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Virus Elimination in Sweetpotato: from Meristem-Tip Culture to Storage Roots Production: A Review

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Abstract

Sweetpotato (*Ipomoea batatas (L.) Lam.*) belongs to the Convolvulaceae family and is an important crop for food security. Sweetpotato is subject to a wide range of pathogen infections. Among them, viral diseases are the major constraints for sweetpotato productions, because viruses can be accumulated and transferred from one generation to the next through the use of infected storage roots for vegetative propagation. There are over 30 viruses worldwide that are known to infect sweetpotato and many of them have been reported in the United States and have caused devastating yield losses. To realize the full potential of this crop, meristem-tip culture based biotechnique has been adopted for producing virus-indexed propagating material in the U.S. This review attempts to assess methodology for sweetpotato virus detection and identification, as well as current biotechnological approaches for virus elimination and disease management. We also provide insights regarding the potential concerns and challenges for sweetpotato industry.

Keywords: Sweetpotato; Meristem-tip Culture; Virus Elimination; Virus Disease; Field Performance

Abbreviations: SPVD: Sweetpotato Virus Disease; SPCSV: Sweet Potato Chlorotic Stunt Virus; SPFMV: Sweetpotato Feathery Mottle Virus; PCR: Polymerase Chain Reaction; RT-PCR: Reverse-Transcription Polymerase Chain Reaction; SPLCV: Sweet Potato Leaf Curl Virus; SPVG: Sweet Potato Virus G; PDR: Pathogen-Derived Resistance; CP: Coat Protein; USDA: United State Department of Agriculture.

Introduction

Sweetpotato (*Ipomoea batatas (L.) Lam.*), a member of the Convolvulaceae (morning glory) family, originated in Central or South America and currently is widely grown in all tropical and subtropical areas of the world [1]. It remains among the 10 most important food crops worldwide on the basis of dry weight produced, yielding about 130 million metric tons per year on about 9 million hectares. As an important root crop and source of industrial raw material worldwide, sweetpotato production constitutes one of the major agricultural business in Southern U.S. states such as North Carolina, Mississippi and Louisiana, where three quarters of the total U.S. production are being produced. Sweetpotato performs well in relatively poor soils and harsh environment with few inputs, has a short growing season and broad adaptability, and is a suitable crop for limited resource farmers. Sweetpotato is subject to a wide range of pathogen infections. Among them, viral diseases are the major constraints in sweetpotato production, because viruses can be accumulated and transferred to the next generation through the use of infected vegetatively propagated sweetpatoto “seeds” (vine cuttings). Cultivar decline due to virus infections in sweetpotato has been observed for decades in the U.S. For example, yield of the widely used cultivar ‘Beauregard’ can decline as much as 25% to 40% due to virus infections. Mixed infection of several

viruses are commonly detected in commercial sweetpotato production fields, and have triggered devastating yield reduction. For example, Sweetpotato Virus Disease (SPVD), caused by coinfection of Sweet potato chlorotic stunt virus (SPCSV) and sweetpotato feathery mottle virus (SPFMV), can reduce yield up to 90%. To realize the full potential of this crop, meristem-tip culture technology has been explored for the production of virus-indexed sweetpotato plants. The technology provides the possibility for producing and maintaining sweetpotato plants free of detectable viruses, and thus have resulted in great yield improvement [2-9].

Virus detection Techniques and Viruses Identified in Sweetpotato

More than 30 viruses from 9 families have been isolated and described on sweetpotato globally [7]. Many antibodies to these viruses are available from Agdia Company that makes the protein-based ELISA assay convenient as a low-cost, large-scale test to those viruses (Elkhart, IN, USA). Additionally, other Ipomoea species, such as *Ipomoea setosa*, which can cause visible symptoms for many of sweetpotato viruses are used as indicator plants in grafting experiment to facilitate detection. Alternatively, the nucleic acid-based polymerase chain reaction (PCR) and reverse-transcription PCR (RT-PCR) are used to achieve higher sensitivity and confirmation at the genomic level of viral species and strains. Primers targeting to conserved regions of the known sweetpotato viruses are used for this nucleic acid based detection. Multiplex PCR were also developed for the simultaneous detection of multiple viruses in one sample. Further, the recent development of high-throughput sequencing technology enables fast genetic identification of known and unknown viruses in sweetpotato [10-12].

Among the viruses identified globally, several viruses have been found in the U.S. and have caused severe yield losses in sweetpotato production. SPFMV, a potyvirus in the family of Potyviridae, has a positive monopartite single strand RNA genome that encodes about 10 gene products. SPFMV is aphid-transmitted and can be seed borne. It is the most widespread sweetpotato virus globally and was first described and characterized in the U.S. in 1978 [13,14]. The Russet Crack strain of SPFMV (SPFMV-RC) can seriously affect sweetpotato yield and quality. Other potyviruses found in the U.S. including Sweet potato virus G (SPVG), Sweet potato virus C (SPVC), Sweet potato virus 2 (SPV2), along with SPFMV, are the four potyviruses that are readily detected in commercial propagation fields in the U.S. Similar to SPFMV, these potyviruses are also aphid-transmitted and the mixed infections are widely observed. Sweet potato leaf curl virus (SPLCV), a begomovirus in the family of Gemeniviridae, has a monopartite single strand DNA genome that encodes 6 mature gene products. SPLCV is transmitted by whitefly (*Bemisia tabaci*) in a persistent manner, and it has been widely identified in fields of commercial sweetpotato growers in the U.S. SPLCV can infect many species in the genus Ipomoea that includes morning glory plants as well as other ornamental sweetpotato cultivars which could potentially serve as reservoir for production field disease epidemic. As a virus commonly infects germplasm accessions and most breeding lines, SPLCV infection alone can reduce yield by at least 26% in the cultivar "Beauregard", the predominant cultivar accounting for ~80% of sweetpotato grown in the U.S. It was speculated that SPLCV will become the most important new emerging virus in sweetpotato in the U.S., just like the Tomato leaf curl begomovirus in tomato [11].

National Clean Seed Programs and Meristem-Tip Culture Technique

Due to the importance of this viral disease issue, sweetpotato foundation clean seed programs were developed long before the advent of technology to produce virus-free propagating materials. One of the earliest seed programs to include virus testing program began in California in the 1960s as a means of managing russet crack disease caused by SPFMV [15]. The use of virus-indexed seed and disease-resistant varieties against a collection of other pathogen groups has resulted in yield increases from 5 tons/acre in 1967 to 12 tons/acre in 2001 in California. In 2015, the National Clean Plant Network for sweetpotato was established under the umbrella of United State Department of Agriculture (USDA). Currently, sweetpotato clean seed programs have been established in California, Louisiana, North Carolina, Mississippi, Arkansas and Hawaii.

Meristem-tip culture is the basic technique adopted by these sweetpotato clean seed programs for regenerating virus-free plantlets. It starts from excision of the organized apex of the shoot from a selected donor plant for subsequent in vitro culture. The excised meristem tip is typically small (often <1 mm in length), which holds the potential that excludes pathogenic organisms that may have been present in the donor plants. Thermotherapy is commonly used before meristem-tip culture to partially deactivate the virus and slow down the movement of virus. The ability to produce and maintain plants free of detectable viruses through meristem-tip culture has greatly improved sweetpotato yields for several decades. However, because viruses can be accumulated and transferred from one generation to the next through the use of infected vegetatively propagated sweetpotato materials (also known as "seeds"), studies indicate that a significant yield and quality reduction will occur due to re-introduction of viruses from previous propagating materials. In two separate studies, 100% of virus-indexed plants were re-infected by SPFMV within the first year in the field, and decline in yield occurred gradually

over several years [4]. Therefore, sweetpotato "seeds", which is the main method to reduce the damage of virus infections currently, is very expensive because farmers must regularly purchase virus-tested seeds due to high reinfection rate in the fields.

Engineered Resistance Approach to Improve Sweetpotato Viral Disease Resistance

Little success has been reported in the breeding for virus resistance in sweetpotato cultivars. Factors such as virus variation, time and expenditure required have mired conventional breeding efforts. Moreover, genetic sources of resistance are scarce and the incorporation of such resistance from the wild diploid Ipomoea spp. species into polyploid sweetpotato is a complicated task. Due to the difficulty of traditional breeding for virus resistance in sweetpotato, genetic engineering has been developed as an alternative tool to target on this agricultural issue since the mid-nineties. In 1986, the first pathogen-derived resistance (PDR) in transgenic tobacco plants had been successfully applied. Then genetic engineering has been established as a powerful tool to generate highly and specific virus-resistant crop varieties at a much faster speed compared to the traditional breeding methods. In sweetpotato, using expression of the coat protein (CP) mediated resistance to SPFMV was obtained, however, the durability of the resistance in the field has not been reported [16-21].

Conclusions

The solution to the problems caused by sweetpotato viruses is to ensure that growers plant virus-indexed clean propagation material or 'seed'. However, by estimate, there is a 72% shortfall of clean plant units needed to cover all sweetpotato acreage (NCPN Network News, May 2018). Thus, there is a huge demand for establishing certified nursery farms to produce clean "seed" in the sweetpotato industry. Besides, the high reinfection rate of viruses in production field requires the farmers frequently to purchase virus-indexed propagation material, which greatly increase their financial input for propagation and production. Using other biotechnological approaches, such as gene transformation or genome editing techniques, to produce virus resistant cultivars, propose another promising strategy to control the virus in sweetpotato.

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