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TUBE GRAFTING REDUCES INCIDENCE AND SEVERITY OF BACTERIAL WILT IN TWO TOMATO CULTIVARS IN ABEOKUTA, NIGERIA

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ABSTRACT

Bacterial wilt, *Ralstonia solanacearum*, causes severe loss of tomato (*Solanum lycopersicum* L.) production in Nigeria. Tube grafting of two tomato cultivars (Beske and UC82-B) on bacterial wilt-resistant tomato landrace rootstock (Tomachiva) was conducted to reduce incidence of bacterial wilt and increase the yield in the two cultivars. The experiments were laid in Randomized Complete Block Design (RCBD). Percentage incidence and Percentage Severity Index (PSI) of grafted tomato were 0.00% each in the early planting season. Incidence (1.70%) and PSI (1.50%) of grafted tomato were observed in late planting season. Non-grafted tomato had higher significant values of 42.50% and 38.80% in incidence and percentage severity index in late planting season. Grafting on Tomachiva effectively reduced the incidence and severity of bacterial wilt in two cultivars of tomato in southwest Nigeria

Key words: Beske, Field, *Ralstonia solanacearum,* Rootstock, *Solanum lycopersicum* L., UC82-B.

INTRODUCTION

Bacterial wilt of tomato is caused by *Ralstonia solanacearum* (Hayward, 1991; Gonzalez and Summers, 1995; Ma and Black, 1999). The bacterium invades and gradually blocks the vascular tissue causing death of affected plant and reducing yield of tomato. Control of bacterial wilt in tomato is very difficult as a result of the wide host range of the pathogen (Lemaga *et al*., 2001) and long-term persistence in soils. Furthermore, this soil-inhabiting bacterium can easily move into and recolonize non-infested areas through infected water, soil and plant material (Kelman, 1998).

Grafting is an alternative approach to reduce crop damage resulting from soil-borne pathogens. Disease-resistant rootstocks have been used by some authors to graft susceptible scions (Vuruskan and Yanmaz, 1990; Marsic and Osvald, 2004). Grafting of tomato scions onto bacterial wilt resistant eggplant rootstocks has been demonstrated at AVRDC to protect the plants from damage caused by waterlogged soils and bacterial wilt (Black *et al.,* 2003). The process saves time and costly breeding procedure. Takahashi (1984) reported that 68% of failure cases in Japanese vegetable production under continuous cropping were caused by soil-borne diseases and nematodes. Bithell *et al.* (2013) reviewed the grafting processes for reducing soil borne pathogens in Australian vegetable production. Grafting is thus an important technique for production of repeated crops of fruit-bearing vegetables grown in greenhouses and on the field.

There are a variety of techniques to graft tomatoes (Oda, 1995; Lee, 2003). In tube grafting, the rootstock and scion seedlings were attached by small silicon tubes or clips when the seedlings were still small (Rivard and Louws, 2006). Although the use of grafted vegetables is associated with disease reduction, yield increase had been attributed to other non disease-related factors (Ruiz and Romero, 1999; Yetisir and Sari, 2003).

Possible mechanisms for increased crop productivity in grafted crops could include increased water and nutrient uptake by vigorous rootstock genotypes. Stomata conductance is known to have improved in tomato with certain rootstock (Fernandez-Garcia *et al*., 2002). Similarly, macronutrient uptake such as phosphorus and calcium can be increased by using certain rootstock genotypes (Leonardi and Giuffrida, 2006; Ruiz *et al*., 1996).

However, grafting was extensively used in Asia to manage bacterial wilt incidence in solanaceous crops (Peregrine and Binahmad, 1982). In Germany, a breeding line (CRA 66) was identified as a resistant rootstock genotype for grafted tomato production (Grimault *et al*., 1994). Ganiyu (2014) reported tomato cultivar Tomachiva as a local small-fruited landrace with high resistance to bacterial wilt. .

The purpose of this study was to examine the effectiveness of tube grafting on the reduction of incidence and severity of bacterial wilt of tomato in open field conditions.

MATERIAL AND METHODS

The experiment was conducted in Plant Tissue Culture Laboratory and DelPHE-5 Field Experimental site, at the Federal University of Agriculture, Abeokuta (FUNAAB), Nigeria during the early and late planting seasons of 2012. The cultivars used as scions were Beske and UC82-B. Tomachiva was used as rootstock. The tomato seedlings were raised on sterilized soil in screen house under natural light condition. Seedlings of rootstock and scions were raised separately. Rootstock was sown two weeks before the scions. Grafting was performed two weeks after the sowing of scions. Stems of scion and rootstock plants were cut at a 45 degree angle. Plastic latex tubes (donated by Johnny's Selected Seeds, Canada), dimension 1 cm long x 2 mm inner diameter, and with side-slits were placed onto the cut ends of the rootstocks. The cut ends of the scions were then inserted into the tubes and were held together with the rootstocks.

The grafted plants were kept in Plant Tissue Culture Laboratory at a temperature of 25.3 - 26.4 ^{o}C and relative humidity of 87-93% for one week. The relative humidity was gradually reduced and the light intensity increased. They were then moved to screen house and left for one week for hardening. The grafted plants were transplanted at a 0.50 m within row and 0.60 m between rows unto raised beds, using a randomized complete block design with three replications.

Each cultivar was either grafted or non-grafted. Normal cultural practices were observed for weeding, staking, tying and insecticide applications.

Disease Incidence was calculated based on the work of Getachew *et al*. (2011) as percentage plants showing symptom of disease using the formula –

$$I = \frac{NPSWS}{NPPP} \times 100$$

Where I= Incidence of bacterial wilt in percentage; NPSWS= Number of plant showing wilt symptoms in a plot and NPPP= Number of plants per plot.

Disease severity scoring was done on a six-point scale (0= No wilt symptom = Immune, 1= One leaf wilted = Highly resistant, 2= 2 or more leaves wilted = Moderately resistant, 3 = all leaves except the tip wilted=Moderately Susceptible, 4= Whole plant wilted = Susceptible, 5= Death (collapse) of the whole plant = Highly susceptible). The six-point scale was proposed by Getachew *et al*. (2011).

Percentage Severity index (PSI) as described by Cooke (2006) were calculated using the formula - Σ [(scores x 100)/ (number of plants rated x maximum scale of the scores)] for each scoring date.

Data were subjected to analysis of variance to examine treatment effects, and means were separated by LSD at $P \leq 0.05$ using GenStat Discovery Edition 4.

RESULTS

Soil texture data (Table 1) showed that sand accounted for 76% of the texture, silt 9% and clay 15% confirming its moderate porosity and sandy loam nature. Organic carbon also indicated some inherent level of porosity of the soil. The pathogen, *Ralstonia solanacearum,* population density at the site of experiment was 1.3 x 10^8 cfu/g soil. There was a gradual increase in rainfall from January (with no rain at all, 0.00 mm) to April (80.1 mm) (Table 2). There was a fall in rainfall in August (36.30 mm), November (49.60 mm) and December (1.30 mm). Much rainfall was experienced in May (115.3 mm), June (225.1 mm), July (155.4 mm), September (181.4 mm) and October (184.7 mm). Relative humidity was relatively high in July (80.90%), August (82.60%) and November (81.90%). Mean daily temperature ranged from 25.5 °C - 29.1 °C. Sunshine hours ranged between 2.7 – 6.1 hr and soil temperature ranged between 25.4 - 29.8 °C in 2012.

Grafted Beske and UC82-B were protected against bacterial wilt as indicated by the reduced incidence and severity of the disease in early and late planting seasons (Table 3). Non-grafted Beske had a range of 36.10 - 50.00% in incidence and percentage severity index. UC82-B was infected only in late planting season of the year. Non-grafted Beske had higher percentage severity indices of 36.10 and 48.30%, while UC82-B had 30.30 and 29.30% in early and late planting seasons, respectively.

There was a progressive significant increase ($P<0.05$) of bacterial wilt incidence in non-grafted tomato in the two seasons (Figures 1A and B). Grafted tomato experienced no bacterial wilt infection in early season except at seventh and eighth week of late planting season when 1.7% incidence was observed.

Table 1: Pre-cropping physico-chemical characteristics of soil and population density of *Ralstonia solanacearum* at the site of experiment.

Soil characteristics	Value
Percent clay	15.00
Percent silt	9.00
Percent sand	76.00
Soil Texture	Sandy loam
pH	5.65
Organic carbon (%)	1.53
Total nitrogen (%)	0.13
Base saturation (%)	98.88
Available phosphorus (mg kg^{-1})	24.98
CEC (Cmol kg^{-1} soil)	8.91
Total Exchangeable Al (Cmol kg^{-1} soil)	0.10
Exchangeable bases (Cmol kg^{-1} soil)	
Ca^{2+}	4.40
Mg^{2+}	3.60
K+	0.38
Na+	0.43
Population density(cfu/g soil)	1.3 x 108

Table 2: Prevailing weather conditions in the planting season, 2012.

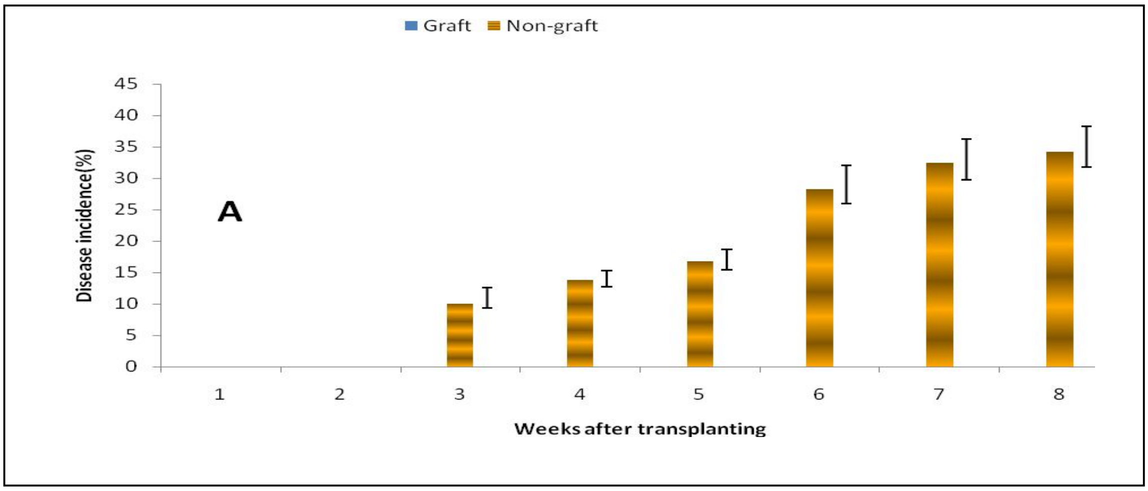
	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
Mean Temp (oC)	27.0	28.8	29.1	28.5	27.7	26.9	26.0	25.5	26.2	27.2	28.2	28.8
Rainfall (mm)	0.00	67.2	67.7	80.1	115.3	225.1	155.4	36.3	181.4	184.7	49.6	1.3
Rel. humidity (%)	75.2	70.5	79.3	79.5	77.3	78.7	80.9	82.6	76.00	77.5	81.9	78.5
Sunshine hours	4.2	4.5	5.1	5.7	4.8	3.9	4.0	2.7	4.0	5.7	5.4	6.1
Soil Temp (°C)	28.5	29.6	29.8	29.4	29.1	27.4	26.6	25.4	26.6	27.1	27.2	28.6

Sourc**e: Agro-meteorological Service, Department of Agro-meteorology and Water Management, Federal University of Agriculture, Abeokuta, Nigeria.**

Table 3: Effects of variety and grafting on incidence and severity of bacterial wilt of tomato

Variety	Treatment	Number of days to the development of visible symptom		Incidence (%)		Percentage severity index (PSI)	
		Early Planting	Late Planting	Early Planting	Late Planting	Early Planting	Late Planting
Beske	Grafted	0.00	0.00	0.00	0.00	0.00	0.00
	Non-grafted	22.80	27.40	36.70	50.00	36.10	48.30
UC82-B	Grafted	0.00	4.10	0.00	3.30	0.00	3.00
	Non-grafted	21.00	27.40	31.70	35.00	30.30	29.30
LSD(5%)							
	Variety	ns	ns	Ns	ns	1.79	7.76
	Grafting	11.98	7.33	6.54	7.32	5.65	9.77
	Variety x Grafting	ns	ns	Ns	10.38	ns	9.85

ns: not significant



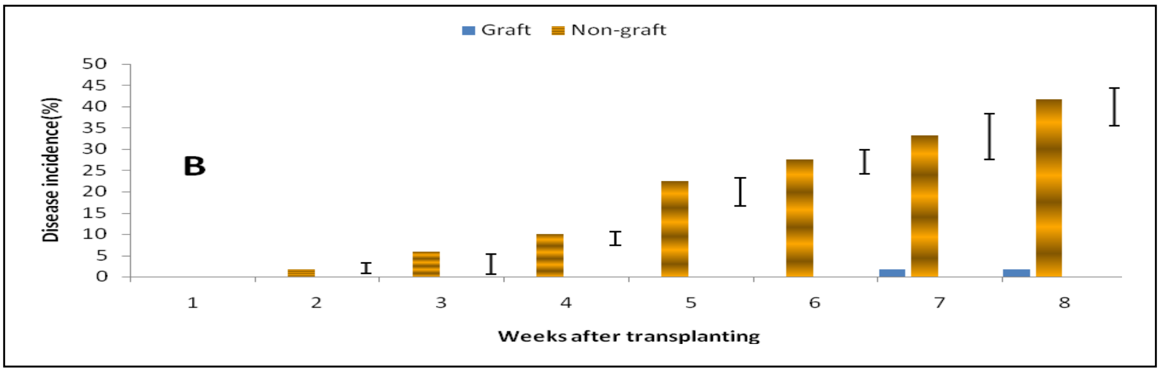


Figure 1: Effect of grafting on incidence of bacterial wilt of tomato during early (A) and late (B) planting

DISCUSSION

This study has shown that in severely infested soils, *R. solanacearum* accounted for high incidence and severity of bacterial wilt and grafting with resistant rootstock Tomachiva significantly reduced the incidence and severity of the disease. Past workers (Buddenhagen and Kelman, 1964; Hayward, 1991) had stressed the frustration associated with the use of resistant varieties to combat bacterial wilt of tomato. This was due to the evolving strains of the organism. Different races and biovars of the pathogen were emerging and they were making resistance to break down in those designated 'resistant' varieties sooner than they were produced (Scott, 1996). Popoola *et al*. (2015) identified the common strains of *Ralstonia solanacearum* in Nigeria as Race 1 Biovar III. Strains of Race 3 Biovar II were isolated in the temperate Jos Plateau of the country. Variation in races and biovars had always been key issues in developing resistant varieties of tomato against bacterial wilt. Grafting, presented here as an alternative method of control, is therefore of relevance.

In this study, grafting with rootstock Tomachiva showed a consistent reduction in disease incidence and severity compared with non-grafted plants across two seasons studied. Thus the rootstock can provide significant protection against severe crop failures caused by bacterial wilt. Other tomato rootstocks have shown efficacy worldwide against bacterial wilt (Grimault and Prior, 1994; Lin *et al*., 2008; Matsuzoe *et al*., 1993; Tikoo *et al*., 1979), but as observed by Rivard *et al*. (2012), little information was available regarding the specific rootstocks utilized in those trials and most of the rootstocks were not commercially available for growers.

Popoola *et al*. (2012) reported an effective use of thymol in the management of bacterial wilt in screenhouse. Ganiyu (2014) described an efficient combination of thymol and acibenzolar – S- methyl in the control of bacterial wilt in severely infested soil. These and other strategies that showed quantitative efficacy against bacterial wilt (Ji *et al*., 2005; Lin *et al*., 2008) could be combined with grafting to further reduce the incidence of bacterial wilt in severely infested soils.

Farmers in southwest Nigeria can cultivate Tomachiva on their field purposely to provide rootstocks for grafting. The variety is prolific and can grow well in most soils. Tomachiva is a landrace cultivar that is resistant to bacterial wilt but whose fruits are so small that it attracts no economic and culinary values among farmers and consumers. Quantitative resistance to diseases in some landrace *Solanum* spp. is well known to breeders, but the utilization of this resistance can be problematic due to unwanted traits, such as small fruit size, being linked to resistance observed in the landrace; this paradigm is particularly acute in the case of bacterial wilt (Opena *et al*., 1990; Wang *et al*., 1998).

Grafting can provide an effective management strategy to help reduce bacterial wilt incidence and subsequent crop loss. This study provides a report of an easily available rootstock cultivar that growers in southwest Nigeria can utilize by grafting to manage bacterial wilt in this region and other coastal wilt-endemic regions of West Africa. From a study on marker-assisted screening of tomato germplasms for resistance to Fusarium wilt, Popoola *et al*. (2012, 2014) showed that the rootstock

Tomachiva contained codominant resistant *I2* gene against Fusarium wilt. It is implied, therefore, that grafting with Tomachiva provides additional protection against wilt caused by *Fusarium oxysporum* f. sp. *lycopersici*.

The findings of this study suggest that the use of resistant rootstocks will be an important component to an integrated pest management program for tomato; the only limitation being that there are no rootstocks that are resistant to all pathogens. Implementation of this technology will depend on further research aimed at accurate, marker-assisted diagnosis of plant disease and selection of resistant rootstocks bearing in mind the race and biovar differentials of the pathogen, especially bacterial wilt pathogen, *Ralstonia solanacearum*.

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