

Identification of *Ralstonia solanacearum* resistant rootstocks for tomato grafting

Kanyua Stella^{*1}, Mwangi Maina¹ and Mbaka Jesca.²

¹Department of Agricultural Science and Technology, Kenyatta University, Kenya.

²Horticulture Research Institute, Kenya Agricultural and Livestock Research Organization, Kenya

*Corresponding author email: ignastella@gmail.com

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1 ABSTRACT

Bacterial wilt, caused by *Ralstonia solanacearum*, causes severe losses to tomato (*Solanum lycopersicum* L). Current management measures are not fully effective. Grafting with resistant rootstocks may be an effective strategy for managing the disease. However, *R. solanacearum* populations maintain considerable diversity, and little information is known regarding the efficacy of available rootstocks for use in grafting to reduce bacterial wilt incidence and subsequent crop loss. Tomato belongs to family Solanaceae which includes other well-known species, such as eggplant (aubergine), peppers, tobacco and potato. The objective of this study was to identify bacterial wilt resistant germplasm that can be used as rootstocks in tomato grafting. The potential candidates for the study included species that belong to solanaceae family. Rootstocks included tomato cultivar Mt56, Eggplant (*Solanum melongena*), Capsicum and Sodom apple (*Solanum incarnum*). Tomato cultivars Anna F1 and Cal J were used as sources of scions. Diseased plants were collected from farmers' fields and bacterial inoculum isolated using CPG Medium with TZC used to identify distinct colonies of *R.solanacearum*. The inoculum was applied on the test plants by injecting into the soil planted with tomatoes. Disease severity data was recorded using 0 to 5 scoring scale. Data was subjected to ANOVA using Genstat version 15 and significantly different treatment means separated using LSD at $P \leq 0.05$. *Solanum melongena*, *Solanum incarnum* and tomato cultivar (Mt56) did not develop infection while tomato cultivar Anna F1 and Cal J (used as positive controls) and Capsicum developed infection. It was concluded that resistant germplasm to bacterial wilt exists and can be utilized to graft susceptible tomato cultivars and contribute to management of bacterial wilt.

2 INTRODUCTION

Bacterial wilt (BW) caused by *Ralstonia solanacearum* is one of the most destructive diseases of solanaceous plants (Mansfield et al., 2012). The disease has a worldwide distribution, affecting crop production in tropical, subtropical, and temperate regions (Martin and French, 1985; Muthoni et al., 2012). *R. solanacearum* persists in soil, weeds, plant debris, rhizospheres, and alternate hosts. It spreads through irrigation water or infected planting

material, thus hindering pathogen eradication (Hayward, 1991; Laferriere et al., 1999; Liu et al., 2016). When living freely in the soil, *R. solanacearum* occurs in a motile form to efficiently find and invade the host through chemotaxis and aerotaxis (Yao and Allen, 2006, 2007). *R. solanacearum* generally enters through wounded roots or natural openings and it infects the intercellular space of the root cortex and vascular parenchyma. It can also invade

xylem vessels and spread to the stem and leaves, where bacterial cell density can reach up to 10^9 cfu·g⁻¹ of host tissue (Yao and Allen, 2006; Álvarez *et al.*, 2010). At this stage, a cell density-dependent (i.e., quorum sensing) conversion occurs from a motile phenotype to a non-motile virulent phenotype, adapted to a plant parasitic lifestyle (Tans-Kesrten *et al.*, 2004). Several resistant rootstocks have been identified and used to control bacterial wilt. However, bacterial wilt has been reported in commercial tomato cultivars grafted on resistant rootstocks in Korea and Japan (Lim *et al.*, 2008; Nakaho *et al.*, 2000). Therefore, there is need to select new resistant germplasm for use in grafting to control bacterial wilt. Grafting is the art of joining together two plant parts (a rootstock and a scion) by means of tissue regeneration, in which the resulting combination of plant parts achieves physical reunion and grows as a single plant (Janick, 1986). Grafting has been used effectively for overcoming environmental stresses including salinity (Estan *et al.*, 2005; Ruiz *et al.*, 2005; Colla *et al.*, 2006), extreme temperatures (Abdelmageed *et al.*, 2004) Black *et al.*, 2003),

3 MATERIALS AND METHODS

The experiment was conducted in the field at Kenyatta University Research and Teaching Farm. The selection criteria for rootstocks were based on botanical relationship to tomato (Solanaceae family) and using available literature. Candidate species included *Solanum incarnum* (Sodom apple), tomato variety Mt56, Capsicum (*Capsicum annum sp.* L) and *Solanum melongena* (Eggplant). Commercial tomato cultivars Cal J and Anna F1 were included in the experiment as positive controls. The seedlings of the selected rootstocks were raised on well prepared nursery beds and all the candidate species seeds were planted on the same day. The seedlings were raised for one month in the nursery. Transplanting was then done in polybags of dimensions 9cm by 5cm by 10cm in sterile soil and manure (2:1) after three weeks in the nursery. *Ralstonia solanacearum* was

and excessive soil moisture (Black *et al.*, 2003). Vegetable grafting has also enhanced resistance to soil-borne pathogens such as fungi, bacteria, viruses and nematodes. It has been used in management of Fusarium wilt on watermelon (Rivero *et al.*, 2003) and tomato (Rivard, 2006); and Verticillium wilt on melon (Bletsos, 2005), eggplant (Ioannou, 2001), tomato (Rivard, 2006) and potato (Tsrer and Nachmias, 1995, Rivero *et al.*, 2003, Tresky and Walz, 1997, Rahman *et al.*, 2005, Draie, 2009). The cultivated tomato, *Solanum lycopersicum* L., belongs to the family Solanaceae, which includes more than 3000 species, occupying a wide range of habitats (Knapp, 2002). The Solanaceae contain many species of economic use, such as food (tomatoes, potatoes, peppers and eggplants), medicines (deadly nightshade, henbane, Datura) and ornamental purposes (petunias). The production and consumption of tomatoes has substantially increased worldwide. This study therefore aimed at finding bacterial wilt resistant germplasm that can be used in tomato grafting for management of Ralstonia wilt.

isolated from fresh symptomatic plants of naturally infected cultivar Kilele plants obtained from farmers' fields. The fresh symptomatic plants were cut into pieces of 10 cm long and cleaned using water to remove debris and soil particles. The infected stems were sterilized in 70% ethanol and then rinsed using sterile distilled water. The stem portions were then suspended in sterile distilled water for bacterial ooze to identify the pathogen (*Ralstonia solanacearum*). Selective medium for *R. solanacearum* isolation was prepared using Casamino acid – peptone - Glucose (CPG) medium (Champoiseau, 2008) consisting of Casamino acid (casein hydrolysate) -1.0g, Peptone – 10g, Glucose – 5g and agar 17.0g per liter. The solution was autoclaved for 15 minutes at 121°C, pH adjusted to 6.5 – 7.0 and the medium allowed to cool; Tetrazolium

Chloride (TZC) was added in the media to identify pure colonies of *R. solanacearum*. Three weeks after transplanting, inoculation was done by drenching the potting media with the *R. solanacearum* suspension of 1.2×10^8 cfu/ml (Prior et al., 1998). The scoring scale for disease severity was 0 - 5; with 0 indicating no disease while 5 indicated all inoculated plants had 100% infection. Pests and diseases such as downy mildew, early blight, late blight, anthracnose, leaf spot and powdery mildew were controlled using pesticides such as Imidacloprid (against whiteflies, thrips and aphids) and fungicides such as Metalaxy 1 - M + Mancozeb. Standard agronomic practices for tomato production were deployed and included; watering done

three times a week and crop nutrition with NPK 23:23:0 (10gms per hole or 50 kgs/ha) applied during transplanting, NPK 17:17:17 (10gms per hole or 50 kgs/ha) used as top dressing fertilizer one month after transplanting, and foliar fertilizers .Boom flower® (Nitrobenzene) (1ml/ litre of water) at flowering alternated with Easy grow® and Calcium Nitrate used as foliar fertilizers at 2g/L of water. Inoculated plants were observed daily until appearance of the first wilt symptoms. Ten plants were used for each treatment and data on disease incidence was recorded based on the number of plants showing wilting symptoms.

4 RESULTS

Colonies of *R. solanacearum* were visible after 48 hours of incubation at 28°C. Colonies of the normal (virulent type) were pink colored, irregularly- round, fluidal and opaque

(Champoiseau, 2008). Sub- culturing was done to get pure cultures which were used to prepare the inoculum used on experimental plants.

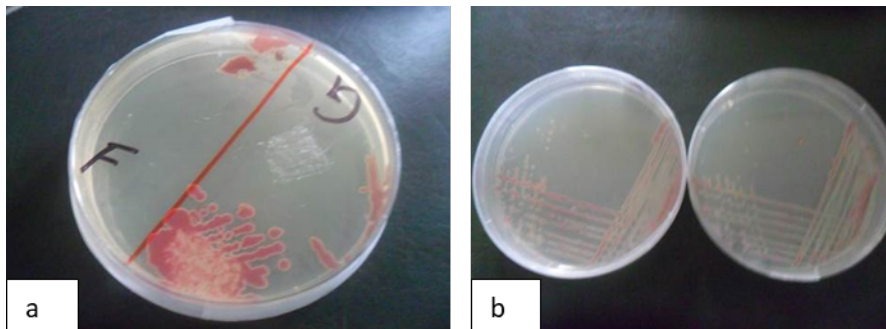


Plate 1: Isolated colonies of *R. solanacearum* on TZC Medium (a) (F – non-virulent and G – virulent colonies) and Sub- cultured colonies of *R. solacearum* (b).

During pathogenicity testing, *Solanum melongena*, *Solanum incarnum* and tomato Mt56 were not infected and thus were classified as resistant/tolerant. Tomato cultivars Cal J, Anna F1 and Capsicum showed wilting symptoms

that were observed in week 2 and by week 5 plants had died (Table 1). Expression of symptoms on the Anna F1 and Cal J and Capsicum confirmed virulence of the bacterial isolate.

Table 1: Mean number of surviving (uninfected) plants following inoculation with *R. solanacearum*.

Treatment	7 days	14 days	21 days	35 days
Cal J	10a	2.7a	5.0a	0.0a
Capsicum	10a	2.3a	4.3a	0.0a
Anna F1	10a	2.0a	4.3a	0.0a
Eggplant	10a	10.0b	10.0b	10.0b
Mt56	10a	10.0b	10.0b	10.0b
Sodom apple	10a	10.0b	10.0b	10.0b

NB: Values within column followed by same letter are not significantly different at $P \leq 0.05$.

5 DISCUSSION

Using genetically resistant cultivars is the most effective and environmental-friendly method to control bacterial wilt. Several resistant rootstocks have been identified and used to control bacterial wilt (Sang *et al.*, 2016). Therefore, selecting additional resistant sources can contribute to improved control of bacterial wilt. The findings of this study demonstrate resistant rootstocks can be an important component in integrated pest management of bacterial wilt on tomato. Quantitative resistance to diseases and pests in wild *Solanum* spp. is known to breeders but its utilization can be problematic because of associated unwanted traits, such as small fruit size (Opena *et al.*,

1990; Walter, 1967; Wang *et al.*, 1998). This study provides information of available rootstock sources that nursery operators can utilize in grafting to manage bacterial wilt in tomato. The resistance to bacterial wilt among potential rootstocks seen in this study and others (Freeman *et al.*, 2009) could complement other integrated pest management strategies to reduce bacterial wilt incidence in severely infested soils. Further study is recommended to evaluate farmer preferred tomato cultivars when grafted onto the resistant rootstocks to determine disease reduction, compatibility and performance of the grafted plants.

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