

## **The Insertion Method: a new and efficient technique for intra- and interspecific hybridization in *Phaseolus* beans**

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Successful hybridization techniques are important for the utilization of the genetic diversity of *Phaseolus* gene pools to transfer useful and desirable genes within and between species.

Different techniques for the crossing of beans have been described (Bliss, 1980; Buishand, 1956; CIAT, 1982; Lorz, 1957; Okonkwo and Clayberg, 1984; Strand, 1943; Wade, 1943; Wester and Jorgensen, 1950; Wester and Marth, 1949; Wigton, 1959). The rubbing and hooking methods of Buishand (1956) are widely used in breeding programs. Considerable success has been obtained using Buishand's techniques. For example, in the breeding program at CIAT, 50% efficiency has been reported using a combination of the rubbing and hooking methods under controlled conditions (CIAT, 1982). Buishand himself reported 30% and 70% success for the rubbing and hooking methods, respectively.

Whatever the pollination technique used for bean hybridization it must be easy, quick and efficient. The efficiency largely depends on the fact that the stigma and pollen should be in very close contact as long as possible to ensure fertilization, as occurs with the keel in natural selfing. The efficiency also depends on the environmental factors such as temperature and humidity, amongst others, since they are responsible for the rate of desiccation of the pollen and stigma. Furthermore, the species and genotypes as well as the physiological, phenological and morphological stage of the plants used as female and/or male parents play an important role in the success of reciprocal crosses.

We have developed a new method for crossing beans which largely meet the above requirements. It is described here as *The Insertion Method*.

*Emasculation.* A chosen flower bud, usually one day prior to anthesis, is held firmly but gently between the thumb and middle finger of one hand, with the forefinger putting some pressure on the top to encourage opening of the standard. Using the other hand, the standard is then completely opened and pushed backwards with forceps to expose the wings. Still with forceps, both wings and the keel petals are carefully removed exposing the coiled style-stigma and the stamens. The exposed stamens are then easily removed with forceps.

*Pollination.* A flower from the desirable male parent is chosen at anthesis when freshly opened. From this flower, the twisted style-stigma and stamens enclosed in the keel petals, are removed from just above the ovary with forceps. Pollination is effected by placing the "keel" (containing the style-stigma and stamens) from the male parent over the style-stigma of the female. In this way the female style-stigma is inserted into the "keel" of the male. Finally, the standard is returned to its original position to cover the pollinated flower. In this position the pollinated flower very much resembles a natural selfed-flower and even after pod formation the male flower parts remain inserted in the tip of the developing pod.

This method keeps the anthers and the pollen bearing style-stigma of the male flower in very close contact with the stigma of the female flower, a period long enough to ensure fertilization. It also reduces the problem of desiccation. These two aspects are the most important factors to be considered in the crossing of beans. Therefore, the pollinated flowers need no artificial

protection from desiccation or insects, which is usually needed when using any other method of pollination. Furthermore, no repeated pollinations are needed to ensure fertilization as considered by Buishand (1956). This may be due to the fact that with this method the anthers dehisce gradually as they do in a natural selfed-flower. This method also has the advantage of being easy, quick and efficient, and is less dependent on environmental conditions for success.

This method ensures from 68% up to a 100% efficiency in interspecific crosses between *P. vulgaris* (female) and *P. acutifolius* (male) and from 90% to 100% efficiency in intraspecific reciprocal crosses in *P. vulgaris* where no initial genetic incompatibilities are apparent. Hybridization efficiency is the number of successful pollinations/total number of pollinations x 100; a successful pollination is considered as one in which pod growth is normal on the maternal plant just before removal for embryo rescue (2-3 weeks after pollination), or one left on the maternal plant for further development.

With this new method, emasculation and pollination can be carried out at any time of the day provided that female flower buds at the right stage for emasculation and pollination are available as well as male flowers at anthesis. Unpollinated flowers and/or flower buds should be continuously removed from the female parents to eliminate competition from selfed-flowers during the development of the hybrid pods.

#### References

- Andrade-Aguilar, J. A. 1987. Interspecific hybridisation between *Phaseolus vulgaris* L. (common bean) and *Phaseolus acutifolius* A. Gray (Tepary bean) by means of embryo culture. M. Sc. dissertation. Department of Plant Biology. University of Birmingham.
- Bliss, F. A. 1980. Common bean. In: Fehr, W. R. and Hadley, H. H. (Eds.). Hybridization of crop plants. American Society of Agronomy. Crop Science Society of America Publishers. Madison, Wisconsin.
- Buishand, Th. J. 1956. The crossing of beans (*Phaseolus* spp.). *Euphytica* 5: 41-50.
- CIAT. 1982. Cruzamiento del frijol; guía de estudio para ser usada como complemento de la unidad audiotutorial sobre el mismo tema. CIAT, Cali, Colombia.
- Lorz, A. P. 1957. Snap and lima bean crossing technique. *Bean Improv. Coop. Ann. Rep.* 1: 14-15
- Okonkwo, C. A. and Clayberg, C. D. 1984. The wrapping method, a new technique for effective crossing of common bean. *Bean Improv. Coop. Ann. Rep.* 27: 156.
- Strand, A. B. 1943. Species crosses in the genus *Phaseolus*. *Proc. Amer. Soc. Hort. Sci.* 42: 569-573.
- Wade, B. L. 1943. An improvement in bean crossing technique. *Proc. Amer. Soc. Hort. Sci.* 43:187-188.
- Wester, R. E. and Jorgensen, H. 1950. Emasculation unnecessary in hybridizing lima beans. *Proc. Amer. Soc. Hort. Sci.* 55: 384-390.
- Wester, R. E. and Marth, P. C. 1949. Some effects of a growth regulator mixture in controlled cross-pollination of lima bean. *Proc. Amer. Soc. Hort. Sci.* 53: 315-318.
- Wigton, R. J. 1959. Snap bean crossing technique. *Bean Improv. Coop. Ann. Rep.* 2: 19-20.