World Journal of Agricultural Research, 2020, Vol. 8, No. 4, 129-133
Available online at http://pubs.sciepub.com/wjar/8/4/4
Published by Science and Education Publishing
DOI:10.12691/wjar-8-4-4

Management of Bacterial Wilt Using Grafting Technique in Tomato (*Ralstonia solanacearum*)

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Received August 14, 2020; Revised September 17, 2020; Accepted September 26, 2020

Abstract Tomato farmers have been facing a major threat of entire crop loss because of bacterial wilt of tomato. Grafting of highly productive scion onto resistant rootstock is one of the best methods to prevent crops from bacterial wilt disease. An experiment was conducted using tomato (RS-101) and eggplant (KER-DC-117) rootstocks grafted with six scion varieties in different combinations to make total of nine treatments replicated three times in completely randomized design in the naturally infected farmer's fields at Dahachok, Nepal in 2019. All the three treatments with eggplant rootstock (KER-DC-117) were found to be resistant. Karma 777, Shrijana and Samjhana scions grafted onto tomato rootstock (RS-101) were moderately resistant, whereas Sarita and Karma 555 grafted onto tomato rootstock (RS-101) were moderately susceptible. Non-grafted Sarita was highly susceptible with 100% disease incidence. There were no bacterial browning and bacterial oozing in treatments with tomato rootstock (RS-101). The yield and number of fruits was maximum with Karma 444 grafted onto eggplant rootstock (KER-DC-117). So, Karma 444 + ER (KER-DC-117) is the best scion-rootstock combination to manage bacterial wilt against tomato at Dahachok, Kathmandu.

Keywords: *bacterial wilt of tomato, resistant rootstock, grafting, management*

Cite This Article: Sudeep Pandey, Utsav Koirala, Prabin Acharya, and Suchit Shrestha, "Management of Bacterial Wilt Using Grafting Technique in Tomato (*Ralstonia solanacearum*)." *World Journal of Agricultural Research*, vol. 8, no. 4 (2020): 129-133. doi: 10.12691/wjar-8-4-4.

1. Introduction

Tomato (*Solanum lycopersicum* L.) is an important contributor in world vegetable production. The vegetable cultivated area and production in Nepal has increased in year 2018/2019 (297,195 h and 4,271,270 ton) [1]. Tomato is one of the most important vegetables contributing to increasing numbers of area and production of vegetables in Nepal. Despite the increase in area and production, it is still insufficient to meet the increasing demands within the country. There are more than one reasons behind insufficient production in the farmers' field. The weeds, insects, and plant diseases are major constraints to the potential tomato yield by 36% and disease alone assist 14% of total [2]. Increase in commercialization and intensive tomato farming system invites different types of diseases.

Bacterial wilt of tomato caused by *Ralstonia solanacearum* is one of the most destructive diseases that might be responsible for up to 91% yield loss [3]. High humidity and temperature are the most favorable environmental conditions for this soil borne pathogen. *R. solanacearum* enters the tomato plants through natural openings or wound in the root, multiplies, and colonizes the xylem, clogging the vascular system. The pathogen causes the wilting of plants, stunting growth, reduced fruit size & number and ultimate collapse of the plant [4]. *R. solanacearum* is a dynamic pathogen and can survive in diverse conditions. It has high mobility to noninfected areas via water, soil, plant materials, weeds, and mechanical tools [5]. Four biovars and three races of *R. solanacearum* shows the diversity of pathogen available [6,7] and posts major challenge in the management practices against the pathogens. Different management practices such as crop rotations, resistant varieties, soil fumigants, etc. have been used over the years. The little success of fumigants and limited availability of proper cultivar to rotate out of solanaceous crops is a major concern opting host resistance as the most prominent option [8]. However, limitations of plant breeders to develop only strain specific resistance while compromising fruit size and yield bails out efficacy and practicality of host [9]. The development of the resistant variety requires significant amount of time that may lead to the excessive yield loss in the farmers' fild until that period. So, the development of a reliable grafting technique prevents the yield loss during the developmental phase of a resistant variety.

These limitations lead to acknowledge the importance of grafting techniques that have been readily used by the farmers of Nepal against bacterial wilt of tomato. Grafting cultivars with optimum fruit type and yield onto bacterial resistant rootstock has been practiced around the world. Due to the unavailability of resistant rootstock and lack of knowledge about compatible cultivars, this approach has not been adopted yet. Since this technique can be of immense importance for the management of bacterial wilt of tomato, it is crucial to identify the resistant rootstock and its compatibility to the available cultivars. Therefore, we came up with an idea to validate proven grafting technology in specific vegetable crop for the specific location in Nepal. For this, we set two objectives for our study (i) assessment of disease resistant ability of rootstocks (tomato and eggplant) grafted with available cultivars in the mid-hill region of Nepal and (ii) evaluate the effect of resistance against pathogen on tomato yield.

2. Materials and Methods

The experiment was conducted from April - September, 2019 at farmers owned greenhouse where tomato cultivation was a failure due to severe infestation of bacterial wilt in previous cropping seasons. The site was located at Dahachok (27.70065, 85.2386), Kathmandu, Nepal at an altitude of 1600 m above the sea level. The trial was conducted as randomized complete design with 9 treatments with 3 replications. Raised bed with good agricultural practices were adopted during experimentation.

2.1. Transplant production and Grafting

The seeds of tomato rootstock RS-101 (TR) and eggplant rootstock KER-DC-117 (ER) were given by Chia Tai Seeds Company Limited, Thailand. Six tomato varieties namely, Sarita, Shrijana, Samjhana, Karma 444, Karma 555 and Karma 777 were used as scion onto the rootstocks. The combinations of scion and stock were used as in Table 1. All the tomato varieties were sown on the same day whereas eggplant seeds were sown 15 days prior in nursery seedling trays at Karma Innovative R & D Station, Dahachok. Healthy tomato seedlings of 30 days old and eggplant seedlings 45 days old were selected for tube grafting. The grafted plants were placed in a chamber with adjusted humidity and light conditions (Figure 1). After 15 days from grafting, the grafted seedlings (15 days old) were transplanted in the field.

Figure 1. Seedlings grafted onto eggplant and tomato rootstock for the management of bacterial wilt of tomato placed in the chamber

Table 1. Scion and rootstock combinations used for the study of impact of bacterial wilt disease at Dahachok during Apr-Sep, 2019.

S. No.	Scion	Rootstock	Treatments
1	Non-grafted Sarita	-	Sarita
2	Sarita	KER-DC-117	Sarita + ER
3	Sarita	RS-101	Sarita + TR
4	Karma 777	RS-101	Karma 777 + TR
5	Karma 777	KER-DC-117	Karma 777 + ER
6	Karma 555	RS-101	Karma 555 + TR
7	Karma 444	KER-DC-117	Karma 444 + ER
8	Shrijana	RS-101	Shrijana + TR
9	Samjhana	RS-101	Samjhana + TR

2.2. Field Layout

The experiment was conducted in the farmer's greenhouse of size of 23m * 4.3m with three rows each of the size of 23m * 1m apart from each other by 0.5m. In each unit plot, plant to plant distance was maintained at 60 cm and row to row 40 cm. Each 2 m^2 experimental plot was accommodated with 8 plants (i.e., 2 rows x 4 plants in each row). The treatment plots were randomly assigned in each replication of the size of 7m * 4.3m and cultural practices were made consistent with the local farmers' practice in the region. The number of harvested fruits and yield were recorded throughout the cropping season and estimated for per hectare production.

2.3. Assessment of Bacterial Incidence and Severity

The disease incidence was assessed based on survival of plants weekly after the first appearance of wilting symptom. All the dead plants were tested for ooze test for the confirmation of bacterial wilt. The disease incidence at 20 days interval were calculated and used for the determination of area under disease progress curve (AUDPC) using formula explained in Shanner and Finney (1977) [10]:

$$AUDPC = \sum_{i=1}^{n-1}\left(\frac{(Y_{i+1}+Y_i)}{2}\right)\times(T_{i+1}-T_i)$$

Where,
Yi = area under bacterial wilt disease incidence on the i^{th} date
Ti = date on which the disease was scored
n = number of dates on which disease was scored.

Similarly, resistance level of the treatments was assigned based on the categorization followed by Mew and Ho (1976) [11]:

Resistant (R) = less than 20% wilt incidence
Moderately resistant (MR) = 21-40% wilt incidence
Moderately susceptible (MS) = 41-60% wilt incidence
Susceptible (S) = 61-100% wilt incidence.

The assessment of disease severity was done by scoring stem browning and bacterial streaming. The wilted and dead plants were cut and scored for stem browning by using a score of 0 - 3, where 0 = no browning, 1 = light brown color restricted to 2 cm from the stem base, 2 = light brown color spread more than 2 cm from the base, and 3 = dark brown color that is wide spread (browning of the vascular tissue) [12]. The bacterial ooze streaming from the stems was also tested by suspending the stems in clear beaker (ooze test) and the ooze rate score of 0 - 3 was given, where 0 = no ooze, 1 = thin strands of bacterial oozing stops in 3 minutes, 2 = continuous thin flow that is unrestricted, and 3 = heavy ooze turning the water turbid in 2 minutes [12].

2.4. Statistical Analysis

Data were encoded and processed in Microsoft Excel for Mac Version 16.16.18. Processed and curated data in excel were then statistically analyzed using RStudio Version 3.6.1.

3. Results

3.1. Evaluation of Rootstock-scion Effectivity against Bacterial Wilt of Tomato

The various combinations of six tomato varieties grafted onto two rootstocks (TR & ER) were planted in farmer's field. The disease incidence was observed only in non-grafted Sarita until 20 DAT (Figure 1). On 40 DAT, the death of plants with tomato rootstocks were observed and the disease incidence was increased in non-grafted Sarita. Disease incidence (%) after 40 DAT was 41.67 which was significantly different from other combinations. No disease incidence was observed in treatments with Karma 444 + ER. Non-grafted Sarita variety was severely infected by bacterial wilt (Figure 2) on 60 and 80 DAT with disease incidence 66.67% and 100% respectively (Figure 3).

All the three treatments with eggplant rootstock were found to be resistant (Table 2). Karma 777, Shrijana and Samjhana scions grafted onto tomato rootstock were moderately resistant, whereas Sarita and Karma 555 grafted onto tomato rootstock were moderately susceptible. Non-grafted Sarita was highly susceptible with 100% disease incidence.

Figure 2. Non - grafted Sarita (left) was severely infected by Bacterial wilt of tomato compared to grafted treatments (right) at Dahachok during Apr-Sep, 2019

AUDPC also increased from 379.17 to 583.33 on 60 and 80 DAT respectively. Similarly, AUDPC of plants with tomato rootstock ranged from 189.58 to 262.50 on 80 DAT (Figure 4). No disease incidence was observed in all treatments with eggplant rootstock throughout the cropping season (Figure 3 & Figure 4). The disease incidence in plants with tomato rootstock was observed between 37% to 46% on 80 DAT. The disease incidence was at increasing rate for all 6 combinations except with eggplant rootstock (Figure 3).

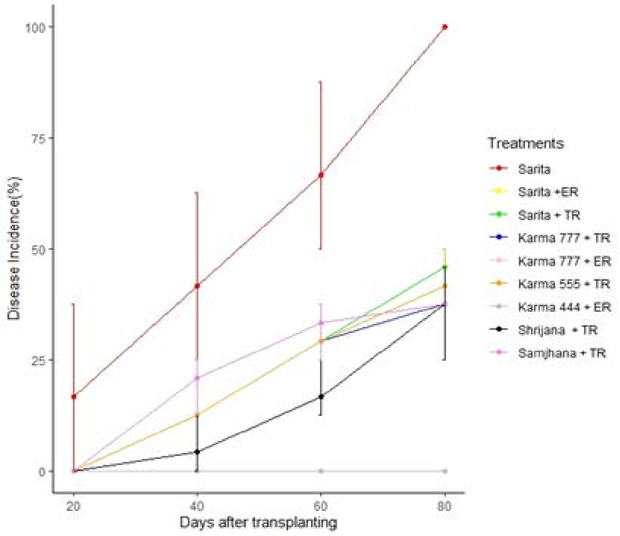

Figure 3. Mean bacterial wilt incidence of non-grafted as well as different combinations of scions and rootstocks at Dahachok, Apr-Sep, 2019. Each data point within disease progress curve represents the observed means of three replications. Each error bar represents the standard error of the mean

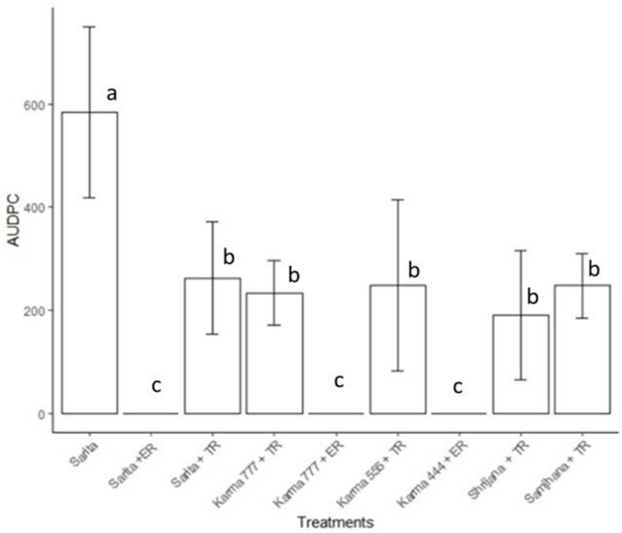

Figure 4. Area under disease progress curve (AUDPC) of non-grafted as well as different combinations of scions and rootstocks at Dahachok, Apr-Sep, 2019. Within each trial, mean AUDPC values represent the mean of three replications and values with same letters are not significantly different based Tukey's mean comparison test ($\alpha = 0.05$)

3.2. Evaluation of Severity of Bacterial Wilt of Tomato

The disease severity was evaluated based on the stem discoloration score and oozing rate. The result of bacterial browning was found to be like that of disease incidence. The non-grafted Sarita was severely infected with bacterial browning with the score of 3 which is significantly different from other combinations. Similarly, bacterial ooze score was maximum (2.4) in non-grafted Sarita compared to all other combinations. The bacterial browning score ranged from 2.0 to 2.7 in treatments grafted onto tomato rootstock. Similarly, bacterial ooze rate score ranged from 1.1 to 1.9 in plants with tomato rootstock. Bacterial browning and ooze were not observed in plants with eggplant rootstock (Table 2).

Table 2. Disease Incidence, Resistance level, Scores of bacterial browning and bacterial ooze rate of different treatments at Dahachok during Apr-Sep, 2019

Treatments	Wilt Incidence (%)	Resistance level	Bacterial browning	Bacterial ooze rate
Sarita	100.00[a]	S	3.00[a]	2.42[a]
Sarita + ER	0.00[c]	R	0.00[d]	0.00[d]
Sarita + TR	45.83[b]	MS	2.67[ab]	1.92[ab]
Karma 777 + TR	37.5[b]	MR	2.11[c]	1.33[bc]
Karma 777 + ER	0.00[c]	R	0.00[d]	0.00[d]
Karma 555 + TR	41.67[b]	MS	2.00[c]	1.11[c]
Karma 444 + ER	0.00[c]	R	0.00[d]	0.00[d]
Shrijana + TR	37.5[b]	MR	2.36[bc]	1.72[bc]
Samjhana + TR	37.5[b]	MR	2.00[c]	1.22[c]
CV%	16.13		10.84	21.29

Note: Mean in the column with same letter are not significantly different at P ≤ 0.001 using Tukey test.

3.3. Impact of Bacterial Wilt on Yield and Number of Fruits

The number of marketable fruits and their yield was recorded from the first harvest up to last marketable harvest. The highest number of fruit production was observed in Karma 444 + ER (3,42,500 per ha) followed by Karma 777 + ER (2,57,292 per ha) and Sarita + ER (2,00,625 per ha). Non-grafted Sarita had the least number of fruits (70,625 per ha) with the total yield of 5.08 t/ha. The treatment Karma 444 + ER with the highest number of fruits had the highest yield (17.37 t/ha) followed by Karma 777 + ER (16.21 t/ha) and Sarita + ER (15 t/ha). The number of fruits and yield in the treatments which had TR in combination (Tomato variety + TR) ranged from 1,61 071 per ha to 1,98,917 per ha with the respective yield of 9.38 t/ha and 11.16 t/ha (Table 3).

We found strong and negative correlation between AUDPC and yield (Figure 5). Which indicated that there is yield penalty due to AUDPC. The decrease in yield of non-grafted Sarita treatment and other treatments are mainly due to high AUDPC.

Table 3. Response of different scion-stock combinations to bacterial wilt disease based on yield and number of fruits at Dahachok during Apr-Sep, 2019

Treatments	Total Number of fruits (10^3/h)	Total yield (t/h)
Sarita	70.625[d]	5.08[c]
Sarita + ER	200.625[c]	15.00[a]
Sarita + TR	161.071[c]	11.16[b]
Karma 777 + TR	183.770[c]	10.59[b]
Karma 777 + ER	257.292[b]	16.21[a]
Karma 555 + TR	173.036[c]	9.99[b]
Karma 444 + ER	342.500[a]	17.37[a]
Shrijana + TR	198.917[c]	9.38[b]
Samjhana + TR	164.375[c]	10.66[b]
CV%	8.04	8.94

Mean in the column with same letter are not significantly different at P ≤ 0.001 using Tukey test.

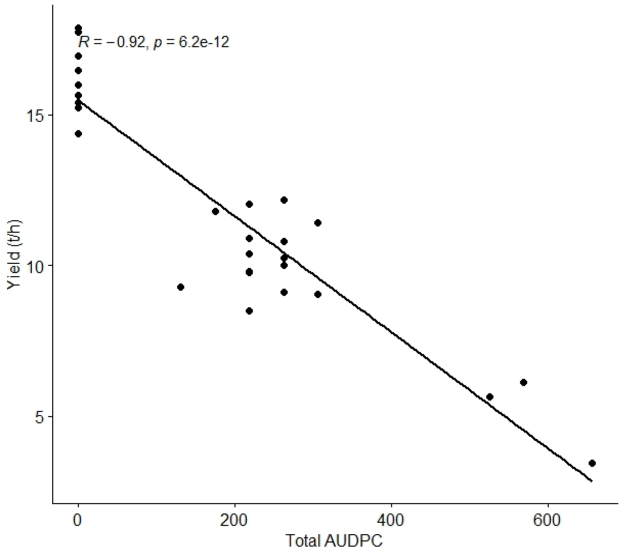

Figure 5. Linear relationship between total AUDPC and yield of different scion-stock combinations at Dahachok, Nepal Apr-Sep, 2019

4. Discussion

Farmers in Nepal are frequently facing severe yield loss of tomato due to bacterial wilt infestation. Management practices such as crop rotation and use of resistant cultivars have been practiced so far in Nepal [13,14]. However, rapidly modifying strains of the bacteria hinder durable management via resistant cultivars [3,15]. In such situation, grafting can be economical, eco-friendly, and durable management strategy against bacterial wilt of tomato [16,17]. Despite, significant success of grafting against bacterial wilt across the world there are no sufficient reports of grafting practice in Nepal.

Bacterial wilt of tomato was significantly controlled by grafting the susceptible tomato variety onto eggplant rootstock (KER-DC-117) compared to the non-grafted susceptible variety in our study. Significant decrease in AUDPC of grafted tomato plants was observed compared to non-grafted. Karma 777, Karma 444 and Sarita tomato varieties grafted onto eggplant rootstock (KER-DC-117) showed no incidence of bacterial wilt. The disease severity was also found zero in treatments with eggplant rootstocks (KER-DC-117) whereas non-grafted treatment had the maximum disease severity. Maximum yield of 17.37 t/ha was obtained with Karma 444 grafted onto eggplant rootstock (KER-DC-117). Here we report scion Karma 444 and KER-DC-117 rootstock as the most economical and compatible grafting for the management of bacterial wilt. Recent study showed grafting with resistant tomato rootstocks RST-04-105-T, Dai Honmei, and DR-BW-NCS2 significantly reduced AUDPC of bacterial wilt disease over non-grafted and self-grafted cultivars [18]. The negative correlation between AUDPC and yield in our study (Figure 4) also supports Lin et. al., 2008 study [18]. Another study suggested that resistant eggplant rootstocks reduced the death of grafted plants to 0-2.8% compared to 92.8% plant death in non-grafted plants [19].

The findings of this study suggest potential control of yield loss due to bacterial wilt by grafting with resistant rootstocks. Further study and extension of this approach will play a crucial role in adaptation of this technology by farmers of Nepal. This study opens the challenges for further research. Study of different combinations of scions and rootstocks effective against bacterial wilt of tomato at different geographical locations will be helpful for farmers from varied regions. Moreover, the study of different strains of pathogen causing this disease can bring drastic changes in approach against the disease. However, this is the first report of management of bacterial wilt of tomato by grafting in Nepal to our knowledge which paves the path for further studies.

5. Conclusion

This study was conducted to validate and understand effectiveness of grafted plant materials against bacterial wilt. Resistant rootstocks of tomato and eggplant were used to graft scions of Sarita, Karma 777, Karma 444, Karma 555, Shrijana and Samjhana tomato varieties. Out of nine combinations including non-grafted Sarita, Karma 444 with eggplant rootstock (KER-DC-117) gave the maximum yield (17.37 t/ha) from 3,42,500 per ha tomato fruits with zero bacterial wilt disease incidence. This is the first report of use of grafting against bacterial wilt disease of tomato in Nepal, which can be used as a platform for future multilocational studies.

Acknowledgements

I would like to acknowledge Chia Tai Seeds Co. Ltd., Thailand for providing seed samples and training on grafting techniques. Thanks to Mr. Suresh Gurung, Chairman of Karma Group of Companies, for financial and logistic support during the study period. Equally thanks to R&D Managers Mr. Arjun Bastola (Agronomist), Mr. Ankit Soti (Entomologist), Ms. Mausami Rana (Soil Scientist), Ms. Januka Dahal (Horticulturist), other staffs of Karma Innovative R&D Center, Dahachok and farmers of Dahachok for their support to accomplish this study.

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