of crosses in 2003. This side by side comparison exposed a very interesting seed quality relationship. The seeds containing F1 embryos produced on petite plants are very small, whereas the seeds containing F2 embryos produced on normal F1 plants are normal size. This size/quality relationship gave the F2s a growth advantage over the F1s such that the F2s appeared slightly more heterotic than the F1s throughout the growth cycle, and the F2s flowered 5-7 days earlier than the F1s. Seed size and quality of seed produced by the F1 plants likely explains these results.

There was not a discrete segregation for petite and normal in the F2 generation among 25 plants studied in the greenhouse. Rather, there was a continuous and normal distribution for plant size with a mean a little more than half way between petite and normal. Interestingly, the partial sterility of Petite-1 also did not segregate since all 25 F2 plants had normal seed set. These results suggest that the Petite-1 phenotype is not due to a single gene. Additional *M. lupulina* plants with small stature have been collected in natural populations and will be used to further study the genetic biology of *M. lupulina*.

In conclusion, Petite-1 was useful in making crosses without emasculation whereby hybrids were identified by normal vigor and size. Seed of Petite-1 is available, and it appears that additional petites can be found in most natural populations.