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Short Communication

Insights into vegetative propagation of moringa (*Moringa oleifera* Lam.)

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Abstract

The present study was conducted at the Department of Vegetable Science, College of Agriculture, KAU with a view to examine suitability of various moringa rootstocks for grafting the high-yielding variety, 'Anupama'. The impact of length and maturity of stem cuttings were also studied. Among the four rootstocks utilized for grafting 'Anupama' scions, the highest success rate was observed when 'Anupama' was used as a rootstock, followed by *Moringa stenopetala*. This study presents the first documentation of graft compatibility between *M. oleifera* and *M. stenopetala*. Wound healing was observed within three weeks post-grafting by histological analyses of the graft union. There was a significant decrease in the yield gap in second year between grafted plants and those propagated from cuttings. Moringa propagation using hardwood cuttings ranging from 55 to 60 cm in length demonstrated comparable sprouting percentage, root and sprout growth to 1.5 m long cuttings. Grafting proves to be an efficient technique for successful vegetative propagation of moringa, similar to traditional limb cuttings, which can produce maximum vegetative propagules. The stem cuttings obtained from mother plant was increased to two or three-fold by using shorter hardwood cuttings.

Keywords: Limb cuttings, *Moringa oleifera*, *Moringa stenopetala*, wedge grafting.

Introduction

Moringa (*Moringa oleifera* Lam.), a drought-resistant, nutritionally abundant tree, commonly known as drumstick is indigenous to Himalayan foothills. It is mostly cultivated in the southern states of India and some African countries. Global interest has been drawn to *M. oleifera* due to its extensive range of culinary, nutraceutical, pharmacological and phytochemical qualities that offer treatments for a wide range of diseases and have applications in the production of biofuel, environmental management and water purification (Jattan et al., 2021; Oni et al., 2021).This offers investors a wide range of business prospects for the production of different functional foods and non-food products from moringa.

Moringa stenopetala is another species of the Moringa genus which has many characteristics in common with the more popular *M. oleifera.* However, *M. stenopetala* has better drought tolerance, more vigorous growth and produces larger leaves and seeds when compared to *M. oleifera* (Abay et al., 2015).

India dominates the worldwide market for moringa and supplies more than 80% of the demand (APEDA). In Kerala, moringa is generally cultivated as a perennial vegetable (Vani et al., 2021). 'Anupama' a high-yielding perennial variety released by Kerala Agricultural University is in high demand among farmers of southern India. However, the availability of planting material, which is traditionally 1-1.5 m long semi-hardwood cutting is a limitation. There is an urgent need to increase the production of planting material to meet the rise in demand. Since *M. oleifera* is a cross-pollinated crop, propagation *via* seeds is not recommended as the progeny will not be true to type (Meena et al., 2021). Cutting and grafting are reliable methods of propagation of moringa. Grafting requires identifying potential rootstocks, scion and other parameters for the successful standardization and commercialization of moringa variety (Sumathi et al., 2023). Standardization of the size of smaller cutting and efficient grafting techniques for the cultivation of the 'Anupama' variety of *M. oleifera* are the objectives of this study.

Material and methods

The study was carried out at Department of Vegetable Science, College of Agriculture Kerala Agricultural University, Vellanikkara, Thrissur

Evaluation of rootstocks for grafting moringa

The seeds of two accessions of *Moringa oleifera*(AD-4 and RSPM) , one variety of 'Anupama' and *M. stenopetala* were raised, and 35-40 days old seedlings were used as rootstock

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for grafting. Four grafting combinations *viz*., 'Anupama' rootstock with 'Anupama' scion, AD-4 rootstock with 'Anupama' scion, RSPM rootstock with 'Anupama' scion and *M. stenopetala* rootstock with 'Anupama' scion were experimented and compared with other methods of vegetative propagation such as cutting and seed propagation. Young shoot tips of girth matching with the girth of rootstocks at the height where grafting was carried out were selected as scion, for the experiment. The rootstocks were raised in polyethylene bags of size 7 cm x 11 cm. A potting mixture in the ratio of 1:1:1 of soil, sand, and farm yard manure was used as growth media. Wedge grafting (Buoso & Loschi 2019) was adopted for the present experiment. Grafted seedlings were maintained in a mist chamber, providing optimum conditions of temperature (26 to 28°C) and humidity (above 90%). The experiments were carried out for two seasons and the average values were statistically analysed.

Histochemical studies on tissue regeneration at graft union:
The advancement in tissue regeneration at the graft union was determined by histochemical observations taken sequentially 7 days, 14 days and 21 days after grafting. Thin transverse sections of the stem were cut at the point of graft union, stained with safranin and observed under the microscope.

Field evaluation of grafted seedlings:
A field experiment was carried out after successful grafting of the seedlings, in a randomized block design with four replications. The grafted plants were planted along with cuttings and seedlings of the 'Anupama' variety. The seedlings were planted at a spacing of 3×3 meters and the crop was grown as per the standard package of practices recommended by Kerala Agricultural University. The treatments were observed in field for the two consecutive years, and parameters *viz*., weight, length and girth of fruit, fruits/plant, and yield were recorded. Four replications were maintained in this experiment.

Evaluation of stem cuttings of different length and maturity for propagation:
Stem cuttings of various length and maturity (hardwood, semi-hardwood) of 'Anupama' variety were evaluated for its vegetative propagation. The cuttings were raised in polythene bags (25 cm x 35 cm). The potting mixture was prepared as mentioned in the experiment on 'evaluation of rootstocks for grafting moringa'. Hardwood (girth 14-16 cm) and semi-hardwood (girth 10-12 cm) cuttings of three sizes *viz*. 35-40 cm, 55-60 cm, and 1.2-1.5 m were selected for the study. The experiments were carried out for two seasons and the average values were statistically analysed.

Results and discussion

Evaluation of rootstocks for grafting moringa:
In cross pollinated perennial moringa types vegetative propagation is most promising as it ensures genetic fidelity of the progeny through generations. Seedling raised moringa trees are reported to produce poor quality fruits (Ramachandran et al., 1980). Cutting and grafting are the most common methods of propagation for moringa in India (Leone et al., 2015).

Improvement of graft success depends on the growth attributes of rootstock (Mng'omba et al., 2010). Among the four rootstocks used for grafting with the scion of 'Anupama', *M. stenopetala* seeds resulted in the least number of days required for germination (6.05 days) followed by seeds of RSPM rootstock (6.13 days) as shown in Table 1.

Table 1. Performance of different rootstocks for grafting of moringa.

Rootstocks	Days to germination	Germination percent	Days to reach graftable size	Graft success percent
RSPM	6.13^{c}	69.13^{b}	22.73^{c}	47.13^{b}
AD4	7.13^{b}	77.43^{a}	27.08^{b}	35.23^{d}
'Anupama'	8.43^{a}	53.63^{d}	31.98^{a}	51.30^{a}
M. stenopetala	6.05^{c}	56.60^{c}	21.25^{c}	44.70^{c}
CV	6.86	2.86	3.88	2.43
SE(d)	0.34	1.30	0.71	0.77

Means with different alphabets are significantly different at á=0.05

The seeds of AD4 and 'Anupama' accessions required 7.13 days and 8.43 days respectively which were significantly higher than the days to germination observed in *M. stenopetala* and RSPM accessions. Days to germination of seeds of moringa accessions was earlier reported to range from 6 to 12 days as compared the range of 6 to 8.4 days recorded in the present study (Elavarasan et al., 2021). Germination per cent was highest in the seeds of rootstock AD4 (77.43). In a similar study Sumathi et al. (2023) have also reported a minimum germination percentage of 53.9 per cent for moringa seeds of a local accession.The rootstock *M. stenopetala* required only 21.25 days to reach graftable size followed by RSPM (22.73 days) for attaining graftable size. In an earlier report the days taken to graftable size ranged from 25.4 days to 52.5 days in perennial types and 20.5 days for annual type moringa (Sumathi et al. 2023). The graft success was highest for the graft with rootstock and scion, both from 'Anupama' variety. The percentage of graft success recorded in grafts with rootstocks from 'Anupama' was 51.30. The compatibility among the same variety might have attributed to the high graft success percent of 'Anupama' rootstock. However, it was observed that the days to germination was higher, and the germination percentage was lower in seeds of 'Anupama', than the other accessions. It also recorded longest duration to reach

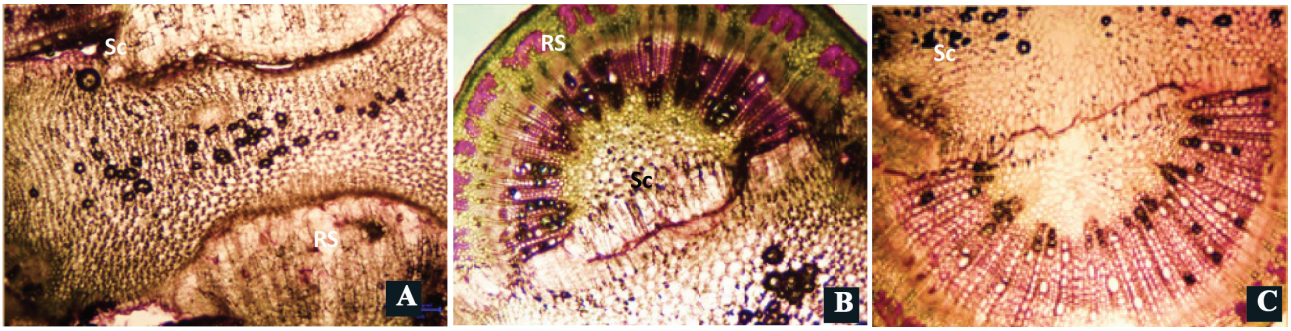

Figure 1. Histochemical studies in grafted moringa seedlings. Transverse section at graft union (A) 7 days after grafting (B) 14 days after grafting (C) 21 days after grafting

graftable size among the other rootstocks. *M. stenopetala* rootstock exhibits better result for parameters, days taken for germination and days to attain graftable size, which reflects the vigorous nature of *M. stenopetala* seedlings.

Histochemical studies on tissue regeneration at graft union:
The observations on the advancement of tissue regeneration at the point of union of the rootstock and scion revealed (Fig. 1) necrotic layer and callus formation in the initial stages of healing. As the wound healing progressed the necrotic layer slowly faded away and was replaced by newly formed callus tissue which ensured connectivity of tissues of rootstock and scion. In bittergourd grafted on pumpkin callus production and vascular bundle differentiation was noticed at 21st day after grafting (Tamilselvi, N. A. and Pugalendhi, 2017).

The wound healing process progressed in a similar manner in grafts involving rootstocks of *M. stenopetala* and *M. oleifera*. No incompatibility was observed when *M. stenopetala* was used as rootstock for grafting the scions of 'Anupama'

Field evaluation of grafted seedlings:
The grafted seedlings were evaluated in the field for two seasons (first and second year) (Table 2). The moringa grafts were compared with the ones propagated via cutting and seedling method. Yield, average fruit weight and fruits per plant observed was higher in case of cuttings of 'Anupama'. Grafts with 'Anupama' rootstock resulted in maximum yield, fruits per plant, weight, and length of fruit among other treatments, followed by graft with RSPM rootstock. The yield of grafts with 'Anupama' (first year: 285.43 g; second year: 2273.75 g) was comparable with that of the stem cuttings of 'Anupama' (first year: 465.35 g; second year: 3561g) during both seasons. The parameters *viz*., yield and fruits per plant recorded for the propagation of moringa via seedling was poor compared to all other treatments. In an earlier study on mango and avocado it was reported that the seedlings flowered less heavily than their grafted counterparts, which could be attributed to the residual juvenility of the seedlings (Lahav et al., 1995). Graft treatments recorded considerably low yield to that of stem cutting treatment in the initial year. However, by the second year, the yield gap between grafted seedlings and plants from cuttings reduced remarkably (Table 2.). A long period (of about 3 years) is required for the yield stabilization in perennial plants like moringa (Waterman et al., 2021). It might be possible that the graft yield might outdistance the yield produced by stem cutting treatments in subsequent years.

Evaluation of stem cuttings of different length and maturity for propagation:
Stem cuttings of different maturity was used for the study, namely, semi-hardwood (SHD) and hardwood cuttings (HWD) (Table 3). Different lengths of stem cuttings including small (35-40 cm), intermediate (55-60 cm) and long (1.2-1.5 m) were used as treatments. Long HWD stem cuttings recorded highest values for the various parameters

Table 2. Field evaluation of grafted seedlings for two seasons

	Fruit weight (g)		Fruit length (cm)		Fruit girth (cm)		No. of fruits/plant		Yield (g)	
Treatment	I st year	II nd year	I st year	II nd year	I st year	II nd year	I st year	II nd year	I st year	II nd year
RSPM-A*	44.68^{d}	97.88^{b}	36.85^{c}	51.01^{a}	6.53^{ab}	6.53	3.75^{c}	21.5^{b}	165.83^{c}	2090.5^{b}
AD4-A	41.2^{e}	95.9^{bc}	36.28^{c}	51.93^{a}	6.30^{b}	6.48	2.75^{d}	16.75^{c}	104.5^{d}	1569.5^{c}
A-A	57.18^{b}	98.43^{b}	43.53^{a}	53.40^{a}	6.23^{b}	6.30	5.00^{b}	23.0^{b}	285.43^{b}	2273.75^{b}
Ms-A	46.18^{cd}	94.2^{cd}	37.33^{c}	45.80^{bc}	6.33^{b}	6.78	2.50^{d}	16.75^{c}	100.08^{d}	1626.00^{c}
A-cutting	62.28^{a}	101.5^{a}	40.48^{b}	50.35^{ab}	6.80^{a}	6.73	8.00^{a}	34.75^{a}	465.35^{a}	3561.00^{a}
A-seedling	48.8^{c}	92.58^{d}	35.7^{c}	42.18^{c}	6.85^{a}	6.73	1.00^{e}	2.50^{d}	51.05^{e}	229.75^{d}
CV	4.31	5.52	3.38	6.35	4.59	4.18	13.75	14.17	7.81	14.88
SE(d)	1.53	3.76	0.92	2.21	0.21	0.2	0..37	1.925	10.8	198.97

* Graft combinations (rootstock-scion) were RSPM-'Anupama'; AD4-'Anupama'; 'Anupama'-'Anupama'; *M. stenopetala*-'Anupama'. Means with different alphabets are significantly different at á=0.05.

Table 3. Effect of length and maturity of stem cuttings on shoot and root parameters for vegetative propagation of moringa ('Anupama' var.).

Treatment	Survival percent	No. of sprouts	Length of sprouts (cm)	No. of main roots	Length of roots (cm)	Root diameter (cm)
SHD1*	13.88^{d}	2.33^{d}	13.97^{e}	2.8^{b}	10.87^{b}	1.17
SHD2*	36.11^{c}	2.67^{cd}	24.53^{d}	3.63^{a}	14.50^{b}	1.20
SHD3*	44.44^{c}	3.00^{bcd}	31.67^{c}	4.1^{a}	21.40^{a}	1.30
HWD1*	61.11^{b}	3.33^{bc}	31.67^{c}	2.8^{b}	19.50^{a}	1.30
HWD2*	77.78^{a}	3.67^{ab}	39.30^{b}	3.87^{a}	22.87^{a}	1.37
HWD3*	80.56^{a}	4.33^{a}	44.43^{a}	3.97^{a}	21.40^{a}	1.37
CV	13.01	16.36	9.09	9.04	11.54	11.02
SE(d)	5.56	0.43	2.3	0.26	1.75	0.12

*SHD1- Semi-hardwood cuttings-small; SHD2- Semi-hardwood cuttings-intermediate; SHD3- Semi-hardwood cuttings- long; HWD1- Hardwood cuttings-small; HWD2- Hardwood cuttings- intermediate; HWD3- Hardwood cuttings- long. Means with different alphabets are significantly different at á=0.05.

observed *viz*., survival percentage (80.56%), length and number of sprouts (44.43 cm and 4.33 respectively), and diameter of main roots (1.37 cm). However, the intermediate length HWD were on par with that of long HWD for the crucial parameters like survival percentage, number of sprouts and main roots and root length. The maturity of stem cutting is crucial for sprouting. Other studies have also reported higher rooting in hardwood cuttings than softwood cuttings. This can be mainly due to the ability of hardwood cuttings to withstand abiotic factors viz., humidity, soil temperature, and light intensity (Antwi-Boasiako & Enninful, 2011). The hardwood cuttings have better lignification which enhances their ability to withstand moisture loss and other adverse growth conditions. They also have higher levels of stored food to nourish and sustain the developing sprouts and roots until the shoots become self-sustaining (Hartman et al., 1990). Hence hardwood cuttings have a high potential to be used as planting material for moringa.

Conclusion

Among the rootstocks studied, 'Anupama' showed maximum success percentage of grafting followed by *M. stenopetala* and AD4. Wound healing was observed within three weeks of grafting on weekly histological studies of the graft union. The wound healing process progressed in a similar manner in grafts involving rootstocks of *M. stenopetala* and *M. oleifera*, indicating no incompatibility reactions for grafting seedlings of these two species of Moringa. There was a significant decrease in yield gap between grafted plants and plants raised from cuttings in the second year. This gap would be further narrowed in the years to come, hence grafting could be a viable alternative to vegetative propagation of moringa by cuttings. In the second study, propagation of moringa with 55-60cm hardwood cuttings were found to be on par with cuttings of 1.5 m length with respect to percentage of sprouting, roots and growth of sprouts.Number of cuttings obtained from mother plant were increased to two or three-fold by using hardwood cuttings of shorter length, without compromising the growth and development of the cuttings.

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