Preliminary studies of inheritance of autogamy in *Lotus corniculatus* L.

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Abstract

Lotus corniculatus L. is a tetraploid, cross-pollinated perennial forage legume that requires insect-pollination to set seed. An exception to this is a self-pollinated, early flowering, and photoperiod-insensitive mutant (RG-BFT) released in 2001 by USDA-ARS. This autogamous character is useful to reduce the need for pollinators in large-scale seed production; however, its inheritance is unknown. To ascertain if we could utilize this character in plant breeding we studied the inheritance of autogamy by hand-crossing three non-autogamous accessions (SA 25252, SA 25295, SA 833) with RG-BFT as the pollen donor. One hundred F_1 seeds were grown per cross, together with the parents in an insect-proof screenhouse, and only RG-BFT set seed. Molecular studies were conducted on a random sample of 12 individual F_1 plants per cross, and results confirmed hybridization was achieved in all three crosses. From this study we concluded that autogamy is a recessive character in *L. corniculatus* as the confirmed F_1 hybrids did not set seed in the absence of pollinators. It was also observed that those crosses resulted in only 27% of inflorescences producing seed which were 40% of the weight of normal *L. corniculatus* seeds, indicating some incompatibility level when crossing non-autogamous by autogamous types. In 2006, a new cycle of hand crossing will commence using 5 confirmed F_1 hybrids per cross. These will be backcrossed (BC) to each respective parent and crossed among themselves. The proportion of autogamous BC_1 and F_2 plants will provide an estimate of the mode of inheritance of autogamy.

Key Words

Inheritance of autogamy, *Lotus corniculatus*, molecular markers, SSR

Introduction

The breeding system of a species has major implications for plant breeding procedures (Comstock 1996) and seed production techniques. Most genotypes within a species will have the same breeding system and hence are classified for example as self-pollinated, cross-pollinated or apomictic species. However, there are exceptions and it is not unusual to find sexual plants in apomictic species or self-pollinated plants in cross-pollinated species, or cross-pollinated plants in self-pollinated species. The cross-pollinated species *Lotus corniculatus* also has a genotypes capable of self-pollination (Beuselinck and McGraw 1986). This rare trait could be very useful in the breeding and selection of a commercial cultivar of *L. corniculatus* for southern Australia

A self-pollinated *L. corniculatus* cultivar selected for homogenous flowering characteristics could have significant seed production advantages by increasing the number of non-shattered and ripe pods ready for harvest at a given date thereby reducing seed losses from over- and under-ripe seed pods. It would also eliminate the reliance on bee pollination for seed set which is important, as in Australian conditions low bee density and activity can limit seed production. Conversely a pure line would be genetically less diverse than an outcrossing line and this may make a self-pollinated line more vulnerable to disease attack or perhaps less broadly adapted. However, in order to utilize the autogamy characteristics in plant

breeding programs, it is necessary to know the way in which it is inherited. This study reports the preliminary results of the inheritance of autogamy in *L. corniculatus*.

Methods

Thirty clones of each of the three cross-pollinated accessions (SA 25252, SA 25295, SA 833) and the self-pollinated RG-BFT accession (Steiner and Beuselinck 2001) were obtained in 2004. In 2004/5, 90 inflorescences of each of the cross-pollinated accessions were emasculated and subsequently pollinated with RG-BFT. Successful crosses were recorded and thousand seed weight (TSW) of the crosses were measured.

In 2005, a random sample of 100 F_1 seeds were planted per cross in an insect-proof screenhouse, together with the parent plants. To confirm that the F_1's were hybrids, molecular studies were conducted with 15 microsatellite markers (SSR), 10 from lupin and 5 from *Lotus japonicus* genome sequencing project website (http://www.kazusa.or.jp/lotus/). These SSR markers were evaluated on the four parent plants to detect the best polymorphic primer to subsequent utilize in the screening of a random sample of 12 individual plants from each of the three crosses to identify positive cross-pollination.

Results and Discussion

F_1 hybrids and their parents SA 25252, SA 25295, SA 833 did not produce seed under insect free conditions while RG-BFT did. This indicates that the autogamy character for self-pollination is a recessive trait. Interestingly the hand-crosses conducted in 2004/5 between the cross-pollinated mother plants and the pollen donor RG-BFT, only had 27% of the inflorescences that produced pods and their seeds were only 40% of the normal seed weight. This indicates that there is some incompatibility between the two types of *L. corniculatus*.

These accessions of *L. corniculatus* have no phenotypic markers that can be used to identify a positive cross, therefore molecular markers (SSR's from lupin and *L. japonicus*) were examined. We found that none of the lupin SSR's were successful discriminators and three primers from *L. japonicus* successfully discriminated all four parents. Primer TMO744 was selected for further screening of the crosses because it produced the most different banding patter for each of the 4 parent plants (Figure 1). From the 12 plants tested in each of the 3 crosses we identified 67%, 50%, and 100% successful crosses from SA 25252, SA 25295 and SA 833 respectively. Twelve F_1's between SA 25252 and RG-BFT are presented in Figure 1.

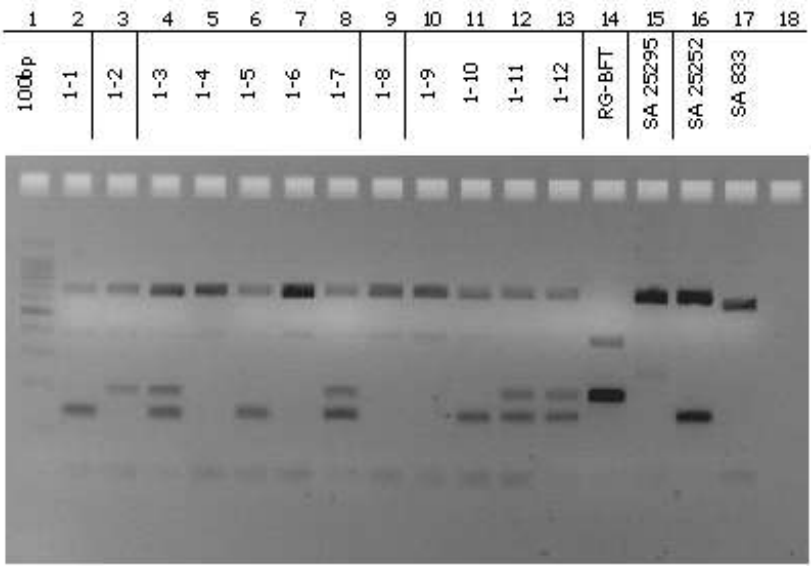

Figure 1. Twelve F_1 crosses between SA 25252 and RG-BFT evaluated with the molecular marker TMO744.

In 2006, four confirmed hybrids of each of the 3 crosses will be selected and back-crossed to their respective parents and among themselves, to determine in the F_2 or BC_1's the proportion of autogamous plants in the population.

Conclusion

The genes coding for autogamy in *Lotus corniculatus* are recessive and the number of genes involved will be determined by the proportion of autogamous progeny resulting from the 2006 crosses.

References

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