

Characterization and maintenance of novel source of male sterility in ridge gourd *Luffa acutangula* (L.) Roxb

Ridge gourd (*Luffa acutangula* (L.) Roxb.) is an important cucurbitaceous vegetable crop widely grown in tropical and subtropical parts of the world. In addition to culinary properties, it has therapeutic properties and is used for extraction of fibres¹. Ridge gourd, being predominantly monoecious, is a cross-pollinated crop and provides ample scope for utilization of the hybrid vigour². Male sterility is defined as the failure of plants to produce functional anthers, pollen or male gametes³. The phenotypic manifestations of male sterility are very diverse ranging from complete absence of male organs, failure to develop normal sporogenous tissues (no meiosis), abortion of pollen and to the absence of anther dehiscence³.

An off type was detected in a population of ridge gourd which was characterized by the production of rudimentary male flowers in racemes (Figure 1a) in contrast to the bright yellow flowers in male fertile plants (Figure 1b). On comparison of the anthers of the suspected male sterile line with those of monoecious ridge gourd variety named *Haritham*, a striking difference was noticed with respect to the morphology of anther lobes. In the male sterile line it was flat, highly pubescent and with few, shrunken pollen grains (Figure 1c) whereas in *Haritham*, it was plump and filled with abundant, large fertile pollen grains (Figure 1d).

Failure to develop normal microspore mother cells in the pollen sac and abnormal microsporogenesis resulting in deformed or inviable pollen are reported in other cucurbits like watermelon and muskmelon⁴. Male sterility observed in ridge gourd is a form of sporogenous male sterility where stamens form, but viable pollens are absent (Figure 1e). No fruit set was observed when male buds from male sterile plant were used for pollinating the female flowers of male sterile and normal monoecious line. Because male buds in racemes failed to open, buds were used for pollination. Pollen from male sterile lines was poorly stained with acetocarmine (1% solution) as compared to the pollen from fertile line (Figure 1f). Meiotic analysis of pollen mother cells (PMCs) in the male ster-

ile line revealed normal meiosis (Figure 1g) but the microspores of the male sterile line did not take up stain in later stages and showed abnormality in morphology such as shrunken nature, reduction in size (Figure 1h) and sterility compared to those from normal fertile flowers.

The genetics of male sterility can be elucidated after a series of crossing with different genotypes and advancing the generation. Female flowers of the male sterile line were crossed with normal monoecious variety *Haritham* and the F₁ population was observed for sex expression. All the plants of F₁ population were found to exhibit male sterility characterized by shrunken and sterile microspores, indicating the heritability and dominant nature of the character.

Like musk melon, male sterility can be exploited in heterosis breeding programme and development of F₁ hybrids in ridge gourd. Maintenance of male sterile line is a major challenge and for genetic dissection of male sterility, it has to be crossed with different pollen parents and F₁, F₂ and back cross populations have to be created.

Micropropagation was attempted for maintaining male sterile plant. Tissue

culture techniques were applied successfully in cucurbits for maintenance of elite plant types⁵. Promising establishment media identified for monoecious plant were used for inoculating the nodal cuttings of field grown male sterile plant⁶ (MS + BAP 0.5 mg l⁻¹ and MS + IAA 1.5 mg l⁻¹ + BAP 2 mg l⁻¹). Explant response was average with 60% establishment in the medium, MS + IAA 1.5 mg l⁻¹ + BAP 2 mg l⁻¹ (Figure 2a) and 45% in the medium, MS + BAP 0.5 mg l⁻¹. Shoot length after 45 days was maximum in the medium MS + IAA 1.5 mg l⁻¹ + BAP 2 mg l⁻¹ (7.5 cm) followed by medium MS + BAP 0.5 mg l⁻¹ (5.3 cm). Cuttings (2–3 nodes) from *in vitro* shoots were used for inoculating in the multiplication medium. The highest number of shoots and nodes was observed in the medium, MS + BAP 1 mg l⁻¹ (Figure 2b). The shoots from multiplication stage were used for rooting in MS medium (half strength) fortified with IBA (1 mg l⁻¹) and charcoal (200 mg l⁻¹). A high percentage of rooting (95%) and continued shoot growth were observed in this medium (Figure 2c). The rooted plants were transferred to mist house in polybags where it was kept for one month before transplanting to the main

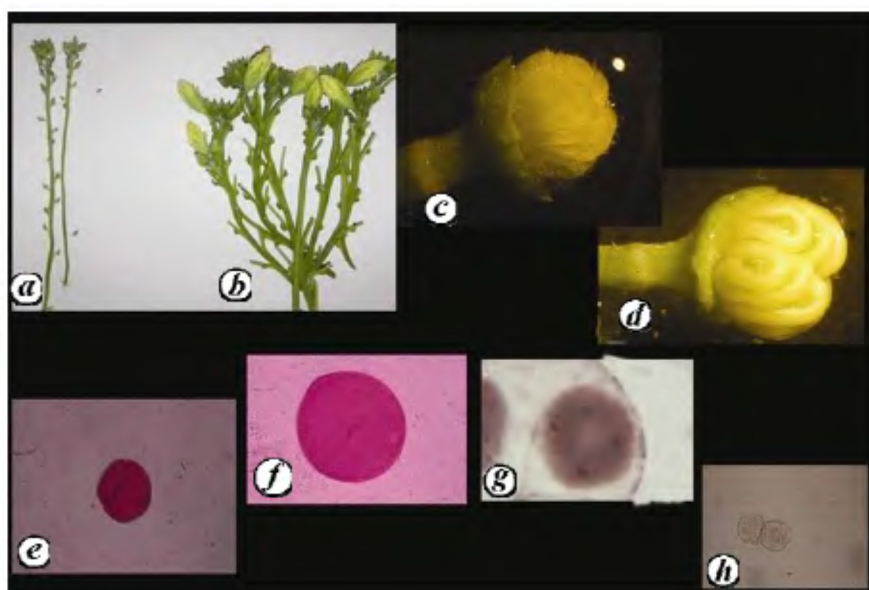


Figure 1. a, Sterile male flowers in raceme; b, Male fertile and male sterile flowers; c, Anther of male sterile plant; d, Anther of male fertile plant; e, Pollen of male sterile plant; f, Pollen of male fertile plant; g, Meiosis of pollen mother cells (PMCs) in male sterile plant; h, Microspore of male sterile plant.

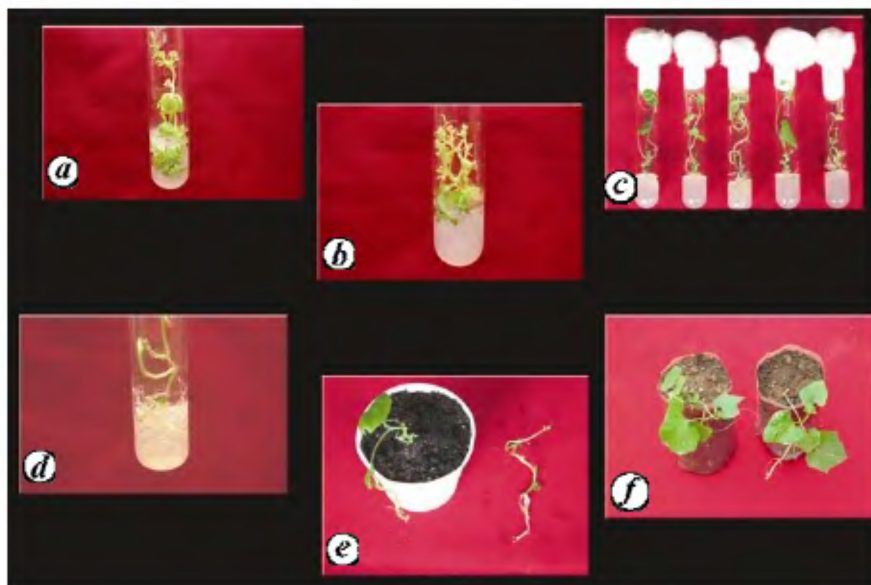


Figure 2. *a*, Regeneration of male sterile plant from the field in establishment medium, MS + IAA 1.5 mg l^{-1} + BAP 2 mg l^{-1} ; *b*, Multiple shoot formation of male sterile line; *c*, *d*, *In vitro* rooting and shoot growth of male sterile line; *e*, *f*, *Ex vitro* establishment of male sterile line

field. But the rate of survival during hardening was found to be low (40%). Different rooting media were thus tried to induce healthier roots among which hormone-free medium with $\frac{1}{2}$ MS salts alone was found to give the best results (Figure 2*d*). There were numerous healthy and strong roots in the basal medium which helped the plants to establish efficiently during the hardening process

(Figure 2*e*). The rooted plants were transferred to the mist house in polybags where they were kept for a month before transplanting to the main field (Figure 2*f*). Tissue culture plants took 50 days after transplanting into the field for flowering and were all male sterile in nature. This protocol can be used for maintenance and multiplication of male sterile ridge gourd plants.

1. Seshadri, V. S., In *Vegetable Crops in India* (eds Bose, T. K. and Som, M. G.), Naya Prokash, Calcutta, 1990, pp. 91–164.
2. Choudhary, B. and Thakur, M. R., *Indian J. Genet. Plant Breed.*, 1966, **5**, 188–197.
3. Kaul, M. L. H., *Male Sterility in Higher Plants*, Springer-Verlag, Berlin, 1988.
4. McCreight, J. D., Nerson, H. and Grumet, R., In *Genetic Improvement of Vegetable Crops* (eds Kalloo, G. and Bergh, B. O.), Pergamon Press, UK, pp. 287–294.
5. Barnes, L. R., Cochran, F. D., Mott, R. L. and Henderson, H. R., *Cucur. Genet. Coop. Rep.*, 1978, **1**, 21–22.
6. Pradeepkumar, T., Krishnaprasad, B. T., Sujatha, R. and Johnkutty, I., In *Proceedings of the 20th Kerala Science Congress*, 28–31 January, Kerala State Council for Science, Technology and Environment, Thiruvananthapuram, 2008, pp. 30–32.

Received 16 February 2009; revised accepted 24 September 2010

T. PRADEEPKUMAR*
V. C. HEGDE
R. SUJATHA
T. E. GEORGE

*College of Horticulture,
Vellanikkara,
Trichur 680 656, India*

**For correspondence.
e-mail: pradeepkau@gmail.com*

Assessment of *Pongamia pinnata* (L.) – a biodiesel producing tree species using ISSR markers

Pongamia (Indian beech) is a non-edible oil-producing tree legume (Syn: *Pongamia glabra* Vent.), that has recently gained importance for its oil utility as biodiesel¹. Various parts of *Pongamia* are used in medicinal and other applications. To date, there is little information regarding the germplasm of this tree. *In vitro* regeneration protocols have been standardized for clonal propagation^{2,3} and for application in gene-transfer technologies towards its improvement. However, for commercialization of the protocol, the genetic fidelity of the *in vitro*-raised clones needs to be assessed using molecular markers. Moreover, the wide range of variability among accessions of

Pongamia in oil content, seed and pod morphology, *in vitro* response, etc. necessitates the understanding of its germplasm using molecular markers. Hence the present work was undertaken to study the fidelity of the *in vitro*-raised clones and the variability among the selected trees using ISSR markers.

Among the propagules raised *in vitro* from mature plant-derived axillary buds^{2,4}, 12, 13 and 12 propagules from three mother trees identified as P1, P2 and P3 respectively, were tested for fidelity analysis. Ten trees of the same height (~12 ft) from the natural population were identified randomly as source of explants in our earlier study². Of

these, six trees that were from the population at location-A (Range hills) and four trees from the population at location-B (Pashan), were tested for variability studies using ISSR markers.

Total DNA from fresh leaves was isolated from samples for both studies using miniprep method. All 100 UBC-ISSR primers were screened with the samples for amplification products. ISSR-PCR reaction was performed with 20 ng template DNA; *Taq* buffer with 1.5 mM MgCl_2 ; dNTPs (1 mM/ μl); *Taq* polymerase (3 units/ μl); ISSR primer (1.5 pm/ μl); Spermidine (20 mM) and reaction volume of 25 ml. Amplifications were performed on a Stratagene Robo-