Evaluation of Different Rootstocks for their Graft Compatibility and Resistance to Bacterial Wilt on Tomato

Sudhir Shrestha[1]

ABSTRACT

Four eggplant and nine tomato rootstocks were evaluated for their resistance to bacterial wilt and compatibility with tomato scion 'Ogata fukuju' in Tsukuba International Training Center, Tsukuba from June to October 2010. Grafting was conducted two times and the method was tube grafting. All combinations were evaluated in terms of graft success, plant growth, stem thickness, evapotranspiration and incidence of bacterial wilt in naturally infested soil. Success percent in the two grafting batch was not similar in terms of different graft combinations. Eggplant rootstocks contributed for the overgrowth on stem diameter of scion, however no wilt symptoms due to incompatibility could be observed. Plants grafted on Tolban vigor, Tonasim, B-barrier and BF-Okitsu showed no bacterial wilt symptoms whereas that on Doctor-K, Taibyo Shinko No.1 and non-grafted control resulted in 100% disease incidence. Remaining rootstocks showed partial wilt incidence. Grafting thus can be taken as an important tool for managing bacterial wilt disease. However, further investigation is recommended for evaluating graft incompatibility.

Key words: grafting, incompatibility, *Ralstonia solanacearum*, rootstock, scion

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetable crops of Nepal. It is commercially grown in plain and hills of Nepal for fresh consumption as well as for processing. Statistics show that in Nepal, tomato was grown in 15,572 ha of land and the total production was 219,194 M.ton in 2008/09 with the average yield of 14.1 M.ton/ha. (VDD, 2009) which was quite low as compared to other countries. Among the reasons, diseases caused by fungi, bacteria, virus and nematode play a major role. Bacterial wilt caused by *Ralstonia solanacearum* is one of soil borne diseases that causes for the yield decrease in tomato. The disease can bring about almost total destruction of the crop during summer and rainy season.

Mid-hills of Nepal has good potential for off-season tomato production under plastic house during rainy season. However, there are many problems faced by the farmers growing rainy season tomato under plastic houses. One of the major problems is the soil borne disease bacterial wilt. The problems of this disease are more common in non-flooded uplands where solanaceous vegetables are grown continuously without crop rotation. However, control of bacterial wilt with crop rotation is still difficult because of the wide host range of the pathogen and long-term persistence in soils (Adhikari and Basnyat, 1998). Even when crop rotations could be practiced, the long intervals required between similar crops result in an economic loss to the grower.

Control of soil-borne pathogens by fumigation with methyl bromide inside greenhouses is widely practiced in many countries (Besri, 2002; Bletsos, 2005). But in case of Nepal it is not common and is not affordable for the small scale farmers. The use of resistant variety to control bacterial wilt in the field has been very difficult due to the nature of the pathogen and host resistance in tomato (Scott *et al.,* 2005). The complex diversity of pathogenic

[1] Senior Vegetable Development Officer, Subtropical Vegetable Seed Production Center, Rukum

Ralstonia strains has led to the development of resistant lines, which are effective in some growing regions and not effective in others (Scott, 1996). It is generally accepted that resistance from various sources is controlled multi-genetically and usually the resistance is not complete and breaks down to some extent when conditions favor the pathogen (Hartman and Elphinstone, 1994).

Although the integration of resistance genes into modern tomato cultivars has been difficult, the use of grafted transplants has historically been very effective for managing bacterial wilt in the field worldwide. Grafting vegetable crops have been used extensively in greenhouse and tunnel production not only to manage soil borne diseases but also to achieve greater crop yields, higher salinity tolerance, increased heat and cold tolerance, and enhanced drought and flood resistance (Black, 2003; Estan *et al.,* 2005; Lee, 1994; Oda, 1999). Investigations on the mechanisms for disease resistance suggested that resistant rootstocks physically limit the movement of the bacteria from the growing media to the scion (Grimault and Prior, 1994).

A successful graft union requires the formation of new connections between vascular strands at the callus graft interface via differentiation and lignification (Fernandez-Garcia *et al.*, 2004). Failure of a graft union to successfully develop may be due to a lack of cellular recognition, the growing stage of the respective plants, interference of the wounding response or growth regulators, incompatibility toxins, or an unfavorable grafting environment (Andrews and Marques, 1993; Davis *et al.*, 2008). Many reports can be found on rootstock/scion incompatibility, which induces undergrowth or overgrowth of the scion, leading to decreased water and nutrient flow through the grafted union, causing wilting. However, overgrowth or undergrowth alone cannot be taken as a specific because this can also occur due to genetic differences in relative growth rate between the scion and the stock (Westwood, 1988).

Graft incompatibility as reviewed by Andrews and Marquez (1993) is differentiated from graft failure, which often results from environmental factors or lack of skill of the grafter. Oda *et al.* (2005) reported delayed graft incompatibility in tomato and the symptom being sudden wilting of grafted plant after a long-term normal growth. Oda *et al.* (2005) found symptoms of delayed graft incompatibility when tomato plants were grafted onto scarlet eggplant (*Solanum integrifolium*). The stem diameter of rootstocks was smaller than that of tomato scion and tomato stem swelled immediately above the union. Symptoms of delayed graft-incompatibility can be found in many of the fruit trees. Grafted tree after 20 or more years of normal growth and bearing fruits, begins with a thin layer of cambium and phloem cells died at the graft union. The necrosis develops around the trunk until tree becomes girdled and dies soon (Hartmann *et al.,* 1990). The mechanism of graft-incompatibility in vegetable crops is still a complex one because of its short growing period.

The objective of this study was to evaluate the graft compatibility and disease resistance of some rootstocks for tomato.

MATERIALS AND METHODS

The experiment was carried out at Tsukuba International Training Center (TBIC), Japan from June to October 2010. Thirteen rootstocks were selected for evaluation (Table 1). To ensure similar stem diameters at the grafting time, seeds of Tolban vigor and Tonasium were sown one month before sowing the seeds of scion and seeds of Daitaro and Akatora were sown 15 days before scion. Similarly, all tomato rootstocks were sown 3 days before seeds of scion. Grafting was done two times. First batch grafting was done on second week of June and second batch grafting was conducted on second week of October. The following rootstocks and scion were used for this experiment.

Table 1: Different combinations between rootstocks and scion (treatments)

S. N.	Rootstocks	Scion	B. wilt resistance*	Fusarium resistance*		TMV resistant gene*
				Race1	Race2	
1.	Tolban Vigor (*S. torvum*)	Ogata fukuju	√			
2.	Daitaro (eggplant)	Ogata fukuju	√			
3.	Tonasium (*S. torvum*)	Ogata fukuju	√			
4.	Akatora (eggplant)	Ogata fukuju	×			
5.	Helper M (tomato)	Ogata fukuju	√	√	√	TM1
6.	Anchor T (tomato)	Ogata fukuju	√	√	√	TM2a
7.	B-barrier (tomato)	Ogata fukuju	√	√	√	TM2a
8.	Doctor K (tomato)	Ogata fukuju	×	√	√	TM2a
9.	BF Okitsu (tomato)	Ogata fukuju	√	√	√	TM2a
10.	Volante (tomato)	Ogata fukuju	√	√	√	TM2a
11.	Taibyo Shinko No. 1 (tomato)	Ogata fukuju	×	√	√	TM1
12.	LS-89	Ogata fukuju	√	×	×	TM1
13.	Gardener	Ogata fukuju	√	√	√	TM2a

* : based on claimed by the seed company (Takii Seed Co)

Grafting method

The cleft grafting using tube method was employed for the experiment. Grafted seedlings were put inside plastic insulated tunnel and kept under shade with high humidity for about 3 days for the healing of the graft union. After 3 days of healing process, the plastic tunnel was gradually opened and after 8th day, fully open to get acclimatized to outside environment. Number of grafts was 40 in the first and 20 in the second batch. Success percentages of grafting were recorded.

Transplanting to the poly-pot

All the non-grafted seedlings of scion varieties were transplanted to 12cm poly-pots in June 28. The grafted seedlings were transplanted in the 1st week of July. The poly-pots were filled with sterilized soil. Length of the stem at one month interval was recorded for five plants in each treatment.

Stem thickening

Diameter of the stem 3cm above and below the graft interface was measured two months after grafting on five plants in each treatment. Ratio of rootstock and scion diameter was calculated and compared with non-grafted control. Diameter at the thickest part of the stem was also measured.

Measurement of evapotranspiration

Two months after grafting, five potted plants of each treatment were watered fully and after 2 hours, weight of the potted plants was recorded. After 24 hours, again the weight was measured. The weight difference was calculated as evapotranspiration of 24 hours. Evapotranspiration was taken as indicator for the water movement through the graft union. Higher value of evapotranspiration was expected with highly compatible rootstock-scion combinations.

Transplanting to the infested soil

The experimental seedlings were transplanted to the field in Arakawaoki, Tsuchiura City, Japan on July 23. Some test plants were transplanted in that field in first week of June 2010 for the confirmation of the presence of pathogen on that soil. Wilting symptoms were observed in 2nd week of June and finally all the test plants were found wilted. The stem of wilted plants were cut and dipped into the test-tube filled with water for bacterial oozing test. All the wilted plants showed positive results towards the test, which confirmed the presence of the pathogen *Ralstonia solanacearum* in that field.

Field experiment was carried out in Randomized Complete Block Design with 12 treatments replicated three times. There were two plants in each plot. Transplanting was done on July 23, 2010. Fertilizer was applied at the rate of NPK 20:15:20 kg/10a. Silver color plastic mulch was used to protect the plants from aphid. All plants were trained as single vine system. Other cultural practices were applied as per standard. The number of wilted plants was recorded at regular time interval.

Statistical analysis

All data were analyzed using analysis of variance (ANOVA) to examine treatment effects and means separated by Tukey's multiple range test at $P<0.05$ by using software developed by Dr. Mitate Yamada, Technical Advisor, TBIC, Japan.

RESULTS AND DISCUSSIONS

Grafting

Table 2 shows the percentage of successful graft-combinations after 7-8 days of acclimatization from two successive batch of grafting. The overall percent of successful graft was 50% in first batch and 83% in second batch. The combination of Akatora as rootstocks showed the highest percent of graft success followed by BF-Okitsu in first batch. The rootstocks Gardener showed the lowest percent of success followed by LS 89. Similar kind of result was observed by Valdez (2008) when rootstock LS 89 was grafted with scion variety Momotaro. Result of second batch was not similar with that of first batch. Tonasium resulted highest success followed by Daitaro, Akatora, and Volante. Lowest success was found with BF Okitsu. The lower success rate of grafting in first batch may be due to the skill of the participant and higher temperature on the month of June. This inconsistent result of graft success, therefore, could not be accounted for graft-incompatibility. Because of the low percentage of success rate of grafting on LS 89 and Gardener with Oogata fukuju in the first batch, these two rootstocks were not included for further investigation.

Table 2: Rootstocks performance on graft-success

S.N.	Combinations		% Successful Grafting	
	Rootstocks	Scion	1st batch (June)	2nd bach (October)
1	Tolban vigor	Ogata fukuju	60.0	100.0
2	Daitaro	Ogata fukuju	65.0	95.0
3	Tonasium	Ogata fukuju	47.5	90.0
4	Akatora	Ogata fukuju	75.0	95.0
5	Helper M	Ogata fukuju	60.0	80.0
6	Anchor T	Ogata fukuju	47.5	90.0
7	B-barrier	Ogata fukuju	32.5	75.0
8	Doctor K	Ogata fukuju	62.5	75.0
9	BF Okitsu	Ogata fukuju	75.0	60.0
10	Volante	Ogata fukuju	50.0	95.0
11	Taibyo Shiko No. 1	Ogata fukuju	57.5	75.0
12	LS 89	Ogata fukuju	17.5	70.0
13	Gardener	Ogata fukuju	0.0	85.0
	Total		50.0	83.5

Plant growth

No Significant difference was observed on the growth of plant height during one month, among the grafted combinations and non-grafted Ogata fukuju (Table 3). However, maximum increase in height was obtained by Taibyo Shikno No.1 (24cm) followed by Helper M (22.4cm). Lowest growth was obtained from Doctor K (14.4cm). Significant growth differences in diameter of the rootstock and scion was observed among the treatments. All eggplant rootstocks contributed highest stem thickening on the scion as compared with the tomato rootstock (Fig. 1). However diameter of rootstock was lowest in eggplant rootstocks as compared with the tomato rootstocks. The ratio of rootstock to scion indicates the non-uniformity of stem growth on rootstock and scion part of the seedling which was found higher in eggplant rootstocks. The result indicated that eggplant rootstocks resulted in overgrowth of scion diameter and undergrowth of rootstock diameter itself. Oda *et al.* (2005) also found similar kind of result when tomato plants were grafted onto scarlet eggplant. Hartman and Kester (1975) stated such kind of overgrowth as a characteristic of graft incompatibility which is associated with poor connection of vascular bundles between scion of rootstock. However, no adverse effect on plant growth or wilt symptoms was observed due to such abnormal stem thickening.

Evapotranspiration

Mixed results were obtained from the evapotranspiration record. Highest value was obtained from Tolvan vigour followed by non-grafted control. It showed that evapotranspiration depends mostly on the overall plant growth. The highest value obtained from Tolban vigor rootstock attributed to its highest plant height (45.4cm) and highest stem diameter (8.5 mm). On the other hand, non-grafted control resulting second highest value in evapotranspiration in spite of its comparatively poor growth proved that graft union creates a barrier for the water flow through the xylem vessels to some extent. However, this parameter also could not be used as indicator for graft-incompatibility.

Table 3. Plant growth and evapo-transpiration affected by different rootstocks

Treatments	Plant height (cm)			Stem diameter (cm)				Evapo-transpiration in 24 hrs (ml.)
	12-Jul	12-Aug	Growth	Scion (S)	Thickest part (G)	Rootstock (R)	Sion Rootstock Ratio (S/R)	
Taliban vigor	23.2 ab	45.4	22.2	8.5 a	11.1 a	5.8 abc	1.5 abc	330 a
Daitaro	25.6 a	41.8	16.2	7.9 ab	9.9 ab	5.1 bc	1.5 ab	203 bcd
Tonasium	20.9 ab	39.2	18.3	7.8 abc	11.2 a	4.6 c	1.7 a	245 abcd
Akatora	23.4 ab	44.0	20.6	7.1 abcd	9.4 abc	5.5 bc	1.3 bcd	290 ab
Helper M	18.6 b	41.0	22.4	6.4 bcd	7.6 cd	5.9 abc	1.1 d	232 abcd
Anchor T	21.8 ab	40.8	19.0	6.3 d	6.7 d	5.7 abc	1.1 d	255 abc
B-barrier	18.0 b	38.2	20.2	6.4 cd	7.7 cd	5.3 bc	1.2 cd	150 cd
Doctor K	21.4 ab	35.8	14.4	6.4 bcd	6.8 d	5.5 abc	1.2 cd	157 cd
BF Okitsu	18.8 ab	35.8	17.0	6.7 bcd	8.2 bcd	6.1 ab	1.1 d	145 d
Volante	19.8 ab	38.0	18.2	7.0 abcd	8.3 bcd	5.7 abc	1.2 bcd	225 abcd
Taibyo Shiko No. 1	19.6 ab	43.6	24.0	6.7 bcd	7.7 cd	5.8 abc	1.2 cd	240 abcd
Non-grafted	20.0 ab	36.4	16.4	6.8 bcd	8.0 cd	6.8 a	1.0 d	303 ab
HSD 5%	6.83	n.s.	n.s.	1.49	1.83	1.32	0.32	0.10

Mean followed by the same letters are statistically not significant according to Tukey's multiple range test *($P<0.05$)*

Incidence of bacterial wilt

Eighteen days after transplanting to the infested field, DoctorK, Taibyo Shinko No. 1 and non-grafted control showed first wilt symptoms (Table 4). In the final observation, 11 weeks after transplanting, they were completely destroyed by the pathogen. Yamakawa (1982) also reported the rootstock Taibyo Shinko No. 1 as susceptible to bacterial wilt.

Both rootstock Taibyo Shinko No. 1 and Doctor K was also referred as susceptible by the seed company (Table 1). After 11 weeks, disease incidence on resistant rootstocks Daitaro, HelperM, AnchorT and Volante were 33.3, 83.3, 50.0 and 33.3 percent respectively. Besides, the tomato plants grafted on Akatora which was supposed to be susceptible to bacterial wilt did not show any wilt symptoms until 9 weeks after transplanting. In the final observation, 16.67% plants grafted on Akatora were found infected with the pathogen. Tomato plants grafted on Tolban vigor, Tonasium, B-barrier, BF-Okitsu did not show any wilt symptoms till final observation.

Fig.1: Typical overgrowth of tomato scion grafted on eggplant rootstocks

Table 4: Percentage of bacterial wilt incidence on different rootstocks

Rootstocks	Percentage of wilted plants								
	18-DAT	24-DAT	34-DAT	38-DAT	45-DAT	53-DAT	57-DAT	64-DAT	77-DAT
Tolban vigor	0.00 b	0.00 b	0.00 b	0.00 c	0.00 c	0.00 b	0.00 c	0.00 c	0.00 d
Daitaro	0.00 b	0.00 b	0.00 b	0.00 c	33.33 abc	33.33 ab	33.33 bc	33.33 bc	33.33 cd
Tonasium	0.00 b	0.00 b	0.00 b	0.00 c	0.00 c	0.00 b	0.00 c	0.00 c	0.00 d
Akatora	0.00 b	0.00 b	0.00 b	0.00 c	0.00 c	0.00 b	0.00 c	0.00 c	16.67 cd
Helper M	0.00 b	16.67 ab	33.33 ab	50.00 ab	50.00 abc	50.00 ab	66.67 ab	66.67 ab	83.33 ab
Anchor T	0.00 b	0.00 b	0.00 b	0.00 c	0.00 c	33.33 ab	50.00 abc	50.00 abc	50.00 bc
B-barrier	0.00 b	0.00 b	0.00 b	0.00 c	0.00 c	0.00 b	0.00 c	0.00 c	0.00 d
Doctor K	33.33 ab	50.00 a	66.67 a	66.67 a	66.67 ab	66.67 a	83.33 ab	83.33 ab	100.00 a
BF Okitsu	0.00 b	0.00 b	0.00 b	0.00 c	0.00 c	0.00 b	0.00 c	0.00 c	0.00 d
Volante	0.00 b	0.00 b	0.00 b	16.67 bc	16.67 bc	33.33 ab	33.33 bc	33.33 bc	33.33 cd
Taibyo Shiko No. 1	50.00 a	50.00 a	50.00 a	83.33 a	83.33 a	83.33 a	100.00 a	100.00 a	100.00 a
Non-grafted	16.67 ab	16.67 ab	50.00 a	66.67 a	66.67 ab	66.67 a	66.67 ab	83.33 ab	100.00 a
HSD 5%	34.70	34.70	34.70	49.07	54.87	60.10	54.87	54.87	49.07

Mean followed by the same letters are statistically not significant according to Tukey's multiple range test (*$P<0.05$*)

From the results of field performance of the different rootstocks; it is clear than the resistant rootstocks viz. Daitaro, Akatora, Anchor T and Volante were not found fully resistant to the pathogen, hence they can be referred as moderately resistant. As in cases of Tolban vigor, Tonasium, B-barrier and BF Okitsu, which did not show any wilt symptoms till final observation, could be categorized as highly resistant rootstocks. The above result proved that disease resistance by rootstocks is a complex phenomenon and is strongly influenced by environmental conditions. In heavily infested soils and under extremely unfavorable environments, the so-called resistant plants become diseased. Intensive successive cropping of the same rootstock provide opportunity for the occurrence of new pathogenic types with increased virulence qualitatively and quantitatively (Yamakawa, 1982). The reason of Akatora rootstock showing less wilt incidence as compared to other so-called resistant rootstocks could not be understood.

CONCLUSION

Tomato when grafted on suitable resistant rootstocks could overcome the problem of bacterial wilt incidence. However, resistance of the rootstocks is not absolute phenomenon. Resistance of rootstocks depends on wide range of environmental factors and field conditions. Therefore, it is highly advisable that the rootstocks are needed to be tested on different environmental and soil conditions for their resistance levels before making recommendation to the farmers. The occurrence of incompatibility in terms of graft success was not clearly observed in the experiment. The success or failure of grafting seemed to be more affected by the environmental conditions and skill of the grafter. Symptoms of delayed graft incompatibility could not observe due to short duration of this study. As far graft-incompatibility is concerned, it is recommended to conduct a series of experiments under various environmental conditions and long duration field test under uninfested soil condition.

REFERENCES

Adhikari, T. B. and R. C. Basnyat, 1998. Effect of crop rotation and cultivar resistance on bacterial wilt of tomato in Nepal. Can. J. Plant Pathol., 20:283–287.

Andrews. P. K. and C. S. Marquez, 1993. Graft incompatibility. Hort. Rev. (Amer. Soc. Hort.Sci.), 15:183-232

Besri, M.,2002, Alternatives to Methyl Bromide for tomato production in the Mediterranean area. Proceedings of International Conference on Alternatives to methyl Bromide, Seville, Spain, 5-8 March 2002, Pp.162 – 166.

Black, L., 2003. Grafting tomatoes for production in the hot-wet season. International Cooperators Guide. AVRDC. Pub. #03-551.

Bletsos, F. A., 2005. Use of grafting and calcium cyanamide as alternatives to methyl bromide soil fumigation and their effects on growth, yield, quality and fusarium wilt control in melon. Journal of Phytopathology, 153:155-161.

Davis, A. R., P. Perkins-Veazie, R. Hassell, A. Levi, S. R. King and X. Zhang, 2008. Grafting effects on vegetable quality. HortScience, 43(6):1670-1672.

Estan, M. T., M. M. Martinez-Rodriguez, F. Perez-Alfocea, T. J. Flowers and M. C. Bolarin, 2005. Grafting raises the salt tolerance of tomato through limiting the transport of sodium and chloride to the shoot. J. Exp. Bot., 56(401):703-712.

Fernandez-Garcia N., V. Martinez, A. Cerda and M. Carvajal, 2004. Fruit quality of grafted tomato plants grown under saline conditions. J. Hort. Sci. Biotechnol., 79:995–1001.

Grimault, V. and P. Prior, 1994. Grafting tomato cultivars resistant or susceptible to bacterial wilt analysis of resistance mechanisms. J. Phytopathol., 141:330-334.

Hartman, G. L., and J. G. Elphinstone, 1994. Advances in the control of Pseudomonas solanacearum race 1 in major food crops. In Bacterial Wilt: the disease and its causative agent, Pseudamonas solanacearum, edited by A. Hayward and G. L. Hartman. Cambridge: CAB International.

Hartmann, H. T. and D. E. Kester, 1975. Plant propagation: principles and practice. 337-347. Prentice-Hall Inc., Englewood Cliffs.

Hartmann, H. T., D. E. Kester, and F. T. Davies, 1990. Theoretical aspects of grafting and budding. p. 305-348. **In**: Plant Propagation. 5th ed. Prentice Hall, Englewood Cliffs, NJ.

Lee, J., 1994. Cultivation of grafted vegetables I. Current status, grafting methods, and benefits. HortScience., 29(4):235-239.

Oda, M., M. Maruyama, and G. Mori, 2005. Water transfer at graft union of tomato plant grafted onto Solanum rootstocks. J. Japan. Soc. Hort. Sci., 74(6):458-463

Oda, M., 1999. Grafting of vegetable to improve greenhouse production. Food and Fertilizer Technology Center for the Asian and Pacific Region. Extension Bulletin no. 480.

Oda, M., 1995. New grafting method for fruit-bearing vegetables in J. Agri. Res. Q., 29:187-194

Scott, J. W., 1996. Tomato improvement for bacterial disease resistance for the tropics: a contemporary basis and future prospects. 1st International Symposium on Tropical Tomato Diseases, November 21-22, 1996. Recife, Pemambuco, Brazil.

Scott, J. W., J. F. Wang, and P. M. Hanson, 2005. Breeding tomatoes for resistance to bacterial wilt, a global view. I International Symposium on Tomato Diseases, 25 November 2005. Orlando, FL, USA

Valdez, E. P. M., 2008. Evaluation of different rootstocks for grafting tomato to control wilt disease. Farming Japan, 42(1):34-41

VDD, 2009. Annual Book 2008-09. Vegetable Development Directorate. Department of Agriculture, Nepal

Westwood, M. N., 1988. Temperate Zone Pomology, Revised Edition. Timber Press, Portland, Oregon.

Yamakawa, K., 1982. Use of rootstocks in solanaceous fruit-vegetable production in Japan. J. Agri. Res. Quart., 15(3): 175-179