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Research Article

Expression Analysis of Sweetpotato Sporamin Genes in Response to Infection with the Root-Knot Nematode *Meloidogyne incognita*

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Abstract Sweetpotato (*Ipomoea batatas* [L.]) is a globally important root crop cultivated for food and industrial processes. The crop is susceptible to the root-knot nematode (RKN) *Meloidogyne incognita*, a major plant-parasitic RKN that reduces the yield and quality of sweetpotato. Previous transcriptomic and proteomic analyses identified several genes that displayed differential expression patterns in susceptible and resistant cultivars in response to *M. incognita* infection. Among these, several sporamin genes were identified for RKN resilience. Sporamin is a storage protein primarily found in sweetpotato and morning glory *(Ipomoea nil)*. In this study, transcriptional analysis was employed to investigate the role of sporamin genes in the defense response of sweetpotato against RKN infection in three susceptible and three resistant cultivars. Twenty-three sporamin genes were identified in sweetpotato and classified as group A or group B sporamin genes based on comparisons with characterized sweetpotato and Japanese morning glory sporamins. Two group A sporamin genes showed significantly elevated levels of expression in resistant but not in susceptible cultivars. These results suggest that the elevated expression of specific sporamin genes may play a crucial role in protecting sweetpotato roots from RKN infection.

Keywords defense signaling, root-knot nematodes, sweetpotato, sporamin, transcriptome

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Introduction

Sweetpotato [*Ipomoea batatas* (L.) Lam)] is a globally important root crop, particularly in Asia and Africa (Afuape et al. 2014), with 91.8 million tons produced from a harvested area of 7.7 million hectares worldwide. Sweetpotato is an important food crop, providing valuable minerals and vitamins, and is also used as a raw material in the production of pigments, alcohols, processed foods, and animal feeds. Sweetpotato cultivation thus forms a significant component of sustainable agriculture (Diaz et al. 2014; Grace et al. 2014). Sweetpotato production is affected by a wide range of pathogenic virus and fungi, and is also subject to attack by parasitic nematodes (Palomares-Rius and Kikuchi 2013). Root-knot nematodes (RKNs), members of the genus *Meloidogyne*, pose a major threat to many agricultural crops, including sweetpotato (Castagnone-Sereno et al. 2013). *Meloidogyne incognita* is a destructive RKN that is the most globally widespread and abundant parasitic nematode species found in agricultural areas. *M. incognita* is able to establish sophisticated feeding sites within the soil near to plant vasculature and has a broad host range encompassing a variety of crop species (Jones et al. 2013). Sweetpotato is a highly susceptible host of *M. incognita*, and infection by *M. incognita* can cause severe damage to the root system and storage roots of developing sweetpotato crops (Bridge and Starr 2010).

Sporamin is the major storage protein in sweetpotato storage roots, accounting for 60-80% of the total soluble protein in the storage roots (Yeh et al. 1997a). Tissue-specific expression of sporamin genes is mainly observed in storage roots, with minimal or no expression in stems and leaves under normal conditions (Hattori et al. 1990). Sporamin proteins are encoded by various genes, classified into sporamin A and B groups according to their sequence similarities (Hattori et al. 1989). Recent studies showed that

expression of sporamin genes was induced by wounding, pathogen exposure, and nematode infection, as well as treatment with plant hormones and sugars (Cai et al. 2003; Senthilkumar and Yeh 2012). This suggests that sporamin expression is closely related to defense responses in sweetpotato storage roots. Supporting this, a protective role for sporamin against herbivorous damage was observed in transgenic tobacco overexpressing the sporamin gene *SpTI-1* against tobacco cutworm (*Spodoptera litura*) (Yeh et al. 1997b). Sporamin expression also correlates with protease inhibitor activity: large amounts of both trypsin and serine protease inhibitors were observed in sweetpotato storage roots (Yao et al. 2001; Yeh et al. 1997a). In sugar beet (*Beta vulgaris* L.), sporamin-mediated resistance to the beet cyst nematode (*Heterodera schachtii Schm.*) produced a sporamin-dependent increase in trypsin inhibitor activity in root hairs (Cai et al. 2003), suggesting a link between protease inhibitor and sporamin-related defense responses.

Our previous research reported the results of proteome and transcriptome analysis of susceptible and resistant *M. incognita*-infected roots in two sweetpotato cultivars (Ha et al. 2017; Lee et al. 2019). Recently, additional transcriptome analysis was performed using six sweetpotato cultivars showing susceptibility and resistance to *M. incognita* infection (Lee et al. 2021). This study identified several candidate genes that may contribute to protection against RKN infection. Among the various genes identified, specific expression changes of some sporamin genes were seen (Lee et al. 2021). However, the responses of sweetpotato sporamin genes to RKN infection have not been characterized in detail. In this study, transcriptome-based expression profile analysis of sporamin genes was conducted in resistant and susceptible sweetpotato cultivars during RKN infection.

Materials and Methods

Plant materials

Six sweetpotato cultivars (*Ipomoea batatas L. Lam*) were obtained from the Bioenergy Crop Research Center of the National Crop Research Institute (Rural Development Administration, Muan, Jeonnam). RKN-susceptible cultivars Dahomi, Shinhwangmi, and Yulmi, and RKN-resistant cultivars Danjami, Pungwonmi, and Juhwangmi, were used in both this study and the previous research (Lee et al. 2021). Sweetpotato plants were inoculated with *M. incognita* as described by Lee et al. (2021). Fifteen plants of each cultivar were planted in an autoclaved sterile sand:soil mixture (50:50) in perforated 500 ml clay pots. Plants were grown in a greenhouse maintained at 25-30°C. Two weeks after planting, approximately 3,000 *M. incognita* eggs were applied to the soil in each pot and covered with a layer of moist sand. Roots were collected four weeks after inoculation and the number of eggs was visually evaluated.

Sequence analysis

Identification and similarity of sequences were determined using NCBI BLAST multiple sequence alignments performed using the BioEdit program. Phylogenetic analysis was performed using the Maximum Likelihood method in the Molecular Evolutionary Genetics Analysis program (MEGA11) (Tamura et al. 2013).

Total RNA isolation and real-time PCR analysis

Total RNA was isolated from RKN-treated fibrous roots of sweetpotato samples using TRIzol reagent (Invitrogen) and treated with RNase-free DNaseI to remove genomic DNA contamination. Real-time reverse-transcriptase PCR analysis was performed using a Bio-Rad CFX96 thermal cycler (Bio-Rad) with EvaGreen fluorescent dye according to the manufacturer's instructions. Linear data were normalized to the average threshold cycle (Ct) of the ADP-RIBOSYLATION FACTOR (ARF) reference gene (Park et al. 2012). Gene-specific primers are listed in Table 1.

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA). The subsequent multiple comparisons were examined based on the least significant difference (LSD)

Table 1 Oligonucleotide primers used for qRT-PCR analysis

Transcript ID	Primer sequence (5'-3')		PCR product (bp)	Sporamin group
G13675|TU22356	Forward primer	CATCTGCCACCATGAAAGCC	189	A
	Reverse primer	CTATGTAGTAGTTCCCGCCGG		
G34382|TU56396	Forward primer	CCCCAACCCAACTCATTCCA	200	A
	Reverse primer	CGCATTCGTTCGAGGAGGAA		

and Duncan's multiple range test. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS 12), and statistical significance was set at $P < 0.05$.

Results

Identification of differentially expressed sporamin genes in pairwise sample comparisons

Transcriptome analysis was previously conducted on *M. incognita*-infected RKN-susceptible (Dahomi (DHM), Shinhwangmi (SHM), and Yulmi (YM)) and RKN-resistant (Danjami (DJM), Pungwonmi (PWM), and Juhwangmi (JHM)) sweetpotato cultivars. Transcriptome comparison during RKN infection identified several candidate genes that likely contributed to protection against RKN in sweetpotato (Lee et al. 2021). Sporamin genes, which encode sporamin storage proteins, were identified in the candidate gene set and were further investigated in this study. In total, 23 unique sporamin transcripts were identified as differentially expressed genes (DEGs) in pairwise sample comparisons among cultivars (Fig. 1A). Characterized sporamin gene sequences from sweetpotato and Japanese morning glory *(Ipomoea nil)* were used to classify the sporamin unigenes into two ortholog groups (Fig. 1B). The sporamin A group contained nine unigenes and the sporamin B group contained 14 unigenes. Susceptible cultivars (SCs) exhibited low sporamin expression levels, regardless of RKN infection.

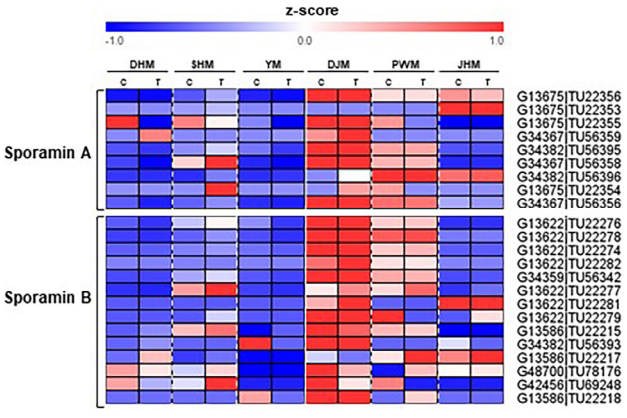

Fig. 1 Relative expression of sweetpotato sporamin unigenes in response to *M. incognita* infection. Samples were collected from susceptible (DHM, SHM, and YM) and resistant (DJM, PWM, and JHM) sweetpotato cultivars infected with *M. incognita* (treated; T), and uninfected controls (C). DHM, Dahomi; SHM, Shinhwangmi; YM, Yulmi; DJM, Danjami; PWM, Pungwonmi; JHM, Juhwangmi.

Among resistant cultivars (RCs), DJM exhibited high expression levels and JHM had low expression levels.

Expression of sweetpotato sporamin unigenes in response to RKN infection

To investigate RC-specific and SC-specific expression patterns, transcription of sweetpotato sporamin genes was examined in SCs (DHM, SHM, and YM) and RCs (DJM, PWM, and JHM) during RKN infection (Fig. 3). Of the 23 candidate sporamin genes, two genes (G13675|TU22356 and G34367|TU56356), both in the sporamin A group, exhibited higher expression in RCs than in SCs.

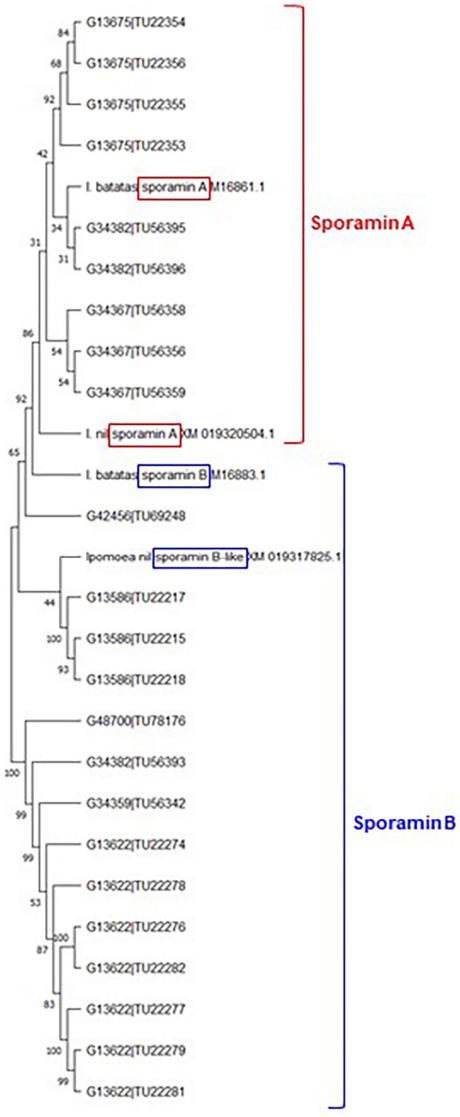

Fig. 2 Phylogenetic analysis of sweetpotato sporamin genes. Sporamin unigenes were assessed alongside characterized sporamin genes from sweetpotato (*Ipomoea batatas*), sporamin A (M16861.1), and sporamin B (M16883.1), and morning glory (*Ipomoea nill*), sporamin A (XM019320504.1), and sporamin B (XM019317825.1).

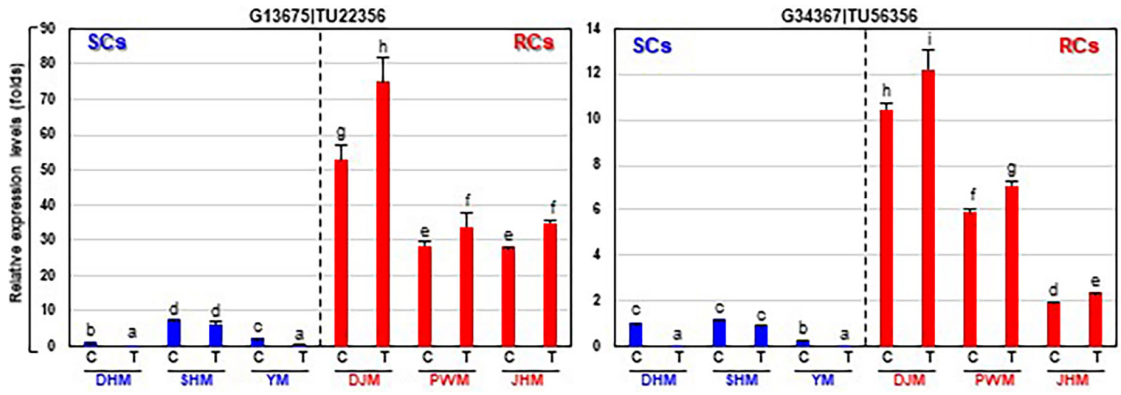

Fig. 3 Relative expression of two sporamin genes in sweetpotato cultivars in response to *M. incognita* infection. Expression of sporamin genes G13675|TU22356 and G34367|TU56356 in susceptible (SC) and resistant (RC) cultivars of plants infected with *M. incognita* (treated; T) and in uninfected controls (C). Data represent the average of three replicates from each of two experiments, and bars labeled with the same letter are not significantly different (*P > 0.05) according to Duncan's multiple range test. DHM, Dahomi; SHM, Shinhwangmi; YM, Yulmi; DJM, Danjami; PWM, Pungwonmi; JHM, Juhwangmi.

Transcriptional changes in two sweetpotato sporamin genes during RKN infection

Transcriptional changes of G13675|TU22356 and G34367|TU56356, which were elevated in RCs but not SCs during infection, were examined during infection progression in YM (susceptible) and JHM (resistant) cultivars using quantitative RT-PCR analysis (Fig. 4). In both infected plants and uninfected controls, both genes had higher expression levels in JHM than in YM until 4 weeks after infection, after which expression levels decreased to very low levels in both YM and JHM by 8 weeks post-infection. In JHM, in both treated and untreated groups, expression of both genes was highest 1 week after infection, dropping by about half by 4 weeks post-infection. Expression levels were similar between RKN-treated and untreated plants. In YM, expression of both genes was lower in RKN-treated plants than in untreated controls at both 1 week and 4 weeks after infection.

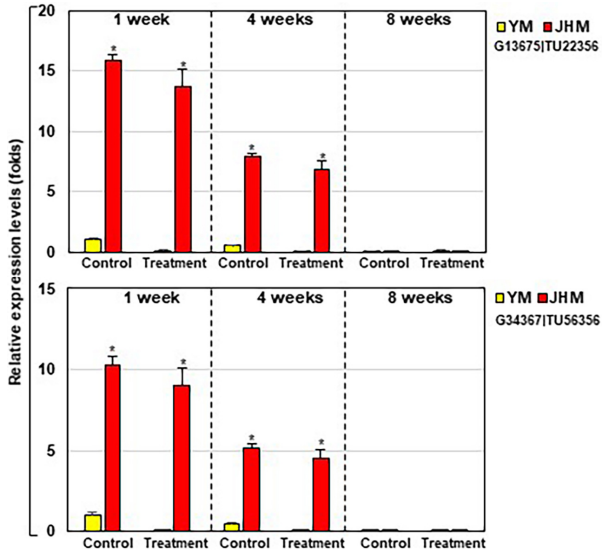

Fig. 4 Relative expression of two sweetpotato sporamin genes during the development of *M. incognita* infection. Time course analysis of sporamin genes G13675|TU22356 and G34367|TU56356 in susceptible (YM) and resistant (JHM) sweetpotato cultivars over 8 weeks in infected (treated; T) and control (C) plants. Data represent the average of three replicates from each of two experiments, and error bars indicate SD of the means. Statistical significance of the differences between the control and treatment groups was determined by one-way ANOVA with the LSD post hoc test (*P < 0.05). YM, Yulmi; JHM, Juhwangmi.

Discussion

The role of sporamin genes in resistance to infection with the RKN *M. incognita* in sweetpotato was examined in this study. Transcriptome data from the sweetpotato transcriptome database identified 23 candidate sporamin genes, and these were classified as sporamin group A and group B genes according to their similarities to characterized sweetpotato and Japanese morning glory *(Ipomoea nil)* sporamins. Expression levels of the 23 genes in susceptible (DHM, SHM, and YM) and resistant (DJM, PWM, and JHM) sweetpotato cultivars were assessed in control plants and plants infected with *M. incognita* (Fig. 1). Generally, RCs exhibited higher expression than SCs, and DJM exhibited particularly high sporamin gene expression (Fig. 1). Two of the group A sporamin genes, G13675|TU22356 and G34367|TU56356, had RC-specific expression patterns (Fig. 3). Of the RCs, gene expression increased in response to RKN infection in DJM, but not in PWM and JHM. Low expression levels were generally observed in

SCs, with expression in RKN-infected plants lower than in controls in DHM and YM. In an 8 week time course following infection, expression of the two genes decreased in JHM (representative RC) (Fig. 4). Previous transcriptome analysis revealed that few genes, regardless of cultivar susceptibility, exhibited higher expression levels in response to RKN infection. However, a large number of genes were identified that had substantially elevated expression in RCs compared with SCs, regardless of RKN infection (Lee et al. 2021). This suggests that many RC genes may be activated at high levels by constitutive defense responses. It can be inferred that the sporamin genes identified in this study may be constitutively activated and that maintaining high levels may provide a defense advantage during sweetpotato infection with RKN.

Tubers and storage root crops contain abundant storage proteins (Shewry 2003). Storage proteins such as patatin from potato, sporamin from sweetpotato, and dioscorin from yam display enzymatic activity in response to external pathogen infection. A study of purified enzymes from potato tubers revealed that patatin displayed enzymatic activity that catalyzed the deacylation of several lipid substrates (Galliard 1971). Subsequent studies demonstrated that the acyl hydrolase activity was due to patatin, which also acted as an esterase (Racusen 1986). The specificity of acyl hydrolases was later studied in more detail (Anderson et al. 2002), especially their activity as phospholipases for phospholipid and lysophospholipid substrates (Hirschberg et al. 2001; Senda et al. 1996). Another type of hydrolytic activity for patatin, as acidic β-1,3-glucanase (Tonon et al. 2001), was recently described. β-1,3-glucanases are thought to contribute to plant defense against fungal pathogens by digesting β-1,3-glycans in the hyphal cell wall, forming part of a pathogenesis-relevant (PR) protein response (Van Loon and van Strien 1999), suggesting that patatin might play a role in defending potato tubers. Sporamin from sweetpotato also displayed enzymatic activity, catalyzing the activation of trypsin inhibitor (Yeh et al. 1997a). Hou and Lin (1997) reported that sporamin also possessed antioxidant activity through acting as a dehydroascorbate reductase and as a monodehydroascorbate reductase associated with intermolecular thiol/disulfide exchange. Sporamin can also scavenge both 1,1-diphenyl-2 picrylhydrazl radicals and hydroxyl radicals (Hou et al. 2001). The biological significance of these observations is not clear. An in vivo role in regulating protease activity is suggested by the observation that sporamin inhibits endogenous serine proteinases in sweetpotato storage roots (Hou and Lin 2002). In particular, a role for sporamin in the resistance response, which protects plants from damage by herbivores and parasitic nematodes, was suggested by an increase in trypsin inhibitor activity when sporamin was overexpressed during attack by tobacco cutworm larvae (*Spodoptera litura*) and beet cyst nematode (*Heterodera schachtii Schm.*) (Cai et al. 2003; Yeh et al. 1997b). The sporamins showing RC-specific expression in this study may play a role in the inhibition of RKN infection through the constitutive defense response in resistant cultivars.

This study describes the first transcriptome-based analysis of the sporamin gene family in sweetpotato, a genetically complex and agronomically important food crop. Sporamin genes were identified in sweetpotato and their expression profiles were compared between RKN-resistant and -susceptible sweetpotato cultivars. These results increase our understanding of the role of sporamin in plant defense, particularly in response to RKN infection.

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