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## Techniques for Artificial Hybridization in the Self-Pollinated Forage Legume *Bituminaria bituminosa* and the Cross-Pollinated Forage Legume *Hedysarum coronarium*

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## Techniques for artificial hybridization in the self-pollinated forage legume *Bituminaria bituminosa* and the cross-pollinated forage legume *Hedysarum coronarium*

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**Key words:** *Bituminaria bituminosa*, *Hedysarum coronarium*, Sulla, hand-crossing techniques

**Introduction** To artificially cross two parent plants to recombine their desirable characteristics into their progeny is a highly desirable tool for breeding purposes. In this paper we would outline the techniques for the artificial hybridization of the self-pollinated *Bituminaria bituminosa* (L.) Stirt. (*B. bituminosa*) (Juan et al., 2003) and the cross-pollinated *Hedysarum coronarium* L. (Sulla) (Chriki et al., 1984).

**Materials and methods** In November 2006, 2 pots of each of 15 accessions of *B. bituminosa* and 50 pots of *H. coronarium* had their flowers removed and placed inside an insect-proof glasshouse. New emerging flowers were tagged and checked if they set seed without the aid of pollinators or wind. Florets of both species were dissected under a microscope to determine the stage of floral development when the anthers start to dehisce, latest stage for emasculation. Three methods of emasculation were tried: removing petals and immature anthers with a pair of tweezers; opening the floret and removing the immature anthers with a suction pump; and removing the anthers by gently pulling the closed petals in the immature floret that came out with the anthers, leaving the female organs undamaged. After emasculation, hand-pollination took place at 24, 48 or 72 hours.

**Results and discussion** All the accessions of *B. bituminosa* were able to set seed inside the insect-proof glasshouse confirming that they are predominantly self-pollinated. However, many non-pollinated flowers dropped, indicating that insect pollination might improve seed production. The anthers started to dehisce when the corolla had emerged to the level of the tip of the calyx teeth. From the 3 methods of emasculation, removing the anthers either with a pair of tweezers or the suction pump were too destructive to the floret and caused abortion. The third method of pulling all the petals with anthers attached did not damage the female organs. After emasculation, 48 hours was the optimum length before hand-pollination. *Hedysarum coronarium* did not set seed inside the glasshouse, therefore, did not require emasculation, confirming its protandrous nature (Yagoubi and Chriki, 2000). To hand-pollinate, petals should not be removed and a pollen blend (several pollen ages) should be gently placed on top of the stigma 1-2 days after anthers dehisce. Closing the floret immediately after pollination will avoid desiccation of the extremely fragile 15mm long by 1.5mm diameter style.

**Conclusions** The key step in the technique for hybridizing *B. bituminosa* is to emasculate before the anthers dehisce by gently pulling the petals with anthers attached to avoid damaging the female organs. For Sulla, the key steps in the technique are using a blend of pollen and to close the floret immediately after hand-pollination to avoid desiccation of the style.

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