New Source of Male Sterility in Ridge Gourd (Luffa acutangula (L.) Roxb.) and its Maintenance Through In Vitro Culture

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Introduction

Ridge gourd (*Luffa acutangula* (L.) Roxb.) is an important warm-season vegetable crop, having a long history of cultivation in tropical countries of Asia and Africa (Seshadri, 1990). Though cultivars of ridge gourd are monoecious, different sex forms were reported in this species, and the genetics of inheritance have been studied extensively (Choudhary and Thakur, 1966). So far, no male sterility has been reported in ridge gourd. An offtype was detected in a population of ridge gourd which was characterized by the production of rudimentary male flowers in racemes. Like muskmelon (Rudich *et al*., 1970), male sterility can be used to produce hybrids to capitalize on heterosis in a breeding program. Maintenance of the male sterile line is a major challenge. In order to study the genetics of male sterility, the line must be crossed with a different pollen parent to produce F_1, F_2 and backcross generations. Micro-propagation is the best approach we have found to maintain this unique source, since the genotype can be fixed without any genetic change.

Methods

The male flowers of the suspected male sterile line were subjected to microscopic analysis using a stereo microscope. and the fertility of the pollen was tested by staining with acetocarmine. Pistillate flowers of the male sterile offtype were crossed using pollen from 'Haritham'. The F_1 was generated and evaluated for pollen fertility. Micro-propagation was attempted to maintain male sterile plants. In order to standardize the establishment medium, explants were collected from two week old seedlings of monoecious 'Haritham' plants and cultured on Murashige and Skoog (1962) medium. Auxin (IAA) and cytokinin (BAP) were used individually or in combination at concentrations of 0, 0.5, 1.0, 1.5 and 2.0 mg l^{-1}.

Promising establishment media identified for monoecious plants were used for tissue culture of field-grown male sterile plants. The established shoots were used as the mother stock. For further multiplication, shoot tips and nodal portions were excised from the mother stock and cultured on MS medium supplemented with different BAP concentrations (0, 0.5, 1, 1.5 and 2 mg l^{-1}). The *in vitro* derived shoots were rooted on Murashige-Skoog medium using half strength fortified with IBA at 1.0 mg l^{-1} and charcoal at 200 mg l^{-1}. Plants were acclimatized for 30 days before they were transferred to the field.

Results

The male sterile line had rudimentary male flowers in racemes, but no fruit set after self-pollination. However fruit set was observed when pollinated using staminate flowers of the monoecious cultivar Haritham. The anthers of the suspected male sterile line were compared to those of 'Haritham' and had a marked difference with respect to the appearance of anther lobes. In the experimental line the lobes were flat and more pubescent whereas in 'Haritham', they

were was plump and filled with large fertile microspores. The microspores of the suspected male sterile line were shrunken, small and

sterile compared to those from the normal flowers. All plants in the F_1 population obtained by crossing with 'Haritham' had the male sterile character indicating its heritability.

Micropropagation has been applied successfully in cucurbits for maintenance of elite plant types (Barnes *et al*., 1978). Auxin:cytokinin ratio plays a pivotal role in determining the *in vitro* response of most of the cucurbits (Trulson and Shahin, 1986). Among the combinations, the highest explant response was observed using Murashige-Skoog medium with IAA at 1.5 mg l^{-1} + BAP at 2.0 mg l^{-1}. IAA:BAP combinations with the highest level of BAP (2 mg l^{-1}) induced profuse callus formation. Single shoots with short internodes were observed in all cultures developed from axillary meristems. The longest shoot was observed on Murashige-Skoog medium with BAP at 0.5 mg l^{-1} (9.0 cm) and with IAA at 1.5 mg l^{-1} + BAP at 2.0 mg l^{-1} (9.1 cm).

These two media were used for inoculating nodal cuttings of male sterile line collected from the field. Explant response was average, with 60% establishment in the medium, Murashige-Skoog medium with IAA at 1.5 mg l^{-1} + BAP at 2.0 mg l^{-1} (Fig. 2a) and 45% on Murashige-Skoog medium with BAP at 0.5 mg l^{-1}. Here also callus formation was observed from the base of the nodes. Shoot length after 45 days was maximum on Murashige-Skoog medium with IAA at 1.5 mg l^{-1} + BAP at 2.0 mg l^{-1} (7.5 cm) followed by BAP at 0.5 mg l^{-1} (5.3 cm). Cuttings (2 to 3 nodes) from *in vitro* shoots were used for inoculating in the multiplication medium. The highest number of shoots and nodes were observed in the medium with BAP at 1.0 mg l^{-1}.

Incorporation of BAP at 1.5 and 2.0 mg l^{-1} gave a diminishing effect on shoot multiplication. Callus formation was observed from the multiplication clumps which later transformed into shoots. The shoots from multiplication stage were used for rooting in MS medium (half strength) fortified with IBA at 1.0 mg l^{-1} and charcoal at 200 mg l^{-1} (Fig. 2c). A high percentage of rooting (95%) and continued shoot growth were observed in this medium (Fig. 2d). The rooted plants were transferred to a mist house in polyethylene bags where they were kept for one month before transplanting to the field (Fig. 2e). Tissue culture plants took 50 days after transplanting in the field for flowering and were all male sterile. Male sterility of the plants can be confirmed only after flowering and at this stage, chemical application for induction of male flowers as in cucumber and muskmelon will not be effective. This protocol can be used for maintenance and multiplication of male sterile ridge gourd plants.

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2a
RIDGE GUARD GYNOECIOUS LINE - ELONGATION
2b
2c
2d 2 division = 1 cm
2e