Crop Protection 29 (2010) 757–765

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Field evaluation of yield effects on the U.S.A. heirloom sweetpotato cultivars infected by *Sweet potato leaf* curl *virus*

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ARTICLE INFO

Article history:
Received 24 September 2009
Received in revised form
12 February 2010
Accepted 18 February 2010

Keywords:
Begomovirus
Ipomoea
Bemisia tabaci
Real-time PCR
Meristem tip culture
SPLCV

ABSTRACT

The incidence of *Sweet potato leaf* curl *virus* (SPLCV), a Begomovirus, on sweetpotato, *Ipomoea batatas* (L.) Lam. (Convolvulaceae), in South Carolina, U.S.A. has increased rapidly in recent years. This is likely due to the use of infected propagating materials and the increasing population of its vector, the sweetpotato whitefly, *Bemisia tabaci* (Gennadius). In this study, we demonstrated in field experiments that SPLCV infection reduced the yields of most heirloom sweetpotato lines relative to the yields of non-infected plants. Real-time polymerase chain reaction (PCR) technology specific to several common sweetpotato viruses was used to determine the virus infection status in 69 selected accessions of heirloom sweetpotato lines. Meristem tip culture technology was used to regenerate virus-free plants from these materials. To ensure the virus-free status, each mericlone was evaluated using real-time PCR and graft bio-indexing on the indicator species, *Ipomoea setosa* Ker Gawl. Mericlones of 27 cultivars were found to be free of the viruses. The 27 cultivars were included in a field test to determine the effect of SPLCV infection on yield. Yields of virus-free plants of the cultivars ranged from 10 to 80% greater than the yields of SPLCV-infected plants. However, the yield differences between virus-free and infected plants were diminished in the second year of the field experiment due to a rapid re-infection by SPLCV. These results demonstrate the importance of using certified, virus-tested seed roots or cuttings. The rapid re-infection of the virus-tested sweetpotato plants with SPLCV observed in these studies suggests that management of the whitefly population should be a critical element in control of this important virus.

1. Introduction

Sweetpotato, *Ipomoea batatas* (L.) Lam. (Convolvulaceae), is an important global food crop (Woolfe, 1992). According to FAO statistics in 2007 as reported by the International Potato Center (Anonymous, 2007), the vast majority of sweetpotato production is in eastern Asia and 80% of the global crop is produced in China. Sweetpotato is an extremely important food crop for subsistence farmers in the relatively humid areas of sub-saharan Africa, from the coastal west to the central and southern areas of that continent (Anonymous, 2007). Sweetpotato yields vary widely, from over 25 metric tons per hectare with high-input to below 3 metric tons per hectare when grown as a subsistence crop with minimal input. From 2004 to 2008, the United States produced an average of 768,382 metric tons of sweetpotatoes on 37,418 ha per year for an average yield of 20.5 metric tons per hectare (North Carolina Sweetpotato Commission, 2009).

One major limitation to sweetpotato production is the cumulative effect of virus infection on this vegetatively propagated crop. Over 20 viruses are known to infect sweetpotato. Sweetpotato virus disease (SPVD), one of the most important diseases, is induced by a co-infection of two viruses, *Sweet potato feathery mottle virus* (SPFMV), a Potyvirus (Moyer et al., 1980), and *Sweet potato chlorotic stunt virus* (SPCSV), a Crinivirus (Hoyer et al., 1996; Kreuze et al., 2002). SPVD is especially important in East Africa (Karyeija et al., 1997; Gibson et al., 1997, 1998). SPCSV also can induce synergistic interactions with other sweetpotato viruses, such as *Sweet potato mild mottle virus* (SPMMV) (Tairo et al., 2005), *Sweet potato virus G* (SPVG) (IsHak et al., 2003), and *Cucumber mosaic virus* (CMV) (Cohen and Loebenstein, 1991). Sweetpotato plants infected with multiple viruses often exhibit more severe disease symptoms and greater yield reduction than plants infected with only one virus.

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doi:10.1016/j.cropro.2010.02.017

SPVD is induced in sweetpotato plants due to the ability of SPCSV to suppress host resistance to co-infecting unrelated viruses (Karyeija et al., 2000; Tairo et al., 2005).

In the United States, SPFMV is widely distributed throughout sweetpotato production areas (Moyer and Kennedy, 1978; Moyer et al., 1980; Cali and Moyer, 1981; Moyer and Cali, 1985; Clark and Moyer, 1988). SPCSV, the other component of SPVD, was identified in the U.S.A. in the heirloom cultivar 'White Bunch' (Pio-Ribeiro et al., 1996). It was recently identified in a sweetpotato production field in North Carolina, but does not appear to be widespread in the United States (Abad et al., 2007). In addition to SPFMV, two other potyviruses, *Sweet potato virus G* (SPVG) and *Sweet potato virus-2* (synonymous for *Ipomoea vein mosaic virus*, IVMV), have been identified in the U.S.A. (Souto et al., 2003).

Sweetpotato leaf curl disease was first observed in Taiwan and Japan (Chung et al., 1985, 1986; Green et al., 1992; Liang et al., 1990; Osaki and Inouye, 1991), and a similar disease was also reported in Israel (Cohen and Loebenstein, 1991). Since its first report in the U.S. A. (Lotrakul et al., 1998; Lotrakul and Valverde, 1999), *Sweet potato leaf* curl *virus* (SPLCV), a begomovirus, has also been observed in Peru (Fuentes and Salazar, 2003), Italy (Briddon et al., 2006), Kenya (Miano et al., 2006) and China (Luan et al., 2006, 2007). A related begomovirus was detected in Spain where it was initially reported as *Ipomoea yellow vein virus* in *Ipomoea indica* (Burm.) Merr. (Banks et al., 1999). A third begomovirus, *Sweet potato leaf* curl *Georgia virus* (SPLCGV) that was first identified in a sweetpotato breeding clone in the U.S.A. (Lotrakul et al., 2003), was also recently identified in India (Prasanth and Hegde, 2008). New begomovirus species of recombinant nature in sweetpotato and *I. indica* have also been identified (Lozano et al., 2009).

In addition to dissemination of the virus through the use of infected propagating materials, SPLCV can also be transmitted by whiteflies, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (Valverde et al., 2004; Simmons et al., 2009). With the presence of SPLCV inoculum in major sweetpotato production areas around the world and the continuing expansion of *B. tabaci* (Simmons et al., 2008, 2009), the impact of SPLCV on global sweetpotato production will probably become important. Recently, it was shown that tall morningglory [*Ipomoea purpurea* L. (Roth)] is a natural host for SPLCV in China (Yang et al., 2009). A host range study through whitefly transmission in a greenhouse environment identified over 36 species of morningglories that could be potential hosts for SPLCV (unpublished data). Various morningglories have been shown to be natural hosts for SPFMV in Louisiana, U.S.A. (Clark et al., 1986) and in Africa (Tugume et al., 2008).

The effect of infection by sweetpotato potyviruses (i.e., SPFMV, SPVG and IVMV) or SPLCV on the predominate cultivar 'Beauregard' were evaluated by researchers in Louisiana (Clark and Hoy, 2006). One of their conclusions was that a single infection with SPFMV, SPVG, or IVMV did not significantly affect yield. However, an infection with SPLCV reduced yield by 26% in comparison to virus-tested (VT) controls in which the virus was not detected (Clark and Hoy, 2006).

Although the distribution of SPLCV in the U.S.A. is not known, the lack of typical leaf curl symptoms by plants in many of the SPLCV-infected sweetpotato cultivars or breeding materials may have resulted in an under-estimation of the importance of SPLCV. On the other hand, the lack of typical leaf curl symptoms of SPLCV-infected plants of various cultivars suggests that some of them may actually be resistant or tolerant to SPLCV infection. Such apparent tolerance to SPLCV infection may have been developed through unintentional selection over the years of sweetpotato cultivars or breeding lines that were evaluated in the field under strong disease pressure and high whitefly populations in South Carolina, U.S.A. The objective of the present study was to test the yield effects of selected heirloom sweetpotato cultivars against SPLCV infection. Herein, we report results from field trials using 27 sweetpotato cultivars during the 2007 and 2008 growing seasons in Charleston, South Carolina.

2. Materials and methods

2.1. Sources of plant materials

In 2005, 69 heirloom sweetpotato cultivars were obtained from the U.S. germplasm collection for sweetpotato (USDA Plant Genetic Resources Conservation Unit, Griffin, Georgia, U.S.A). Many of these were originally submitted to the collection by researchers at the USDA, Agricultural Research Service, U.S. Vegetable Laboratory, Charleston, South Carolina during the 1990s. These cultivars have been maintained *in vitro* through shoot-tip or nodal propagation using MS basal medium (Murashige and Skoog, 1962) with sucrose and agar.

2.2. Determination of virus infection and regeneration of virus-free plants using meristem tip culture

Infection by four RNA viruses (SPFMV, SPVG, SPV-2 and SPCSV) and one DNA virus (SPLCV) in the sweetpotato clones was initially assessed using real-time PCR (Kokkinos and Clark, 2006) with some modifications (described below). The clones were also subjected to meristem tip culture in an effort to regenerate virus-free plantlets following the procedures developed at Louisiana State University (Clark and Hoy, 1999). Briefly, after sterilization in a chlorine bleach solution, aseptically isolated meristem tips (~0.5 mm) were placed onto Petri dishes (10 cm in diameter) containing Meristem Shoot Initiation Medium (1 packet of SIGMA MS salts, 30 g sucrose, 4 mL of 500 μg/mL thiamine HCl and 0.1 g of Myo-inositol, adjusted to pH 5.8, 2.5 g Phytagel/L and 500 μg/mL 6-benzyl-aminopurine/L added after autoclaving). The dishes were placed in a tissue culture room with 16 h/day lighting and 25–30 °C. Newly developed shoots were transferred to Phyta trays in Regeneration Medium (1 packet of GIBCO salts, 5 mL GIBCO vitamins, 50 g sucrose and 2.5 g Phytagel/L) for further shoot growth and root development. Plantlets with roots were then transferred to test tubes with Gibco MS Medium (1 packet of GIBCO salts, 5 mL GIBCO vitamins, 30 g sucrose and 2.5 g Phytagel/L). Status of SPLCV on each newly regenerated plantlet was tested again with the improved real-time PCR as described below. Only those plantlets which tested clean of SPLCV were saved. Finally, the virus-tested status of such plantlets was further confirmed through graft indexing on *Ipomoea setosa* plants. In this study, the virus-tested 'Beauregard' and 'Picadito' plants were developed by Dr. Christopher Clark, Louisiana State University, the virus-tested 'Liberty' plants were developed at North Carolina State University, and virus-tested plants of 'Sumor' and the 23 heirloom clones were generated at the U.S. Vegetable Laboratory as described above.

2.3. Generation of SPLCV-infected materials

The SPLCV culture (isolate SC1) used in this study was obtained through transmission to an *I. setosa* plant by a single whitefly, which was maintained on *I. setosa* in the growth chamber (Simmons et al., 2009). The SPLCV infection of selected heirloom sweetpotato clones for field testing was conducted through whitefly transmission to potted sweetpotato plants in a greenhouse (Simmons et al., 2008). Successful transmission of SPLCV was monitored through symptom expression on the control indicator plants (*I. setosa*) as well as on sensitive sweetpotato cultivars, e.g., 'Topaz'. Similarly, infection to the test heirloom sweetpotato lines by SPLCV was achieved using whitefly transmission in a greenhouse. Confirmation of the SPLCV infection on the test plants was