

Preliminary evaluation of guava selections for guava wilt disease resistance in South Africa

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Keywords: guava wilt, *in vitro* screening, *Nalanthamala psidii*, resistance,

Abstract

Guava wilt disease (GWD), caused by *Nalanthamala psidii*, is a serious disease occurring in the guava-producing areas of the Mpumalanga and Limpopo Provinces of South Africa. Two resistant guava rootstocks TS-G1 and 'TS-G2' were developed by the ARC-ITSC in 1995. In 2009, a renewed outbreak of GWD was reported, which also affected the resistant 'TS-G2' cultivar, placing the guava industry under threat again. The aim of this study was to seek resistant guava selections by means of *in vitro* screening of guava seedlings and subsequently testing the most promising selections in inoculation studies with *N. psidii*. A culture filtrate of *N. psidii* was used to screen guava seedlings *in vitro*. Promising selections were multiplied in tissue culture, hardened-off and planted in bags before inoculation with the GWD fungus in a shade house trial. The number of plants surviving nine months after inoculation, was recorded. Although none of the selections showed complete resistance, selection MS44 showed some tolerance against the G2 isolate of the pathogen obtained from diseased 'TS-G2' trees whilst selection MS70 showed some tolerance against the G1 isolate obtained from diseased TS-G1 trees. These selections were also resistant to the original Fan Retief isolate of the pathogen (FR).

Introduction

Approximately 1200 hectares are currently under guava production in South Africa. Of these, the largest production area is the Western Cape Province (547 ha), followed by the Limpopo

Province (442 ha) and the Mpumalanga Province (140 ha). The total production per annum is approximately 27 000 tons (DAFF 2013). Sixty two percent of the production is used in the processing industry, 12% is canned, 24% is sold on the formal fresh market and a small amount is dried (Anon 2009).

Guava wilt, caused by *Nalanthamala psidii* (Schroers et al. 2005) is a serious disease of guava in the guava-producing areas of the Mpumalanga and Limpopo Provinces. Guava wilt disease was first reported in Taiwan by Kurosawa (1926), but is now also present elsewhere in South-East Asia. In Taiwan the disease is present in all the guava producing areas and has reduced the life expectancy of orchards from over 25 years to between 10 and 12 years (Grech 1986). In Malaysia the disease affected 42% of a commercial planting of 270 ha of the “Beaumont” cultivar (Schoeman 1997). In Thailand guava wilt disease caused great loss to guava growers in several production areas (Athipunyakom and Luangsa-ard 2008). In the Philippines the disease resulted in the discontinuing of production of guavas as early as the fifth year of production (Quimo et al. 1984, Opina 1995).

In South Africa the disease was first reported from the Malelane area (Mpumalanga Province) in 1981 (Grech 1985). At that stage the guava industry relied solely on the Fan Retief cultivar. Within 10 years, guava wilt disease (GWD) had spread throughout the guava-growing areas of the Mpumalanga and Limpopo Provinces, reducing the area planted to guava from approximately 700 ha to 100 ha (Grech 1990). Quarantine measures implemented in 1985 have to date prevented the spread of the disease to the Western Cape Province.

Symptoms of guava wilt disease include wilting of the foliage of the upper branches which subsequently spreads to the whole tree. During rapid decline, leaves tend to shrivel and

die on the trees, which then assume a scorched appearance. When decline occurs more slowly, leaves drop gradually, resulting in complete defoliation (Schoeman et al. 1997).

Effective GWD control measures, other than eradication of diseased trees, do not exist. Two resistant rootstocks, TS-G1 and 'TS-G2', were developed by the Agricultural Research Council's Institute for Tropical and Subtropical Crops (ARC-ITSC) in 1995 (Schoeman 1995). These rootstocks were selected after 30 000 tissue cultured guava seedlings were exposed to a culture filtrate of the GWD fungus (Vos et al. 2000). Resistance to GWD was thereafter confirmed in nursery trials. Of the two rootstocks, only 'TS-G2' was submitted for registration of Plant Breeders Rights (Grant No. ZA 20002283) as field evaluation demonstrated the yield and fruit quality of this rootstock to be commercially acceptable. In 2007 it was estimated that 600 ha of both TS-G1 (which was never registered), and 'TS-G2', were established in the Mpumalanga and Limpopo Provinces (Schoeman 2007). In 2009, a renewed outbreak of GWD was reported from several localities, which also affected the resistant 'TS-G2' cultivar (Schoeman 2011). Losses of up to 500 trees per month in certain months have been reported to the authors from several producers in the Limpopo Province. Within three years, disease incidence in some orchards in the Mpumalanga Province exceeded 30% and many orchards were soon thereafter removed and replanted with other crops. The renewed outbreak suggested that a new virulent strain / race of *N. psidii* had evolved, placing the guava industry under threat once again. Thus it became critical for the South African guava industry to seek new resistant guava selections.

The aim of the current study was to find resistant guava selections by means of *in vitro* screening of guava seedlings against a culture filtrate of *N. psidii* isolated from diseased Fan Retief trees and subsequently testing the most promising selections in inoculation studies with

the various new isolates of the pathogen. These inoculation studies were conducted under shade house conditions.

Materials and methods

A culture filtrate of *N. psidii*, isolate Fan Retief (CMW34343), being the only isolate available at the time, was prepared according to the method described by Vos et al. (2000). The method was slightly modified by replacing Phytigel with agar (12 g/l). *In vitro* guava plants were also produced according to the method described by Vos et al. (2000). Briefly, open pollinated *Psidium guajava* seed originating from escape trees in Fan Retief and 'TS-G2' orchards were obtained. Approximately 12 000 seeds were sterilized in calcium hypochlorite and placed into tissue culture vessels containing autoclaved germination medium. When plants had reached a height of approximately 35 mm five plants were aseptically transferred to sterile culture tubes containing 1 ml of filter-sterilised fungal filtrate of *N. psidii*. The plants were maintained at 27°C, 16 h light/8 h darkness for two months, or until deceased. From an initial 12 000 seeds, 14 surviving plants were obtained (named selections 1-14). These were aseptically transferred onto multiplication medium and sub-cultured monthly. Plants were then transferred to a rooting medium (Vos et al. 2000) and when sufficiently rooted these were transplanted into a bark mix in a mist bed to allow for hardening-off. Finally, the plants were transplanted into four-litre plastic bags containing composted pine bark. Six to 12 plants from each of the 14 selections surviving exposure to the culture filtrate were subsequently subjected to challenge inoculation with *N. psidii*. Inoculum was produced by growing the three different isolates of *N. psidii* on malt extract agar plates for 10 days at 25°C. The three isolates comprised the original Fan Retief isolate (CMW34343), the new isolate obtained from the 2009 GWD outbreak on 'TS-G2' in the Levubu area of the Limpopo Province (CMW34346) and another new isolate from

Nelspruit/Brondal area (CMW34339) obtained in 2009 from GWD outbreaks on TS-G1. The three pathogen isolates were labelled FR, G2 and G1 respectively. Three culture plates of each isolate were macerated in 2 L of sterile water and 100 ml of the resulting suspension applied per plant bag as a drench treatment. Before inoculation, the roots were wounded by thrusting a knife into the root system at three locations around the stem. Susceptible Fan Retief plants inoculated with the FR isolate served as positive controls whereas resistant 'TS-G2' plants inoculated with the FR isolate served as negative controls. Sterile macerated agar provided a second negative control treatment.

Data was recorded on the number and percentage of *N. psidii* inoculated plants that survived up to nine months after inoculation.

Proportions in two independent samples were compared pairwise by Chi-Square with one degree of freedom using 2x2 contingency tables using the CHITEST and CHIINV functions in Excel 2010 (Snedecor 1967). Non-significant ranges are indicated in Table 1 with similar letters next to the values indicating percentage survival.

Results and discussion

Of the fourteen guava selections tested (Table 1) six selections and the control, 'TS-G2', showed 100% survival after inoculation with the FR isolate. None of the Fan Retief cultivar plants survived. Plants of the selections inoculated with macerated agar only also showed 100% survival (data not shown).

Table 1: Survival of various guava selections nine months after inoculation with three different isolates of *Nalanthamala psidii* under shade house conditions. Values within the same column followed by the same letters do not differ significantly at a 5% level of significance.

Selections	FR Isolate			G2 Isolate			G1 Isolate		
	Number inoculated	Number alive	% Survival	Number inoculated	Number alive	% Survival	Number inoculated	Number alive	% Survival
MS44	8	8	100 a	9	7	78 a	8	4	50 abc
MS70	9	9	100 a	9	3	33 abcd	9	8	89 a
MS7	12	12	100 a	12	5	42 abc	12	5	42 bc
MS10A	8	5	63 a	7	0	0 d	8	0	0 d
MS72	12	12	100 a	13	3	23 bcd	14	5	36 cd
G11	9	7	78 a	10	2	20 cd	11	5	45 bc
MS32	9	0	0 b	9	6	67 ab	9	7	78 ab
MS20A	8	6	75 a	7	1	14 d	7	1	14 cd
MS20B	7	6	86 a	8	1	13 d	8	1	13 cd

MS47	10	10	100 a	11	0	0 d	11	0	0 d
G5	8	8	100 a	8	0	0 d	8	0	0 d
MS8B	8	0	0 b	8	3	38 abcd	8	1	13 cd
MS25B	8	5	63 a	8	1	13 d	8	0	0 d
MS18	10	0	0 b	10	0	0 d	10	1	10 cd
TSG2	6	6	100 a	6	1	17 d	6	3	50 abc
Fan Retief	8	0	0 b	10	4	40 abcd	10	2	20 cd

None of the selections inoculated with the G2 isolate showed 100% survival. The two best selections were MS44 and MS32 showing seven of nine (78%) and six of nine (67%) plants surviving, respectively. All plants of selection MS44 also survived when inoculated with the FR isolate. Selection MS44 therefore showed some resistance to both of the pathogen isolates known to be present in the Levubu area (FR and G2). None of the plants of selection MS32 survived after inoculation with the FR isolate and would therefore not be suitable for the Levubu area. Of all the selections inoculated with the G1 isolate, selection MS70 performed best with eight of nine plants surviving (89%) and selection MS32 was second with seven of nine plants surviving (78%). All plants of selection MS70 also survived after inoculation with the FR isolate. Selection MS70 therefore showed some resistance against both known isolates of *N. psidii* in the Nelspruit area (FR and G1).

These promising selections need to be further evaluated for yield and fruit quality. If these prove to be acceptable these selections could be used as alternatives to the 'TS-G2' cultivar in production areas where the new virulent strains of *N. psidii* are not present.

As only the culture filtrate of the FR isolate was used in the *in vitro* screening procedure, it was expected that more selections would survive when inoculated *in vivo*. The *in vitro* screening procedure, to find an increased number of tolerant selections against the only isolate of *N. psidii* present at that stage, started in 2007 during which time the industry was based on only two cultivars. These were the cultivar Fan Retief which was highly susceptible to the original FR isolate of the pathogen and the cultivar 'TS-G2' which was resistant to the original FR isolate of the pathogen. However, when the renewed outbreak of GWD was reported in 2009, the strategy had to be changed and selections surviving the *in vitro* screening procedures had to be tested against all three isolates of the pathogen.

Although no guava selection showed 100% resistance against the new *N. psidii* isolates from the Levubu (G2 isolate) and Nelspruit (G1 isolate) areas, selections MS44 and MS70 show some tolerance to the G2 and G1 isolates, respectively. These two selections were also resistant to the FR isolate and will be tested further and could also be useful as parent material in a breeding programme. Different levels of pathogenicity between the different *N. psidii* isolates were again recorded, confirming observations of earlier studies by Schoeman (2011).

The results (Table 1) indicated a strong correlation between tolerance to the culture filtrate of a specific isolate of the pathogen and resistance to the same fungal isolate during the inoculation study in the shade house. Seventy nine percent (11 of 14) of the guava plants selected *in vitro*, based on resistance to the culture filtrate of the FR isolate were found to be more tolerant than the Fan Retief cultivar. Of these, 43% showed 100% resistance against the FR isolate. Where plants were, however, inoculated with the new virulent isolates of the pathogen (isolates G1 and G2), only 43% (6 of 14) and 21% (3 of 14), respectively, were found to be more resistant than the Fan Retief cultivar. None showed 100% resistance.

Internationally, GWD poses a major threat to guava production in South-East Asia (Quimo et al. 1984, Grech, 1986, Schoeman 1997, Athipunyakom and Luangsa-ard 2008). Resistant plants could potentially be of benefit to affected countries via the relevant agreements, possibly saving their industries from decimation by the disease.

Future research will include screening of a considerably larger seed collection, including imported seed to increase diversity. Maintaining selections in tissue culture allows rapid multiplication of promising selections and quick release to the industry. As fruit quality and yield will only be determined in later studies, resistant selections will be released as rootstocks and

grafted with commercially acceptable cultivars. The ultimate goal will, however, be the release of resistant cultivars with acceptable fruit quality and yield.

The emergence of additional races of the GWD pathogen poses a major hurdle to the development of guava varieties with durable resistance. Accurate identification of the different races of the pathogen as well as knowledge of the genetic variation within the pathogen populations is critically important for the identification, breeding and deployment of resistant guava selections. Future research will include genomic studies to provide insight into the defence and virulence mechanisms involved in resistance or susceptibility of guava to *N. psidii*.

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