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Utilizing grafting techniques with wild and cultivated cucurbit rootstocks: Enhancing crop resilience and performance

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Abstract
Initially, grafting was used to propagate trees with challenging root systems or susceptibility to pathogens. Now, it is widely applied to vegetable crops, especially solanaceous crops and cucurbits. In the case of cucurbits, grafting focuses on finding suitable rootstocks for cucumber, enhancing resistance to soil-borne pathogens, particularly nematodes. Wild cucurbit rootstocks are generally more resistant to diseases due to their robust root systems. This increased resistance, coupled with better nutrient uptake, helps mitigate biotic stress. The rootstocks include, bitter apple (*Citrullus colocynthis*) pumpkin (*Cucurbita moschata*), bottle gourd (*Lagenaria siceraria*), and sponge gourd (*Luffa cylindrica*). Two cucumber scions, Pusa Uday and Swarna Sheetal were grafted using two methods-tube grafting and tongue approach grafting. Among these, tongue approach grafting was found to have a higher success rate for both scions. This method demonstrates the potential to improve disease resistance in cucumber plants while ensuring better growth and survival. Grafting could be a viable strategy for controlling soil-borne pathogens like root-knot nematodes in cucumber production.

Keywords: Grafting, biotic stress, tube grafting, tongue approach grafting, nutrient uptake

Introduction
Grafting is a widely used horticultural technique that involves joining the tissues of two plants to form a single, more robust organism. In cucurbit crops, such as watermelon, cucumber, melon, and squash, grafting is primarily employed to improve plant health, enhance resistance to soil-borne diseases, and increase tolerance to environmental stressors, such as salinity and temperature extremes. The primary motivation for grafting cucurbits is to address challenges associated with soil-borne pathogens, including Fusarium wilt, Verticillium wilt, and nematodes, which can significantly reduce crop yield and quality. By grafting a susceptible scion (the upper part of the plant responsible for fruit production) onto a disease-resistant rootstock (the lower part of the plant), growers can protect crops without relying on chemical treatments. Additionally, grafting can improve water and nutrient uptake, enhance abiotic stress tolerance, and extend the growing season by enabling early planting in cooler soils. As a result, grafted plants are often more vigorous and productive, leading to higher yields and better-quality fruits. The history of grafting in cucurbits highlights significant advancements in research and agricultural practices across different regions. In France, research into grafting began in the 1950s, particularly focusing on cucumber and melon scions grafted onto fig leaf gourd (*Cucurbita ficifolia*) rootstocks to control Fusarium wilt. Around the 1960s, cucumber grafting gained traction in Japan, where it was used to enhance low-temperature tolerance and resistance to Fusarium wilt (Fujieda, 1994) [3]. Spain embraced grafting techniques in cucumbers in the mid-1990s, leading to a substantial increase in the use of grafted plants after recognizing its benefits (Hoyos Echebarría, 2001) [4]. By 1998, grafting had become standard practice in East Asia, with approximately 95% of watermelons and oriental melons in Japan, Korea, and Taiwan being grafted onto squash or gourd rootstocks (Lee and Oda, 2003) [6]. This widespread adoption demonstrates the technique's value in improving crop resilience and productivity.

The adoption of grafting in cucurbits is becoming increasingly common in both commercial and small-scale agriculture due to its numerous benefits in sustainable farming practices. However, the technique requires skilled labor and careful selection of compatible scion and rootstock varieties to ensure successful grafting and optimal plant performance.

In summary, grafting in cucurbits offers a promising solution for enhancing disease resistance, improving crop resilience, and increasing productivity, making it an essential tool in modern vegetable production systems. The present study was to standardize the method of grafting with cucurbitaceous species as rootstocks and cucumber scions, to compare the results on success rate of grafting.

Materials and Methods

The experiment was conducted with four rootstocks *viz*., pumpkin (*Cucurbita moschata*), bottle gourd (*Lagenaria sciceraria*), sponge gourd (*Luffa cylindrica*), bitter apple (*Citrullus colocynthis*) and two cucumber scions Pusa Uday and Swarna Sheetal in completely randomized design with three replications. Initially the seeds of the rootstocks and scions were raised in 50 celled protrays containing sterilized coir compost as media. A total of 60 grafts were maintained per treatment per replication. Two grafting methods *viz*., tongue approach grafting method (TAG) and tube grafting (TG) methods were carried out and they are detailed as below.

Tongue approach grafting method (TAG)

At first true leaf stage, growing point of the rootstock was cut carefully before grafting and was made by using a sharp blade. The grafting cut for rootstock was made in a downward direction and the scion cut in an upward direction at an angle 30° – 40° to the perpendicular axis. Each slit was like a tongue, and were fitted together and sealed with grafting clips to fix the graft position at the graft union site and allowed till healing take place. After healing the rootstock top is removed and the scions are cut

Tube grafting method (TG)

The rootstock with first true leaf was selected to graft (about 7 to 10 days after sowing). One cotyledon and the growing tip was removed. The seedling is cut at a slant from the base of one cotyledon to 0.8 – 1.0 cm below the other cotyledon, removing one cotyledon and the growing tip. The length of the cut on the scion hypocotyl was at 35° to 45° angle. The scion is attached to the rootstock and fixed tightly by a grafting tube. Grafted plants maintained at 25 °C to 30 °C and 95-100% humidity for three days until the junction was healed

Healing and acclimatization

After grafting is performed, healing and acclimatization are very important operations to attain the highest percentage of success. After grafting, the plants were kept under mist chamber for six to seven days at >90% relative humidity, 25-30°C temperature and darkness. Initially, plants were kept under dark for two days, in mist chamber for fast healing of graft union. Then the relative humidity was little reduced for acclimatization. After acclimatization, the plants were transferred to shade net house (75%) for five to six days. The clips were removed after assurance of graft union in tube grafting. The grafted plants in portrays were placed in a tray with water upto 1/4th level. Rootstock sprouts were removed periodically. The observations *viz*., number of days taken for germination, hypocotyl length (cm), hypocotyl diameter (mm), days taken for graftable size, days taken for graft union and grafting success rate 15 and 30 days after grafting (%) are taken and the data were recorded regularly and analyzed statistically following Panse and Sukhatme (1989) [11].

Results and Discussion

Significant differences were observed among the treatments for all the studied parameters, including days taken for germination, hypocotyl length (cm), hypocotyl diameter at the cotyledon stage (mm), hypocotyl diameter at the two-leaf stage (mm), days taken to reach graftable size, days required for graft union formation, and graft success percentage 15 days post-grafting. These variations highlight the influence of different treatments on plant growth, development, and the success of the grafting process.

Growth parameters

The data on number of days taken for germination is presented in Table 1. Rootstocks and scions varied significantly pertaining to number days to germination and the results ranged from 2.90 to 24.22. Among the rootstocks, pumpkin (4.67) followed by sponge gourd (5.90) took the least number of days to germinate. The wild rootstock, Bitter apple took highest number of days for germination (24.22). However, cucumber scions Pusa uday (2.90) took significantly least number of days followed by Swarna sheetal (3.22) for germination.

The wild species Bitter apple has taken the maximum number of days to germinate. Since Bitter apple is a wild species with the hard seed coat and prolonged dormancy of seeds that can lead to unpredictable, low and late germination. However, cultivated cucurbit rootstocks pumpkin and sponge gourd took least number of days to germinate.

Hypocotyl length in cucurbitaceous rootstocks and scions significantly varied from 5.83 to 8.50 cm in tube grafting whereas hypocotyl length varied from 5.01 to 9.45 cm in tongue approach grafting. Among the four different rootstocks longest hypocotyl length of 8.50 and 8.26 cm was recorded by Pumpkin in TG and TAG respectively. Wild cucurbit root stock, Bitter apple recorded significantly shortest hypocotyl length (5.83 and 5.01 cm) in TG and TAG methods respectively. Among the scions Pusa uday (8.20 and 9.26 cm) and Swarna sheetal (8.48 and 9.45 cm) recorded significantly long hypocotyl length with TG and TAG respectively. These findings were in accordance with those of Tamilselvi (2014) [18], Punithaveni (2015) [13], Sanmathi *et al*. (2020) [16], Tamilselvi and Pugalendhi (2018) [17] in bitter gourd, Aydin *et al*. (2022) [1] in cucumber, Paikra and Verma (2022) [10] in solanaceous crops and Verma *et al*. (2024) [19] in tomato.

Grafting is a form of stress for plants and different cucurbit varieties may respond differently to this stress. Some exhibit faster or more robust growth in the hypocotyl as a stress response, while others show stunted growth. Different cucurbit varieties have inherent genetic variations that affect the growth rate and final length of their hypocotyls. Environmental factors like light, temperature, humidity and nutrient availability impact the growth of the hypocotyl. Inconsistent conditions can lead to variations in length.

Higher stem girth at the stage of grafting was observed in the cucurbitaceous rootstocks it may be due their genetic material. The wild species recorded less stem girth at the stage of grafting it may be attributed to its slower growth rate. These results are opposite to the findings of Praveen (2019) [12] where higher stem girth was recorded in wild species, it may be due to variable environmental condition and seed source. It is essential to select the rootstock and scion according to their stem girth, which can be done by adjusting the sowing time of rootstock and scion to get relative stem girth at the stage of grafting, so planning is very important in grafting.

Number of days to graftable size of seedlings varied significantly among cucurbitaceous rootstocks and cucumber scions from 12.83 to 26.97 in tube grafting method. Among the rootstocks sponge gourd (12.83) followed by pumpkin (15.91) took significantly least number of days to attain graftable size. While, bitter apple took maximum number of days (26.97). Among the cucumber scion seedlings, Pusa uday and Swarna sheetal recorded (16.72 and 18.19). Highest number of days taken for graft union was Swarna sheetal grafted onto Bitter apple (11.72) followed by pusa uday grafted onto Bitter apple (11.41).

Higher percentage of graft success was recorded in pusa uday grafted onto pumpkin (68.33 and 58.3 percent) at 15 and 30 days after grafting. The lower percentage of success was recorded by swarna sheetal grafted onto Bitter apple rootstock (35.00 and 23.00 percent at 15th and 30th days after grafting in tube grafting method which showed incompatibility by way of sudden wilting and reduction in initial plant population. Higher average graft success rate was observed in pumpkin rootstock (65.95 and 55.80 percent) and lowest was recorded in the rootstock of Bitter apple (40.00 and 26.65 percent) at 15 and 30 days after grafting.

Higher percentage of graft success was recorded by pusa uday grafted onto pumpkin (71.66 and 61.66 percent at 15 and 30 days after grafting) and lower percentage of graft success observed in pusa uday grafted onto Bitter apple (41.66 and 30.00 percent at 15 and 30 days after grafting) in tongue approach grafting method. Higher average graft success rate in tongue approach grafting method was observed in pumpkin rootstock (69.95 and 58.95 percent) and lowest was recorded in the rootstock of Bitter apple (43.33 and 30.80 percent) at 15 and 30 days after grafting.

Among the graft combinations revealed that the rate of success was more with pumpkin as compared to other species. This is presumed from the fact that the species would have better adaptability to the prevailing climate led to more success and the method followed was cleft grafting which suits more for this species, which is in agreement with the report of Rashid *et al.* (2005) [14], where the highest grafting success of 95% was obtained with tomato on *S. torvum*. Similar findings were reported in tomato grafted on Mate, Hawaii 7998 and Joint as rootstocks by using cleft grafting in Japan (Oda, 1995) [9]. Mohammed *et al.* (2009) also reported that the success percentage of grafting tomato Cecilia F1 scion on either of Beaufort or Heman rootstocks was 99% whereas the success percentage of grafting Cecilia scion on Syrian rootstock was 98%. The percent graft success gradually decreased from 15th to 30th days after grafting in all the rootstocks. Similar reports had been pointed out by Kawaguchi *et al.* (2008) [5] who observed that the growth inhibition and high mortality in tomato on pepper and pepper on tomato grafts were due to discontinuities in the vascular bundles at the graft union. Wilting of grafts was due to physiological incompatibility as a result of lack of cellular recognition, wounding responses, presence of growth regulators or incompatible to grafted onto or an unfavourable grafting environment (Davis *et al*., 2008) [2].

In the present study, thirty days after grafting the leaves turned yellow, withered and finally wilting was occurred in bottle gourd and Bitter apple used as rootstocks. This is a characteristic of graft-rootstock incompatibility, which contributes to poor vascular connection with poor connecting sieve tubes, cambium and grafted onto xylem in tomato on pepper and pepper on tomato grafts (Kawaguchi *et al*., 2008) [5]. Sudden wilting is also due to physiological incompatibility as a result of lack of cellular recognition, wounding responses. (Davis *et al*., 2008) [2].

Table 1: Days taken for germination, hypocotyl length and hypocotyl diameter in cucurbitaceous rootstocks and cucumber scions to be used in grafting

Treatments	Number of days taken to attain graftable size	
	Tube grafting method	**Tongue approach grafting**
Rootstocks		
Pumpkin (*Cucurbita moschata*)	15.91	17.09
Bottle gourd (*Lagenaria siceraria*)	14.89	16.47
Sponge gourd (*Luffa cylindrica*)	12.83	17.83
Bitter apple (*Citrullus colocynthis*)	26.97	21.78
Scions		
Pusa uday	16.72	22.95
Swarna sheetal	18.19	25.89
SEd±	0.15	0.20
CD at 5%	0.32	0.43

Table 2: Number of days taken to attain graftable size by the cucurbitaceous rootstocks and cucumber scions under tube grafting and tongue approach grafting methods

Treatments	Days taken for germination	Hypocotyl length (cm)		Hypocotyl diameter (mm)	
Rootstocks		**TG**	**TAG**	**TG**	**TAG**
Pumpkin (*Cucurbita moschata*)	4.67	8.50	8.26	3.50	2.95
Bottle gourd (*Lagenaria siceraria*)	6.43	7.53	6.25	4.13	3.34
Sponge gourd (*Luffa cylindrica*)	5.90	8.33	8.55	3.95	3.27
Bitter apple (*Citrullus colocynthis*)	24.22	5.83	5.01	2.63	2.10
Scions					
Pusa uday	2.90	8.20	9.26	3.23	2.79
Swarna sheetal	3.22	8.48	9.45	3.31	2.96
SEd±	0.19	0.24	0.22	0.59	0.09
CD at 5%	0.40	0.51	0.45	1.26	0.18

Table 3: Number of days taken for graft union by the cucumber graft combination under tube grafting and tongue approach grafting method

Graft combination	Number of days taken for graft union	
	Tube grafting	Tongue approach grafting
Swarna sheetal grafted onto Pumpkin *(Cucurbita moschata)*	8.73	6.67
Pusa uday grafted onto Pumpkin *(Cucurbita moschata)*	7.90	6.73
Swarna sheetal grafted onto Bottle gourd (*Lagenaria siceraria*)	9.95	8.76
Pusa Uday grafted onto Bottle gourd (*Lagenaria siceraria*)	9.81	8.42
Swarna sheetal grafted onto Sponge gourd (*Luffa cylindrica*)	9.81	6.53
Pusa Uday grafted onto Sponge gourd (*Luffa cylindrica*)	9.37	6.68
Swarna sheetal grafted onto *(Citrullus colocynthis*)	11.72	8.89
Pusa Uday grafted onto (*Citrullus colocynthis*)	11.41	9.32
SEd±	0.26	0.14
CD at 5%	0.45	0.29

Table 4 Effect of tube grafting (TG) method on number of successful grafts and percent graft success at 15 and 30 days after grafting (DAG)

Graft combination	Number of seedlings grafted	Number of successful grafts		Graft success (%)			
		15 DAG	30 DAG	15 DAG	Average	30 DAG	Average
Swarna sheetal grafted onto Pumpkin *(Cucurbita moschata)*	60	38	32	63.33	65.95	53.33	55.80
Pusa uday grafted onto Pumpkin *(Cucurbita moschata)*	60	41	35	68.33		58.33	
Swarna sheetal grafted onto Bottle gourd (*Lagenaria siceraria*)	60	34	29	56.66	54.10	48.33	42.45
Pusa Uday grafted onto Bottle gourd (*Lagenaria siceraria*)	60	31	22	51.66		36.66	
Swarna sheetal grafted onto Sponge gourd (*Luffa cylindrica*)	60	34	25	56.66	55.80	41.66	40.80
Pusa Uday grafted onto Sponge gourd (*Luffa cylindrica*)	60	33	24	55.00		40.00	
Swarna sheetal grafted onto (*Citrullus colocynthis*)	60	27	18	45.00	40.00	30.00	26.65
Pusa Uday grafted onto (*Citrullus colocynthis*)	60	21	14	35.00		23.33	
SEd±		1.57	1.21	1.71		1.59	
CD at 5%		3.24	2.76	3.54		2.78	

Table 5: Effect of tongue approach grafting (TAG) method on number of successful grafts and percent graft success at 15 and 30 days after grafting (DAG)

Graft combination	Total number of seedlings grafted	Number of successful grafts		Graft success (%)			
		15 DAG	30 DAG	15 DAG	Average	30 DAG	Average
Swarna sheetal grafted onto Pumpkin *(Cucurbita moschata)*	60	41	34	68.33	69.95	56.66	58.95
Pusa uday grafted onto Pumpkin *(Cucurbita moschata)*	60	43	37	71.66		61.66	
Swarna sheetal grafted onto Bottle gourd (*Lagenaria siceraria*)	60	38	32	63.33	60.9	53.33	50.80
Pusa Uday grafted onto Bottle gourd (*Lagenaria siceraria*)	60	35	29	58.33		48.33	
Swarna sheetal grafted onto Sponge gourd (*Luffa cylindrica*)	60	37	27	61.66	59.11	45.00	45.80
Pusa Uday grafted onto Sponge gourd (*Luffa cylindrica*)	60	34	28	56.66		46.66	
Swarna sheetal grafted onto *Citrullus colocynthis*	60	27	19	45.00	43.33	31.66	30.8
Pusa Uday grafted onto *Citrullus colocynthis*	60	25	18	41.66		30.00	
SEd±		1.87	1.91	1.09		1.21	
CD at 5%		3.21	3.45	2.45		2.26	

Conclusion

This study highlights the potential of grafting techniques using wild and cultivated cucurbit rootstocks to enhance the resilience and performance of cucumber plants. Tongue approach grafting (TAG) and tube grafting (TG) were evaluated for their effectiveness, with TAG showing superior success rates. Among the rootstocks, pumpkin (*Cucurbita moschata*) consistently demonstrated the highest graft success, particularly when paired with the scions Pusa Uday and Swarna Sheetal. Conversely, bitter apple (*Citrullus colocynthis*), a wild species, exhibited lower germination rates and graft success due to its prolonged seed dormancy and physiological incompatibility.

The study also found that pumpkin rootstock significantly enhanced graft success rates, likely due to better adaptability to environmental conditions and superior vascular connections. Grafts with pumpkin rootstocks had higher success at both 15 and 30 days post-grafting compared to other species, especially when Pusa Uday was used as the scion.

Overall, grafting cucumbers onto pumpkin rootstocks using TAG provides a promising method for enhancing crop resilience, especially against soil-borne pathogens like root-knot nematodes. This technique has significant potential to improve cucumber cultivation and reduce reliance on chemical treatments. However, careful selection of rootstock and scion varieties, along with precise grafting techniques, is crucial for achieving optimal results.

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