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Short communication

Standardisation of grafting in bitter gourd (*Momordica charantia* L.)

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Abstract

A study on grafting bitter gourd on various cucurbitaceous rootstocks was carried out to identify the best rootstocks for grafting bitter gourd and to compare two grafting methods namely, wedge grafting (WG) and tongue approach grafting (TAG). In the study bitter gourd scion (var. Preethi) was grafted on six rootstocks, *viz*., ash gourd, bottle gourd, smooth gourd, pumpkin, oriental pickling melon and self rootstocks by wedge grafting and tongue approach grafting. There was significant difference in graft success among the rootstocks in WG. In WG, best rootstock in terms of graft success was smooth gourd (80%) followed by pumpkin, bottle gourd, ash gourd, OP melon and self rootstock. There was no significant difference among the rootstocks on graft success in TAG. WG (67%) was superior to TAG (15.17%) in terms of final graft success. Graft success had significant positive correlation with relative humidity and significant negative correlation with maximum and minimum temperature. Anatomical studies of the graft union revealed cell proliferation which is the stage prior to callus formation.

Keywords: Grafting, Rootstock, Scion, Tongue approach grafting, Wedge grafting

Bitter gourd (*Momordica charantia* L.) is a popular warm season vegetable belonging to the family Cucurbitaceae. It is cultivated throughout the country for its immature fruits which are used as vegetable. In India, area under bitter gourd is 92,870 ha with an annual production of 10.45 lakh tonnes. In Kerala, area under bitter gourd is 2,880 ha with the production being 42,250 tonnes (NHB, 2016). Short duration and premium market price make it remunerative for vegetable growers in Kerala.

Grafting with resistant rootstocks offer one of the best methods to avoid soil borne pathogens and nematodes. Grafting in vegetables is common in many parts of the world. First attempt in raising grafted vegetable plants was done in Japan and Korea in late 1920s by grafting watermelon onto bottle gourd for imparting resistance to Fusarium wilt (Lee, 1994). In addition to site specific management tool for soil borne insects and pathogens, grafting also imparts resistance to abiotic stresses, increases yield, extends harvest period, manipulates sex expression and improves fruit quality (Pandey and Rai, 2003). Survival rate of grafted plants depends on stock-scion compatibility and post graft management. Commercial use of vegetable grafting is a relatively recent innovation. Information on the effect of various rootstocks on the performance of cucurbits in Kerala is meagre. In this background, the present study was undertaken to identify best rootstocks for grafting bitter gourd, to compare two grafting methods namely, Wedge grafting and tongue approach grafting and to study the anatomy of successful grafts.

Bitter gourd variety Preethi was used as the scion material and six different rootstocks namely, ash gourd (*Benincasa hispida*) variety KAU Local, bottle gourd (*Lagenaria siceraria*) variety Arka Bahar, smooth gourd (*Luffa cylindrica*), pumpkin (*Cucurbita moshchata*) variety Ambili and OP

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melon (*Cucumis melo* var. *conomon*) variety Mudicode were used for standardisation of grafting. The requirements for grafting operation were razor blade, grafting clips and healing chamber with mist system.

Seeds were sown in 98 well pro-trays containing two parts coir pith compost, one part vermicompost and one part soil. Number of days taken for germination and number of days to attain graftable size for rootstocks and scions were noted. Sowing time of rootstock and scion seeds were adjusted such that they attained grafting stage uniformily. Grafting was performed under shade net preferably during morning or evening hours. Immediately after grafting, the plants were transferred to a healing chamber or mist chamber, and they were maintained at high relative humidity by intermittent application of mist to facilitate the formation of graft union. Once the graft union was established, grafts were transferred to beneath shade nets and kept for two to three days for acclimatization before planting in the main field.

The procedure of wedge grafting is demonstrated in plate 1. Growing point of rootstock was removed below the cotyledons and a cleft was made for inserting scion. The scion hypocotyl was cut at both sides and a wedge was made. The prepared scion was inserted into the cleft made in rootstock. The

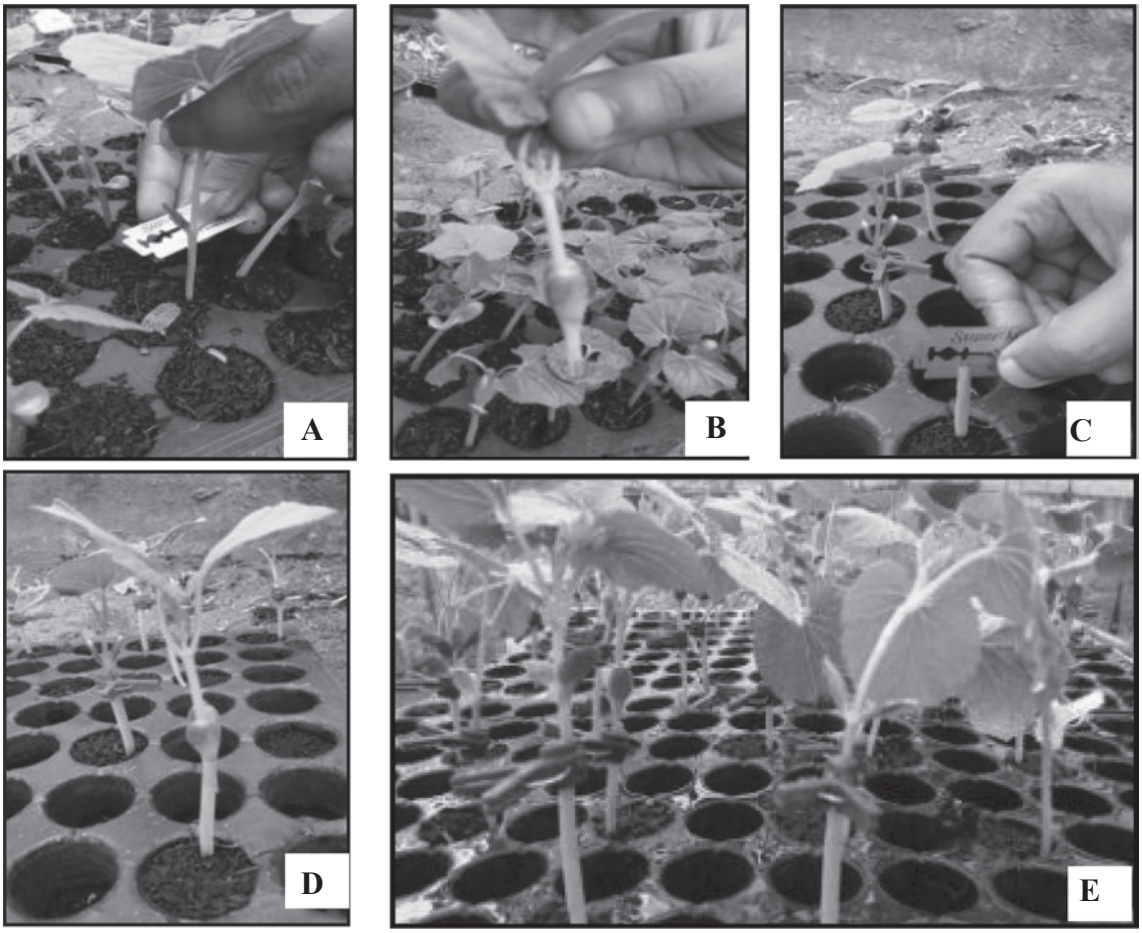

Plate 1. Steps in wedge grafting in bitter gourd
(A) Top portion of scion removed from seedling (B)Scion hypocotyl made in form of wedge (C) Cleft made in rootstock hypocotyl (D) Scion inserted into cleft in the rootstock (E) Graft union fixed using grafting clip

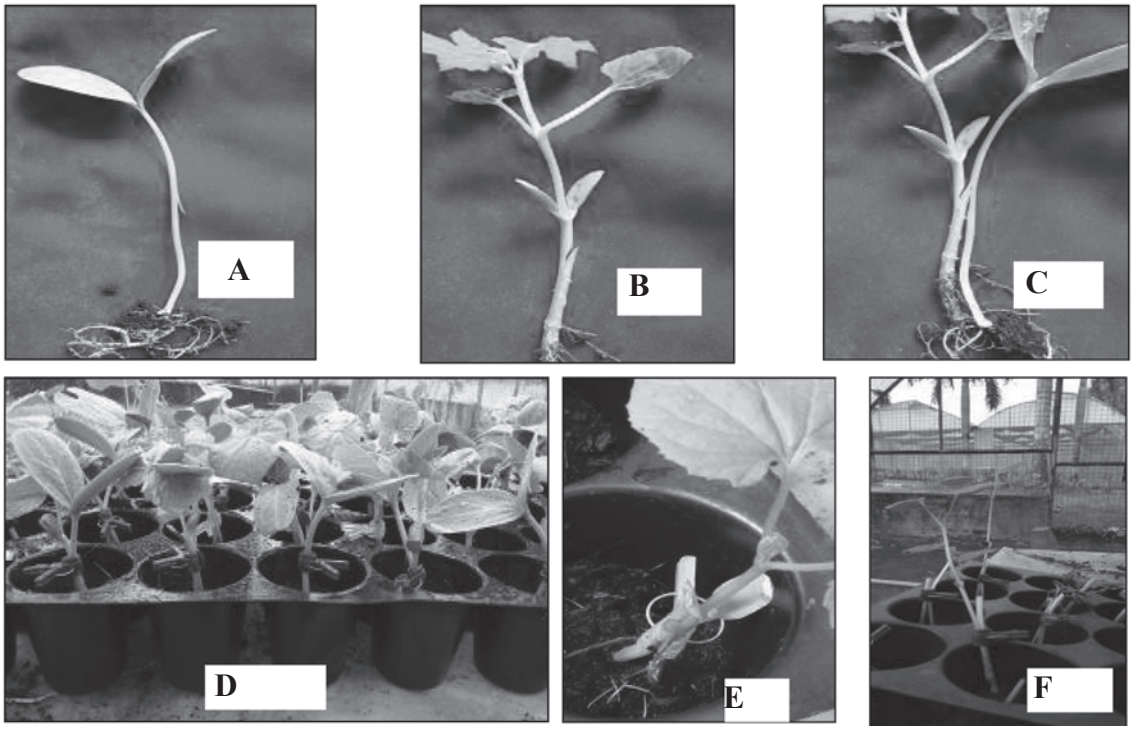

Plate 2. Steps in tongue approach grafting in bitter gourd. (A) Upward cut in rootstock hypocotyl (B) Downward cut in scion hypocotyl (C) Joining the cut surfaces of rootstock and scion (D) Graft union fixed using grafting clip (E) Removal of top portion of rootstock (F)Removal of bottom portion of scion

rootstock and scion hypocotyls were fixed using grafting clip and placed in mist chamber for three days.

In tongue approach grafting, the rootstock and scion were cut through the hypocotyl at 35^0 to 45^0 angle upwards and downwards respectively (or vice versa). The cut surfaces were joined and grafting clip was used to hold the graft union. The procedure is demonstrated in plate 2. The grafts were kept in mist chamber for three days. After three days, the top of rootstock was removed and again kept in mist chamber. The bottom portion of the scion just below the graft union was removed five to seven days after grafting.

To confirm the best age of rootstocks for grafting, wedge grafting was done in six selected rootstocks. The age of scions were kept constant at 7-10 days after germination. In all the six rootstocks, three different age groups viz., 5-7 days, 10-12 days, and 15-17 days after germination were selected. Observation on graft success was recorded 9-12 days after grafting. To study the influence of type of rootstocks and methods of grafting on graft success, percentage success was recorded at three, six, nine and twelve days after grafting. To study the effect of season on success of grafting, wedge grafting was done on every 15^{th} day of each month commencing from May to October. Observation on graft success was recorded 9-12 days after grafting. The grafting experiment was laid out in Completely Randomized Design (CRD) with four replications and 25 plants per replication. In order to study the cellular changes of grafted plants, anatomical studies were carried out at 20 days after grafting. Transverse sections were taken from wedge grafts, stained with saffranine stain and viewed under electron microscope.

Table 1. Days taken by various rootstocks for germination and to attain graftable size for grafting bitter gourd and diameter of rootstock hypocotyl at the time of grafting

Rootstocks	Number of days taken for germination (days)	Number of days taken to attain graftable size(days)	Diameter of rootstock hypocotyl at the time of grafting (mm)
R_1	4.14[bc]	12.99[d]	4.40[d]
R_2	4.43[b]	10.43[e]	9.93[a]
R_3	6.00[a]	17.57[b]	6.90[c]
R_4	3.85[bc]	9.99[f]	7.17[b]
R_5	3.42[c]	18.27[a]	3.13[e]
R_6	6.42[a]	15.42[c]	7.16[b]
C.D. (0.05)	0.825	0.079	0.21

Number of days taken for germination and days to attain graftable size

A preliminary study was conducted to determine number of days taken for germination and number of days to attain graftable size for rootstocks and scion (Table 1). Among the six rootstocks studied, significantly lesser number of days was required for germination of OP melon (3.42 days) compared to other rootstocks. Highest number of days for germination was recorded by bitter gourd (6.42 days) which was on par with smooth gourd (6.00 days). Criteria for the rootstocks and scions to attain graftable size were based on thickness of the stock and scion and their suitability for grafting operation. Pumpkin (9.99 days) and bottle gourd (10.43 days) took less days to attain graftable size due to larger diameter of hypocotyls (7.17 mm and 9.93 mm respectively). Even though oriental pickling melon took less number of days for germination, days taken to attain graftable size was more (17.57 days) due to its slender hypocotyls having diameter of 3.13 mm. Accordingly, for grafting bitter gourd the scion seeds should be sown two, four and five days before sowing the seeds of ash gourd, bottle gourd and pumpkin respectively. The seeds of rootstocks of smooth gourd and oriental pickling melon should be sown three and four days respectively prior to sowing seeds of bitter gourd. The results were supported by the reports of Punithaveni et al. (2014). They reported that fig leaf gourd (10.55 days) and pumpkin (11.52 days) took least number of days to attain graftable size due to higher diameter of hypocotyls while sponge gourd (14.56 days) took maximum number of days, due to smaller diameter of hypocotyls.

Influence of age of rootstocks on graft success

Age of grafting was standardised, based on graft success of each rootstock in different age groups. Age of rootstocks had profound influence on graft success in three rootstocks studied *viz*., ash gourd, smooth gourd and oriental pickling melon and optimum age group was from 10-12 days after germination, which was on par with 15-17 days (Table 2). Graft success in these rootstocks in the age group 5-7 days was low compared to age groups 10-12 days and 15-17 days. This could be due to slender and succulent seedlings with smaller

Table 2. Influence of age of rootstocks on graft success in bitter gourd

Age of rootstocks	Graft success in different rootstocks (%)					
	R_1	R_2	R_3	R_4	R_5	R_6
A_1	50.00[b]	81.00	67.00[b]	79.00	48.00[b]	48.00
A_2	68.00[a]	82.00	80.00[a]	77.00	68.00[a]	51.00
A_3	65.00[a]	79.00	80.00[a]	75.00	75.00[a]	50.00
C. D. (0.05)	11.093	NS	9.032	NS	11.250	NS

A_1: 5-7 days A_2: 10-12 days A_3:15-17 days

R_1: Ash gourd R_2: Bottle gourd R_3: Smooth gourd R_4: Pumpkin R_5: OP melon R_6: Bitter gourd

Table 3. Interaction effect of rootstocks and methods of grafting on graft success in bitter gourd

Rootstocks	Graft success at three days interval (%)											
	3 DAG			6 DAG			9 DAG			12 DAG		
	G_1	G_2	Mean (Treatments)	G_1	G_2	Mean (Treatments)	G_1	G_2	Mean (Treatments)	G_1	G_2	Mean (Treatments)
R_1	73.00^{b}	85.00^{a}	79.00^{cd}	68.00^{cd}	71.00^{c}	69.50^{bc}	66.00^{b}	24.00^{e}	45.00^{bc}	66.00^{b}	*16.00^{d}*	41.00^{abc}
R_2	88.00^{a}	91.00^{a}	89.50^{a}	81.00^{a}	74.00^{ab}	77.50^{ab}	75.00^{a}	26.00^{e}	50.50^{ab}	75.00^{a}	17.00^{d}	46.00^{ab}
R_3	82.00^{ab}	90.00^{a}	86.00^{ab}	82.00^{a}	60.00^{de}	71.00^{b}	80.00^{a}	24.00^{e}	52.00^{a}	80.00^{a}	13.00^{d}	46.50^{ab}
R_4	92.00^{a}	92.00^{a}	92.00^{a}	86.00^{a}	73.00^{c}	79.50^{a}	77.00^{a}	27.00^{e}	52.00^{a}	77.00^{a}	17.00^{d}	47.00^{a}
R_5	70.00^{bc}	90.00^{a}	80.00^{abc}	60.00^{de}	68.00^{cd}	64.00^{d}	56.00^{cd}	24.00^{e}	40.00^{cd}	56.00^{c}	15.00^{d}	35.50^{bc}
R_6	68.00^{bc}	86.00^{a}	77.00^{d}	53.00^{e}	58.00^{de}	55.50^{e}	48.00^{ed}	22.00^{e}	35.00^{d}	48.00^{c}	13.00^{d}	30.50^{c}
Mean (Grafting method)	78.83^{b}	89.00^{a}		71.67^{a}	67.33^{b}		67.00^{a}	24.50^{b}		67.00^{a}	15.17^{b}	
C.D. 0.05)	4.33[1], 7.51[2], 10.62[3]			3.978[1],6.89[2],9.745[3]			3.613[1], 6.258[2], 8.85[3]			3.527[1], 6.109[2], 8.64[3]		

[1]Methods, [2]Treatments, [3]Interaction

R_1 – Ash gourd R_2- Bottle gourd R_3 – Smooth gourd R_4– Pumpkin R_5– OP melon R_6 – Self rootsctock G_1- Wedge Grafting G_2- Tongue Approach Grafting

diameter of hypocotyls. For rootstocks of bottle gourd, pumpkin and bitter gourd, grafting could be performed irrespective of age starting from 5-7 days after germination to 15-17 days after germination. This could be due to sturdy seedlings with higher diameter of hypocotyls even at younger age. In a study by Tamilselvi and Pugalendhi (2017) on days taken by cucurbitaceous rootstocks to attain graftable size, rootstocks of ash gourd, bottle gourd, sponge gourd and pumpkin took 36.50, 24.80, 23.10 and 22.70 days while scion of bitter gourd (CO1) took 26.40 days to attain graftable size. But in the present study considerable graft success was obtained even at younger age of stock and scion.

Influence of rootstocks on graft success

In wedge grafting, pumpkin rootstock recorded highest graft success of 92.00 per cent and 86.00 per cent at three and six days after grafting respectively (Table 3). Later, graft success was reduced to 77 per cent. In bottle gourd, high initial graft success of 88 per cent and 81 per cent was observed at three and six days after grafting respectively but it reduced to 75 per cent when observed at nine days after grafting. The graft success in smooth gourd rootstock was 82 per cent on third and sixth day after grafting. The final graft success was 80.00 per cent in smooth gourd which was highest among all the six rootstocks studied. Graft success was least for homografts at three, six, nine and twelve days after grafting (68%, 53%, 48%, and 48% respectively).

In tongue approach grafting, graft success for the rootstock pumpkin on three, six, nine and twelve days after grafting was 92 per cent, 73 per cent, 27 per cent and 17 per cent respectively (Table 3). Bottle gourd also had similar final graft success (17%). Smooth gourd and bitter gourd rootstocks had final graft success of 13 per cent.

Influence of methods of grafting on graft success

In wedge grafting, per cent graft success gradually decreased from third day (78.83%) to ninth day (67%) after grafting. In tongue approach grafting, even though graft success was high during initial days (89%), it was reduced to 15.17 per cent on 12th day of grafting (Figure 1). Davis et al. (2008) suggested that tongue approach grafting is more suited for rootstocks with solid hypocotyls. Thus

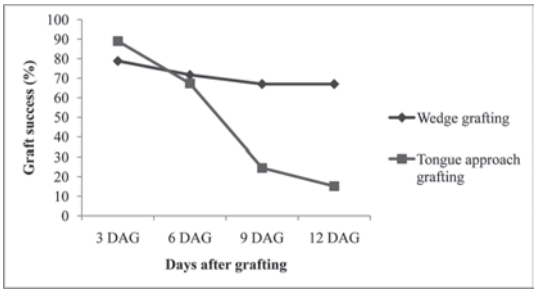

Figure 1. Influence of grafting methods on graft success in bitter gourd

low success per cent could be due to hollow hypocotyls in cucurbitaceous rootstocks.

Interaction effect of rootstocks and methods of grafting on graft success

When interaction effect was studied, the rootstock of pumpkin had graft success of 92 per cent, 79.50 per cent, 52 per cent and 47 per cent respectively on three, six, nine and twelve days after grafting (Table 3). In the case of bottle gourd, initial success was 89.50 per cent while it was reduced to 46 per cent on 12th day of grafting. In smooth gourd, even though initial graft success was less than bottle gourd, final success per cent was 46.50 per cent. At all the intervals studied, bitter gourd as rootstock had least graft success. It was in agreement with the findings of Tamilselvi and Pugalendhi (2017). They reported pumpkin as the best rootstock for grafting bitter gourd with a success per cent of 71.71 followed by smooth gourd (68.26%). Least success (49.24) was observed in mithipakal (*Momordica charantia* var. *muricata*).

Influence of month of grafting on graft success

Weather conditions prevailing in a region influence graft success and it varies from one region to another within a season. Graft success was high in the month of July (68.33%) which was on par with graft success in June and August (67%) (Table 4). Graft success during September and October were 58.83 per cent and 57.83 per cent respectively. Least success was noticed in the month of May with a success per cent of 51.16.

Correlation of weather and graft success in bitter gourd

Data on graft success of different rootstocks were subjected to correlation analysis. The correlation

Table 4. Interaction effect of rootstocks and month of grafting on graft success in bitter gourd

Rootstocks	Graft success in different months of grafting (%)						
	M_1	M_2	M_3	M_4	M_5	M_6	Mean(Rootstocks)
R_1	48.00^{ed}	66.00^{ab}	67.00^{ab}	64.00^{ab}	62.00^{b}	56.00^{d}	60.50^{b}
R_2	56.00^{d}	75.00^{a}	74.00^{a}	74.00^{a}	64.00^{ab}	64.00^{ab}	67.83^{a}
R_3	56.00^{d}	80.00^{a}	80.00^{a}	80.00^{a}	74.00^{a}	72.00^{a}	74.00^{a}
R_4	65.00^{ab}	77.00^{a}	80.00^{a}	77.00^{a}	66.00^{ab}	66.00^{ab}	71.83^{a}
R_5	44.00^{e}	56.00^{d}	59.00^{c}	54.00^{d}	50.00^{d}	52.00^{d}	52.16^{c}
R_6	38.00^{e}	48.00^{ed}	50.00^{d}	53.00^{d}	37.00^{e}	37.00^{e}	43.83^{d}
Mean	51.16^{d}	67.00^{a}	68.33^{a}	67.00^{a}	58.83^{b}	57.83^{bc}	
C. D.(0.05)	4.28[1], 4.28[2], 10.49[3]						

M_1 – May M_2 – June M_3 – July M_4 – August M_5 – September M_6 – October
1: C. D. for comparing month of grafting * Significant at 0.05 level
2: C. D. for comparing rootstocks ** Significant at 0.01 level
3: C. D. for comparing interaction

Table 5. Correlation of weather and graft success in bitter gourd

Rootstocks	Correlation coefficient		
	Relative humidity	Maximum temperature	Minimum temperature
R_1	0.64**	0.74**	0.56**
R_2	0.69**	0.69**	0.63**
R_3	0.61**	0.74**	0.58**
R_4	0.55**	0.49*	0.54**
R_5	0.51**	0.49*	0.59**
R_6	0.52**	0.47*	0.46*

R_1: Ash gourd R_2: Bottle gourd R_3: Smooth gourd R_4: Pumpkin R_5: OP melon R_6: Bitter gourd

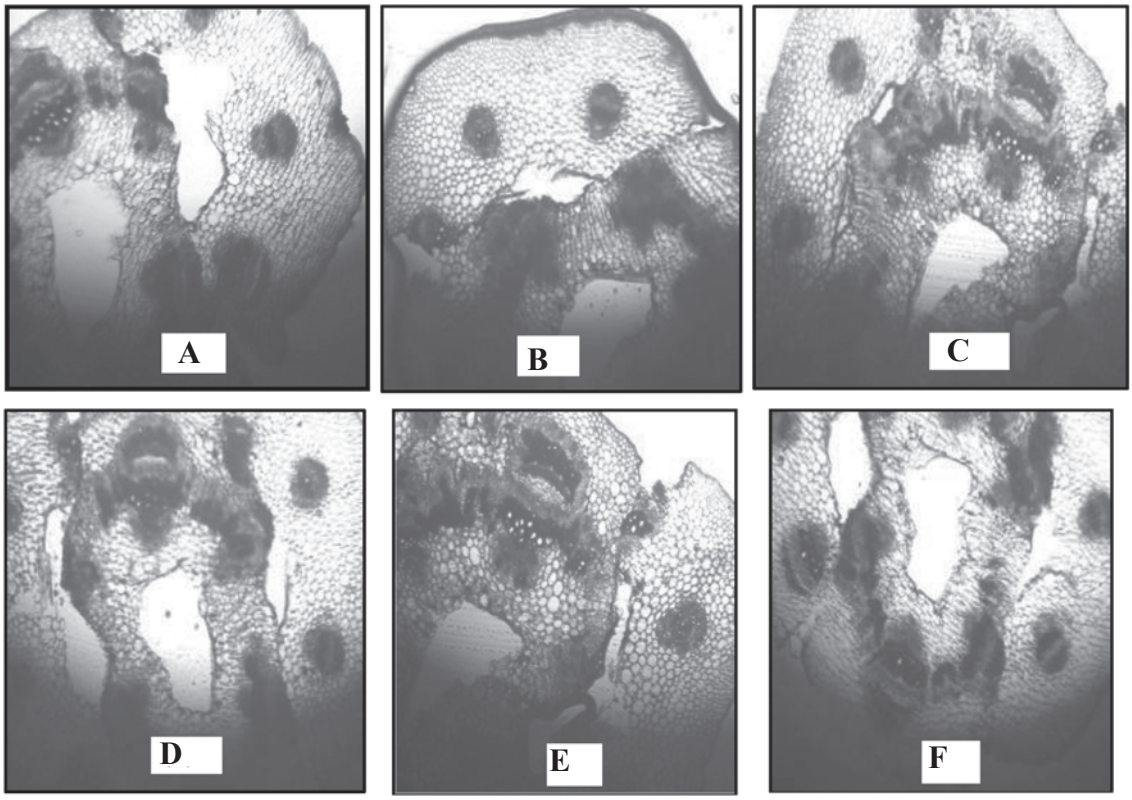

Plate 3. Cross-section of graft union in bitter gourd grafted on
(A) Ash gourd (B) Bottle gourd (C) Smooth gourd (D) Pumpkin (E) OP melon and (F) Self rootstock

coefficients between graft success and monthly mean weather parameters *viz*., relative humidity, maximum and minimum temperature were estimated and presented in table 5. Relative humidity had highly significant and positive correlation with graft success. Maximum and minimum temperature had negative correlation with graft success.

Anatomical studies

Histological observation of bitter gourd at graft interface during the present study revealed formation of necrotic layer in response to wound repair and proliferation of cells of rootstock and scion which is the stage prior to callus formation (Plate 3).

Among the type of rootstocks studied for grafting bitter gourd, in wedge grafting best rootstock was smooth gourd followed by pumpkin, bottle gourd, ash gourd, oriental pickling melon and self rootstock. For tongue approach grafting, the graft success was not significant among the rootstocks. Interaction effect of rootstocks and methods of grafting revealed that best rootstock for grafting bitter gourd was pumpkin followed by smooth gourd, bottle gourd, ash gourd, oriental pickling melon and self rootstock. Wedge grafting was found to be superior over tongue approach grafting in terms of final graft success. Grafting done during the months of June, July and August gave higher graft success per cent compared to May, September and October. Histology of grafts revealed cell proliferation at graft union.

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