The Pharma Innovation Journal 2022; 11(9): 2452-2456

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(9): 2452-2456

www.thepharmajournal.com
Received: 05-06-2022
Accepted: 12-08-2022

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Graft compatibility of bottle gourd scion with different cucurbitaceous rootstock

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Abstract
A study was carried out to determine the graft compatibility of bottle gourd scion (Haruna and Gaurav) with five different cucurbitaceous rootstock *viz*., watermelon, pumpkin OP, Pumpkin F1, Bottle gourd and Muskmelon. The grafting method adopted in this study was slant grafting. Graft compatibility was determined by evaluating the number of days taken to attain graftable size, days taken for graft union, percent of grafting success, graft healing and survival rate of grafted plants after regeneration of vascular bundles over the graft interface and vegetative growth of the scion. There was a significant difference in graft success among different graft combination. Among the rootstocks, pumpkin (*Cucurbita moschata*) and bottle gourd (*Lagenaria siceraria*) recorded significantly less number of days to attain graftable size (17.0) and (17.4) respectively. The rootstock bottle gourd (*Lagenaria siceraria*) took the least number of days (6.6) for graft union with Haruna whereas pumpkin OP (*Cucurbita moschata*) grafted onto Gaurav recorded the highest number of days (8.6). Graft success on 30th days after grafting revealed that the rootstocks bottle gourd (*Lagenaria siceraria*) recorded highest grafting success (85.5) with Haruna followed by water melon (*Citrullus lanatus*), lowest number of days (4.5) was required for graft hardening in Haruna grafted on bottle gourd (*Lagenaria siceraria*) and the maximum survival (86.5) was recorded in the combination of HT1 *i.e* Haruna grafted onto watermelon. This suggest that bottle gourd (*Lagenaria siceraria*) and watermelon (*Citrullus lanatus*) rootstock could be the compatible rootstock for grafting with bottle gourd scion.

Keywords: Compatibility, bottle gourd, cucurbitaceous, rootstock, *Lagenaria siceraria*

Introduction
Bottle gourd, synonymous to white flowered gourd and calabash gourd (*Lagenaria sicereria*) is one of the important vegetable crop belonging to the family cucurbitaceae with a chromosome no. of 2n=22. It is one of the important cucurbits grown throughout the world for its tender fruits (Arvind *et al.*, 2011) [1]. It is grown in summer season as well as rainy season. The crop is cultivated over an area of 187 million hectare (FAOSTAT 2019) [3] in India with an annual production of 1428296 tones and the productivity of 12.21 tones per hectare (Horticulture database, 2019) [2]. To be successful on a global scale, this indigenous crop requires attention from breeders and production system specialists.

Grafting is a common technique in vegetable production in several Asian and European countries (Lee, 2003) [4]. Grafting with compatible rootstock offer one of the best method to avoid biotic as well as abiotic stress, increase yield, extend harvest period, manipulate sex expression, and improves fruit quality (Panday and Rai, 2003). The success of grafting depends on the union of the grafts (rootstock and scion) and compatibility of the rootstock and scion. A successful graft union starts with a series of event such as multiplication of callus from the scion and rootstock, formation of Callus Bridge, vascular separation and production of xylem and phloem (Hartman and Kester, 2011). Additionally, fundamental environmental elements such as temperature, relative humidity must be met to guarantee the successful graft union. Commercial use of vegetable grafting is a relatively recent innovation. The days taken to attain graftable size, days taken for graft union, percent of grafting success, graft healing and survival rate of grafted plants depends on scion-rootstock compatibility. Despite the widespread of grafting, information about scion and rootstock compatibility is still lacking. Information on effect of various rootstock on the performance of cucurbits in Chhattisgarh is meager. Hence, this study was undertaken to access graft compatible of bottle gourd scion with different cucurbitaceous rootstock.

Materials and Method

Site of the experiment

The proposed experiment was conducted and carried out in Factorial Randomized Block Design (FRBD) at experimental farm of Centre of Excellence on Protected cultivation and Precision Farming, Indira Gandhi Krishi Vishwavidyalaya, Raipur, India (latitude 21° 23″ to longitude 81° 65) during the year 2019-20 and 2020-21. Raipur district is located at elevations ranging from 244 to 409 meters above sea level and comes under tropical region. However, in summer the maximum temperature reaches to 45 ºC, and recently temperature has been recorded in the city as high as 44.3 ºC and minimum of 12.5 ºC.

Plant Materials

The experimental materials consisted of six wild and cultivated cucurbitaceous species *viz.*, water melon (*Citrullus lanatus*), Pumpkin OP (*Cucurbita moschata*), Pumpkin (F1 hybrid) (*Cucurbita moschata*) Bottle gourd (*Lagenaria siceraria)* and musk melon (*Cucumis melo*) which were used as rootstocks were collected from different parts of Chhattisgarh, and two scions which is a private sector hybrid *viz.*, Haruna from VNR seed company and Gaurav from Bioseed company, CG.

General conditions of propagation structures

A low cost mist chamber was constructed using locally available iron rods. It consisted of two layers *viz.*, inside made up of high density polyethylene (HDPE) and outside made up of shade net. The relative humidity of 85 to 95% and the temperature of 35 to 40 °C were maintained by cooling the surface of chamber by pouring water and sprinkling of water twice a day. This mist chamber was used for healing of graft union for six to eleven days. A shade net house of 75% allowing only 25% sunlight used for hardening of grafted plants. In this structure relatively low temperature with high humidity was maintained in comparison with the outside environment.

Method of grafting

Slant grafting approach was preferred when rootstocks and scion were having same hypocotyl thickness at the time of grafting (Sakata *et al.*, 2007) [5]. In slant grafting, one cotyledonary leaf of rootstock was removed and scion seedlings of same thickness were cut below the cotyledonary leaves at 10 to 14 days after sowing. True leaves from scion seedlings were removed to avoid over weight. A wound was made by removing outer skin (3-3.5 cm length) of both the stems of scion and rootstock without damaging the vascular bundles. The wounded surfaces of both rootstock and scion (the same size on each) were bound tightly together with grafting clips, where the two wounded areas were brought to in contact.

Healing and acclimatization

Healing and acclimatization are very important operations to attain the highest percentage of success. After grafting, the plants were kept under mist chamber for one week at more than 90% relative humidity, 25-30 ºC temperature and darkness. Initially, for two days, in mist chamber the plants were kept under dark for fast healing of graft union. After acclimatization, the plants were transferred to shade net house for five to six days. The clips were removed after assurance of graft union in side grafting. After this, graft union was completed and the cucumber scions were able to get water and nutrients through the rootstocks. Steps of grafting shown in Plate 1.

Result and Discussion

Earliness in terms of number of days taken for germination was presented in Table 1. Among the five different rootstock used, significantly less number of days (7.8) was required for germination of pumpkin (*Cucurbita moschata*) seeds compared to the other rootstock. However, result is at par with watermelon (*Citrullus lanatus*) seeds (8.17) and the maximum number of days (10.67) taken for germination was recorded by muskmelon (*Cucumis melo*) followed by pumpkin OP (*Cucurbita moschata*) (9.67) and in both rootstock, growth was too slow. In between scion, Haruna required significantly less number (4.17) of days for germination in comparison with Gaurav (4.50).

The data pertaining to days to seedling ready for grafting varied significantly among the rootstock and scions and was presented in Table 1. Among the different rootstocks, pumpkin (*Cucurbita moschata*) and bottle gourd (*Lagenaria siceraria*) recorded significantly less number of days to attain graftable size (16.9) and (17.1) respectively whereas, rootstock muskmelon (*Cucumis melo*) recorded maximum (19.1) number of days to attain graftable size. Both the scion took same number of days to attain graftable size (12.6). The stem thickness of theses rootstock doesn't match the thickness of bottle gourd scion. Hence, it took more number of days for graft union.

The number of days taken for graft union is an important trait, which indirectly effects the yield of the crop. The graft was considered functional in 5 to 8 days after grafting in case of cucurbits and was presented in Table 2. Among the different graft combination used for study, the species bottle gourd (*Lagenaria siceraria*) took the least number of days (5.50) for graft union with Haruna followed by watermelon (*Citrullus lanatus*) grafted onto Haruna (6.33). However, the rootstock muskmelon (*Cucumis melo*) grafted onto Gaurav recorded the highest number of days (8.6) for graft union which was at par with pumpkin OP (*Cucurbita moschata*) grafted onto Gaurav (8.3).

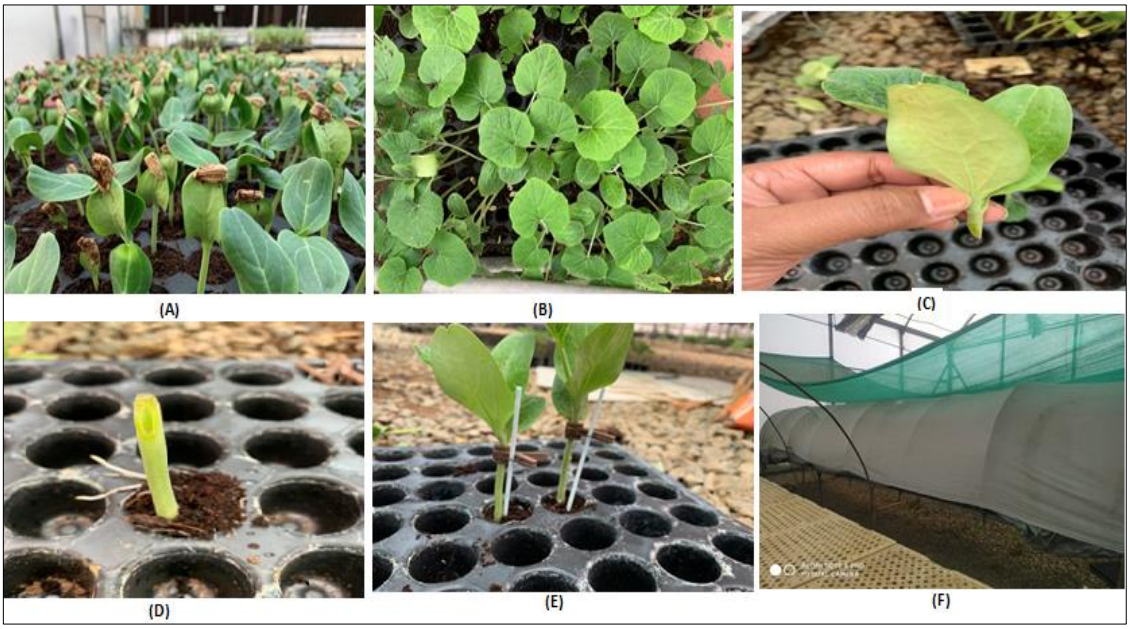
The grafting success depends upon many factor such as size of scion and rootstock, grafting method, culture condition, tissues and structure differences, physiological and biochemical characteristics, growing stages of rootstock and scion. The environment also plays a major role on graft success (Davis *et. al.*, 2008) [4]. Successful grafting was due to cell division in the scion and rootstock at the graft union. However, an increase grafting success was not seen until the rootstock has reached the third leaf stage. Graft incompatibility could occur at early stage when vascular connection could not form properly after grafting. Success percentage of different graft combination varied significantly and was presented in Table 2. Among all the different graft combination, the rootstocks bottle gourd (*Lagenaria siceraria*) recorded highest grafting success (86.17) with Haruna followed by water melon (*Citrullus lanatus*) *i.e* (83.1) DAG. The lowest graft success (73.4) was observed in pumpkin OP (*Cucurbita moschata)* rootstock with Gaurav. In case of pumpkin and musk melon, most of the plants shows quick wilting immediately after grafting. This is the characteristics of graft incompatibility. In graft

incompatibility, the leaves turned yellow and leaves withered (Davis *et. al*., 2008) [4].

In the healing process, the scion and rootstock must establish vascular connection, which is considered the most critical process in the production of vegetable grafted transplants. The complete vascular connection establishment takes approximately five to eight days, during which the scion is unable to uptake water through the rootstock. The data of days taken for graft hardening presented in Table 3, showed there was considerable difference in the number of days it took for graft hardening between the treatments. Lowest number of days (4.5) was required for graft hardening in Haruna grafted on bottle gourd (*Lagenaria siceraria*) which was at par (5.0) with watermelon (*Citrullus lanatus*) rootstock grafted on Haruna. Whereas highest number of days (8.5) was required by muskmelon (*Cucumis melo*) grafted on Gaurav.

Graft compatibility was determined by evaluating the survival rate of the grafted plants after regeneration of vascular bundles over the graft interface and vegetative growth of the scion and was presented in Table 3. Among rootstock, on 30th days after transplanting, significantly more (84.21) survival percentage was recorded by bottle gourd (*Lagenaria siceraria*) whereas less (78.26) survival percentage was observed in pumpkin OP (*Cucurbita moschata*) which was at par with muskmelon (*Cucumis melo*) (78.28). In between the scion, the maximum survival percentage after transplanting was recorded in Haruna (90.6). and among the different graft combinations, the maximum survival (86.8) was recorded in the combination of HT1 *i.e* Haruna grafted onto watermelon followed by HT4 *i.e* Haruna grafted onto Bottle gourd (85.5). Under theses combination the minimum survival percentage (75.4) was observed under the combination GT2 (Gaurav grafted onto pumpkin OP) during the summer season of 2020-21 and 2021-22.

Histological observation at 30th days after grafting showed complete fusion of rootstock and scion. Healing of wound was almost completed. Interaction effect of rootstock and method of grafting revealed that best rootstock for grafting bottle gourd was watermelon followed by pumpkin F1, and self-rootstock.



A) Germinate the rootstock and scion in different portrays
B) Attained graftable size
C) Top portion of scion removed from seedling
D) Slant cut made in rootstock
E) Graft union fixed using grafting clip
F) Placed grafted plants into grafting chamber

Plate 1: Steps in slant grafting method

Table 1: Days taken for germination, and number of days taken to attain graftable size of cucurbitaceous rootstock and scion

No. of days taken for germination				Days taken to attain graftable stage		
	2021	2022	Pooled	2021	2022	Pooled
Root-Stock						
T1	8.33	8.00	8.17	17.53	17.97	17.75
T2	9.00	9.67	9.33	18.83	18.63	18.73
T3	8.00	7.67	7.83	17.07	16.80	16.93
T4	9.67	8.67	9.17	17.40	16.83	17.12
T5	10.67	10.67	10.67	19.10	19.20	19.15
p-value	0.0207	0.0190	0.02	0.0089	0.0026	0.01
Scion						
H	4.33	4.00	4.17	12.73	12.62	12.68
G	5.00	4.00	4.50	13.37	11.95	12.66
p-value	0.1800	1.0000	0.59	0.4289	0.1918	0.31

Table 2: Number of days taken for graft union and percent of grafting success

	No. of days taken for graft union			Percent of grafting success		
	2021	2022	Pooled	2021	2022	Pooled
Rootstock						
T1	6.83	7.17	7.00	78.58	78.01	78.30
T2	7.83	8.17	8.00	80.00	79.51	79.75
T3	7.00	7.50	7.25	79.75	78.64	79.19
T4	6.83	6.50	6.67	83.62	83.32	83.47
T5	8.17	8.33	8.25	75.87	74.71	75.29
S.Em	0.35	0.29	0.32	0.78	0.65	0.71
CD at (LSD)	1.04	0.85	0.95	2.30	1.92	2.11
Scion						
H	5.56	5.83	5.69	68.09	67.33	67.71
G	6.67	6.72	6.69	64.52	64.07	64.29
S.Em	0.222	0.182	0.20	0.490	0.410	0.45
CD	0.66	0.54	0.60	1.46	1.22	1.34
Cont vs Others						
Control	0.00	0.00	0.00	0.00	0.00	0.00
Others	6.98	7.17	7.08	75.77	75.08	75.43
S.Em	0.29	0.23	0.26	0.63	0.53	0.58
CD	0.85	0.70	0.77	1.88	1.57	1.73
Interaction						
HT1	6.00	6.67	6.33	83.56	82.67	83.11
HT2	7.33	8.00	7.67	80.01	79.30	79.65
HT3	6.67	7.00	6.83	81.06	79.65	80.35
HT4	5.67	5.33	5.50	86.79	85.56	86.17
HT5	7.67	8.00	7.83	77.11	76.81	76.96
GT1	7.67	7.67	7.67	73.60	73.36	73.48
GT2	8.33	8.33	8.33	79.99	79.72	79.85
GT3	7.33	8.00	7.67	78.43	77.62	78.03
GT4	8.00	7.67	7.83	80.46	81.09	80.77
GT5	8.67	8.67	8.67	74.62	72.61	73.61
HT6	0.00	0.00	0.00	0.00	0.00	0.00
GT6	0.00	0.00	0.00	0.00	0.00	0.00
S.Em	0.50	0.41	0.45	1.10	0.92	1.01
CD	1.47	1.21	1.34	3.26	2.72	2.99

Table 3: Days taken for graft healing and survival percentage

	Days taken for graft healing			Survival Percentage		
	2021	2022	Pooled	2021	2022	Pooled
Rootstock						
T1	5.67	5.83	5.75	83.58	83.53	83.56
T2	7.83	7.83	7.83	78.57	77.95	78.26
T3	6.67	6.50	6.58	80.20	79.82	80.01
T4	5.83	6.00	5.92	84.30	84.12	84.21
T5	7.83	8.17	8.00	78.53	78.02	78.28
S.Em	0.21	0.23	0.22	0.55	0.49	0.52
CD at (LSD)	0.63	0.67	0.65	1.61	1.42	1.51
Scion						
H	5.11	5.28	5.19	84.11	84.01	84.06
G	6.17	6.17	6.17	81.21	80.64	80.93
S.Em	0.135	0.143	0.14	0.316	0.280	0.30
CD	0.40	0.43	0.41	0.93	0.82	0.87
Cont vs Others						
Control	0.00	0.00	0.00	90.78	90.52	90.65
Others	6.44	6.54	6.49	81.50	81.15	81.33
S.Em	0.17	0.18	0.18	0.43	0.38	0.41
CD	0.52	0.55	0.53	1.27	1.12	1.19
Interaction						
HT1	5.00	5.33	5.17	87.17	86.57	86.87
HT2	7.33	7.67	7.50	81.47	80.77	81.12
HT3	6.67	6.33	6.50	80.50	80.83	80.67
HT4	4.33	4.67	4.50	85.77	85.40	85.58
HT5	7.33	7.67	7.50	79.13	79.13	79.13
GT1	6.33	6.33	6.33	80.00	80.50	80.25
GT2	8.33	8.00	8.17	75.67	75.13	75.40

GT3	6.67	6.67	6.67	79.90	78.80	79.35
GT4	7.33	7.33	7.33	82.83	82.83	82.83
GT5	8.33	8.67	8.50	77.93	76.90	77.42
HT6	0.00	0.00	0.00	90.63	91.37	91.00
GT6	0.00	0.00	0.00	90.93	89.67	90.30
S.Em	0.30	0.32	0.31	0.77	0.69	0.73
CD	0.90	0.95	0.92	2.27	2.01	2.14

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