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ORIGINAL ARTICLE

Comparative proteomic analysis of the response of fibrous roots of nematode-resistant and -sensitive sweet potato cultivars to root-knot nematode *Meloidogyne incognita*

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Abstract As a major root-knot nematode (RKN), *Meloidogyne incognita* causes serious losses in the yield of sweet potato (*Ipomoea batatas* L.). To successfully colonize the host plant, RKNs elicit changes of dramatic physiological and morphological features in the plants. The expression of several genes is regulated as the nematode establishes its feeding site. Therefore, in this study, we analyzed the proteomes in the fibrous roots of sweet potato plants by an infection of RKN to understand the effect of the infection on the plant root regions. This study revealed differences in proteomes of the RKN-resistant sweet potato cultivar Juhwangmi and RKN-sensitive cultivar Yulmi. During plant growth, Juhwangmi plants were shown to be more resistant to *M. incognita* than Yulmi plants. No *M. incognita* egg formation was observed in Juhwangmi plants, whereas 587 egg masses were formed in Yulmi plants. Differentially expressed 64 spots were confirmed by proteomic analysis using 2-D gel electrophoresis with three spots up-regulated in the two cultivars during RKN infection. Of these 64 protein spots, 20 were identified as belonging to such different functional categories as the defense response, cell structure, and energy metabolism. This study provides insight into the molecular and biochemical mechanics of the defense response and metabolism of sweet potato plant during nematode invasion. We anticipate that this study will also provide a molecular basis for useful crop breeding and the development of nematode-tolerant plants.

Keywords Juhwangmi · Proteomics · Root-knot nematode · Sweet potato · Yulmi

Introduction

Plant-parasitic nematodes (PPNs) cause an estimated average crop yield loss of 10.7% in life-sustaining crops and 14% in economically important crop plants (Palomares-Rius and Kikuchi 2013). These losses are usually

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estimated to exceed 100 billion US dollars. Among PPNs, root-knot nematodes (RKNs; *Meloidogyne* spp.) are sessile internal parasites. The most economically significant species are *M. hapla* in cool climates and *M. arenaria*, *M. javanica*, and *M. incognita*. These are considered important RKNs in the major crop plants and can be easily found by the galls or knots in the root regions (Bird 1996; Caillaud et al. 2008). These RKNs usually cause dramatic changes of physiological and morphological features in the plants. In the RKN-infected plan cells, expressions of some genes are repressed by RKNs to establish feeding sites, whereas expression of nematode genes was induced during infection (Williamson and Gleason 2003). They also showed the immune response to nematodes (Goverse and Smart 2014).

Sweet potato (*Ipomoea batatas* L.) is one of the important food crop plants, especially in parts of Asia and Africa. It serves as a nutrient source for humans, providing energy, fiber, and antioxidants, including carotenoids and anthocyanin, and can be used to produce industrial raw materials for animal feed, starch, and alcohol (Diaz et al. 2014; Grace et al. 2014). The major damage agents of sweet potato are fungal and viral diseases (Clark and Moyer 1998; Kreuze 2002). The PPNs can also decrease its productivity up to 10.2–11.4% (Kistner et al. 1993; Palomares-Rius and Kikuchi 2013). The main PPNs affecting sweet potato are RKNs: *M. incognita* Chitwood, *M. javanica* Chitwood, *M. arenaria* Chitwood and *M. hapla* Chitwood (Kistner et al. 1993; Agu 2004). Sweet potato is a highly suitable host especially for the southern RKN, thereby *M. incognita* causes severe damage to the storage roots and occurs worldwide in the tropics (Bridge and Starr 2010).

The molecular genetics and physiological mechanisms of resistance of sweet potato to RKNs are poorly understood. Previous studies indicate a large number of RKN resistance-related genes in sweet potato, suggesting that RKN resistance might be inherited in the form of multiple molecular genetic factors (Jones and Dukes 1980). Cervantes-Flores et al. (2002a, b) evaluated five sweet potato cultivars with respect to resistance to different RKN species. They found that the resistance response of each cultivar differed depending on the nematode species; therefore, different genes may be involved in the sweet potato resistance to RKNs.

The aim of this research was to study the isolation and molecular analysis of RKN resistance proteins of sweet potato during *M. incognita* infestation using proteomic analysis. The study analyzes the resistance and proteomes of RKN-sensitive and -resistant cultivars during an infection with RKN *M. incognita* Chitwood 1949, to determine the role of RKN-resistant molecular mechanisms in sweet potato plants. The results presented herein enhance the understanding of plant defense mechanisms against RKN infestation in RKN-sensitive and -resistant sweet potato cultivars.

Materials and methods

Plant materials

Two sweet potato (*Ipomoea batatas L. Lam*) cultivars used in this study, the most RKN-sensitive cultivar Yulmi and the most RKN-resistant cultivar Juhwangmi, were obtained from Bioenergy Crop Research Center, National Crop Research Institute (RDA, Muan, Jeonnam, Korea).

Plant treatment with *M. incognita*

Sweet potato plants were cultivated and treated with *M. incognita* according to a method of Lee et al. (2012). The Yulmi and Juhwangmi cultivar cuttings (approximately 10 cm long) with three leaves attached were transplanted into plastic pots (24 cm width, 18 cm length, and 12 cm height) filled with sandy loam soil infested with *M. incognita* at a density of 1154 ± 176 s-stage juveniles per 300 g of soil. *M. incognita* infested soil was collected from natural occurrence greenhouse in experimental farm of Gyeongsang National University. As a control, the soil was steam-sterilized at 115 °C and 1.5 atmospheric pressure for 20 min. For treatments under growth chamber conditions, the sweet potato cuttings were grown in a growth chamber conditions (16 h photoperiod, 30/22 °C day/night temperature, and 70% relative humidity) with light (intensity: 200 µmol s^{-1} m^{-2}). The plants were harvested 50 days after planting and washed in tap water, and plant fresh weights and RKN egg masses were then determined. The plants were cultivated in ten replicates.

Analysis of plant RKN resistance

The egg masses formed by *M. incognita* in the fibrous roots of the sweet potato plants were dyed with a Phloxin B solution and counted (Viaene et al. 2012). The design of the experiment was completely randomized, with ten replicates.

Two-dimensional electrophoresis

Total protein was isolated from the fibrous roots of each sweet potato plant using a modified phenol-based method (Hajduch et al. 2005). Two-dimensional SDS-PAGE (2-DE) and isoelectric focusing (IEF) and were performed as described previously (Lee et al. 2012). Total protein extract was separated on Bio-Rad 17 cm immobilized pH gradient gel (IPG) strips (pH 5–8). After IEF, the IPG strips were equilibrated according to the manufacturer's protocol (Bio-Rad, Hercules, CA, USA). SDS-PAGE was performed using PROTEAN II xi Cell (Bio-Rad). The 2-DE gels were stained with colloidal Coomassie brilliant blue (CBB). At least ten independent proteins were isolated from different infected

fibrous root samples and used in 2-DE analysis. After electrophoresis, gel images were analyzed using a PDQuest software (Version 7.2.0; Bio-Rad) and GS-800 Calibrated Imaging Densitometer (Bio-Rad).

MALDI-TOF/TOF MS analysis

The spots were excised from the CBB-stained gels and subjected to reduction, alkylation, and in-gel digestion, as described previously (Kwon et al. 2016). The analyses were carried out using an ABI 4800 Plus TOF–TOF Mass Spectrometer (Applied Biosystems, Framingham, MA, USA). Spectral data (MS and MS/MS) were unpacked using the NCBI (https://www.ncbi.nlm.nih.gov/), Protein Pilot V.3.0 and UniProt database (version 20131104; 30,938,908 sequences), at 100 ppm mass tolerance. Database search criteria with the MS/MS spectra were as follows: a single missing peak, carbamidomethylation of cysteines, and oxidation of methionines.

Gene expression analysis

Quantitative real-time PCR analysis was investigated using a Bio-Rad CFX96 thermal cycler (Bio-Rad, USA) with EvaGreen fluorescent dye according to the manufacturer's instructions. The transcriptional expression levels were analyzed by quantitative RT-PCR using gene-specific primers (Table 1).

Results

Differential RKN resistance of the two sweet potato cultivars

According to the reports of Bioenergy Crop Research Center, Yulmi is the most RKN-sensitive sweet potato cultivar, whereas Juhwangmi is the most RKN-resistant cultivar (Choi et al. 2006). Therefore, we first tested and compared the resistance of Juhwangmi and Yulmi cultivars to *M. incognita*. When the plants were grown in the growth chambers in the soil infested with *M. incognita*, the shoot and root growth of Yulmi plants were inhibited by 38.7 and 34.8%, respectively, compared with uninfested conditions, whereas the shoot and root growth of Juhwangmi plants were not inhibited by RKN treatment (Fig. 1a, b). *M. incognita* formed 587 infested egg masses in Yulmi plants, while no egg formation was observed in Juhwangmi plants (Fig. 1c, d). Therefore, consistent with previous reports, our results demonstrated that, in comparison with the Yulmi cultivar, the Juhwangmi cultivar was highly resistant to *M. incognita* under growth chamber conditions.

2-DE analysis of the sweet potato fibrous root proteomes in response to RKNs

Total protein isolated from the fibrous roots of Yulmi and Juhwangmi cultivars was electrophoresed and compared by 2-DE (Fig. 2). The pIs ranges of the spots are pH 5–8, and the molecular masses of most spots are 14.4–116.0 kDa. To evaluate expressed protein patterns, the spots in all replicate gels were quantitatively compared using PDQuest software. Although the general protein spot patterns of fibrous root remained largely unchanged in the two cultivars, 64 spots showed significant differences in expression in Yulmi and/or Juhwangmi plants under control and *M. incognita* infection conditions. Of these 64 protein spots, the intensity of 48 protein spots in Yulmi samples (red arrows; spots 1, 2, 3, 5, 6, 8, 9–20, 25, 26, 28–44, 46, 47, and 49–57) was specifically increased upon exposure to RKNs compared with control conditions; the intensity of four spots (green arrows; spots 27, 48, 58, and 59) decreased during RKN infection (Fig. 2 and Table 1). The intensity of five spots specific for Juhwangmi (black arrows; spots 4, 7, 21, 22, and 25) also increased after RKN exposure, compared with control conditions. Interestingly, three spots (sky blue arrows; spots 23, 24, and 64) were up-regulated in both cultivars during *M. incognita* infection.

Identification of proteins involved in the response of the sweet potato fibrous roots to RKN infection and their functional categorization

The excised protein spots were analyzed by MALDI-TOF/TOF MS. Unfortunately, 44 out of the 64 spots remained unidentified, with 20 protein spots identified (Table 1). Twelve protein spots were identified in Yulmi samples, including cell division cycle protein (spot 1), heat shock protein 70 (HSP70; spots 2, 3, 5, and 6), alpha-tubulin (spot 10), ascorbate peroxidase (APX; spot 25), sporamin A precursor (spot 27), glycine-rich RNA-binding protein 2 (spot 46), lysosomal alpha-mannosidase (spot 48), mitochondrial ATP synthase (spot 51), and actin 4 (spot 54). Two HSP70 proteins (spots 4 and 7) were identified in Juhwangmi samples, whereas GTP-binding protein Ran 3 (spot 23) was identified in both cultivars. Five nematode proteins were also identified, including enolase (spots 12 and 53), glutathione S-transferase (GST; spot 28), and peroxiredoxins (Prxs; spots 30 and 33).

Using AgriGO software (Du et al. 2010), identified 15 sweet potato proteins were analyzed to Gene Ontology (GO) terms for description and annotation of their predictive biological functions. In the biological process category, GO overrepresentation analysis showed that GO terms "cellular process", "metabolic process", and "response to stimulus" in the domain "biological process" were significantly

Table 1 Differentially expressed proteins in the root-knot nematode-resistant and -sensitive sweet potato cultivar Yulmi and Juhwangmi plants, identified by MALDI-TOF MS analysis after comparing control and nematode infestation conditions

Spot no[a]	Protein name	NCBI accession no.	Organism	Mr[b]/pI[c]		Score	SC[d] (%)	Abundance ratio[e]			
				Theoretical	Observed			CY	TY	CJ	TJ
1	Cell division cycle protein 48 homolog	XP_006344438	*Solanum tuberosum*	91.5/5.23	99.1/6.11	178	21	1.0	5.9	nd	nd
2	Heat shock protein 70	AFU34350	*Ipomoea batatas*	71.7/5.11	93.9/5.75	46	7	nd	5.6	nd	nd
3	Heat shock protein 70	AFU34350	*Ipomoea batatas*	71.7/5.11	92.9/5.7	77	10	nd	10.8	nd	nd
4	Heat shock protein 70	XP_006649853	*Oryza brachyantha*	71.7/5.23	90.8/5.78	146	17	nd	nd	nd	62.8
5	Heat shock protein 70	AFU34350	*Ipomoea batatas*	71.7/5.11	89.1/5.91	84	7	7.5	40.6	nd	Nd
6	Heat shock protein 70	AFU34350	*Ipomoea batatas*	71.7/5.11	90.6/5.83	52	5	nd	7.4	nd	nd
7	Heat shock protein 70	AFU34350	*Ipomoea batatas*	71.7/5.11	89.2/5.61	110	31	nd	nd	77.1	83.7
10	Alpha-tubulin	NP_001237301	*Glycine max*	28.3/4.86	60.2/5.71	156	32	nd	6.1	nd	nd
12	Enolase	XP_003136500	*Loa loa*	47.9/6.77	49.8/6.64	98	15	1.5	nd	nd	nd
23	GTP-binding protein Ran3	ACG34606	*Zea mays*	25.5/6.38	28.9/7.22	148	31	nd	nd	85	166.4
25	Ascorbate peroxidase	AGT80152	*Ipomoea batatas*	27.7/5.32	27.9/6.3	118	25	4.5	11.7	nd	nd
27	Sporamin A	P14715	*Ipomoea batatas*	24.2/5.81	27.1/5.16	108	47	11.7	4.8	nd	nd
28	Glutathione S-transferase-1	ABN64198	*Meloidogyne incognita*	23.7/6.77	25.4/7.8	413	63	nd	17.8	nd	nd
30	Peroxiredoxin	ACZ67203	*Meloidogyne incognita*	22.3/6.74	24.3/7.23	462	56	nd	33.4	nd	nd
33	Peroxiredoxin	ACZ67203	*Meloidogyne incognita*	22.3/6.74	21.9/7.21	110	21	nd	29.8	nd	nd
46	Glycine-rich RNA-binding protein 2	AAC50020	*Nicotiana glutinosa*	15.7/5.56	15.4/6.15	127	30	nd	170.5	nd	nd
48	Lysosomal alpha-mannosidase	XP_002276092	*Vitis vinifera*	115.8/5.62	88.6/6.42	236	13	17.1	6.9	nd	nd
51	Mitochondrial ATP synthase	XP_006344203	*Solanum tuberosum*	59.6/5.65	63.01/5.62	197	38	nd	21.5	nd	nd
53	Enolase	ERG79934	*C. elegans*	46.7/5.56	50.1/6.73	76	12	1.5	18.4	nd	nd
54	Actin 4	ABY19575	*Ipomoea batatas*	41.8/5.38	45.9/5.8	417	42	nd	33.8	nd	nd

CY control Yulmi, *TY* treatment Yulmi, *CJ* control Juhwangmi, *TJ* treatment Juhwangmi, *nd* not detected

[a]Numbers correspond to those in 2-DE gels in Fig. 1

[b]Molecular weight

[c]Isoelectric point

[d]Sequence coverage

[e]The abundance ratios of each spot were measured using a densitometer (Bio-Rad)and then compared to control Yulmi (CY) of spot 1 and the other identified spots

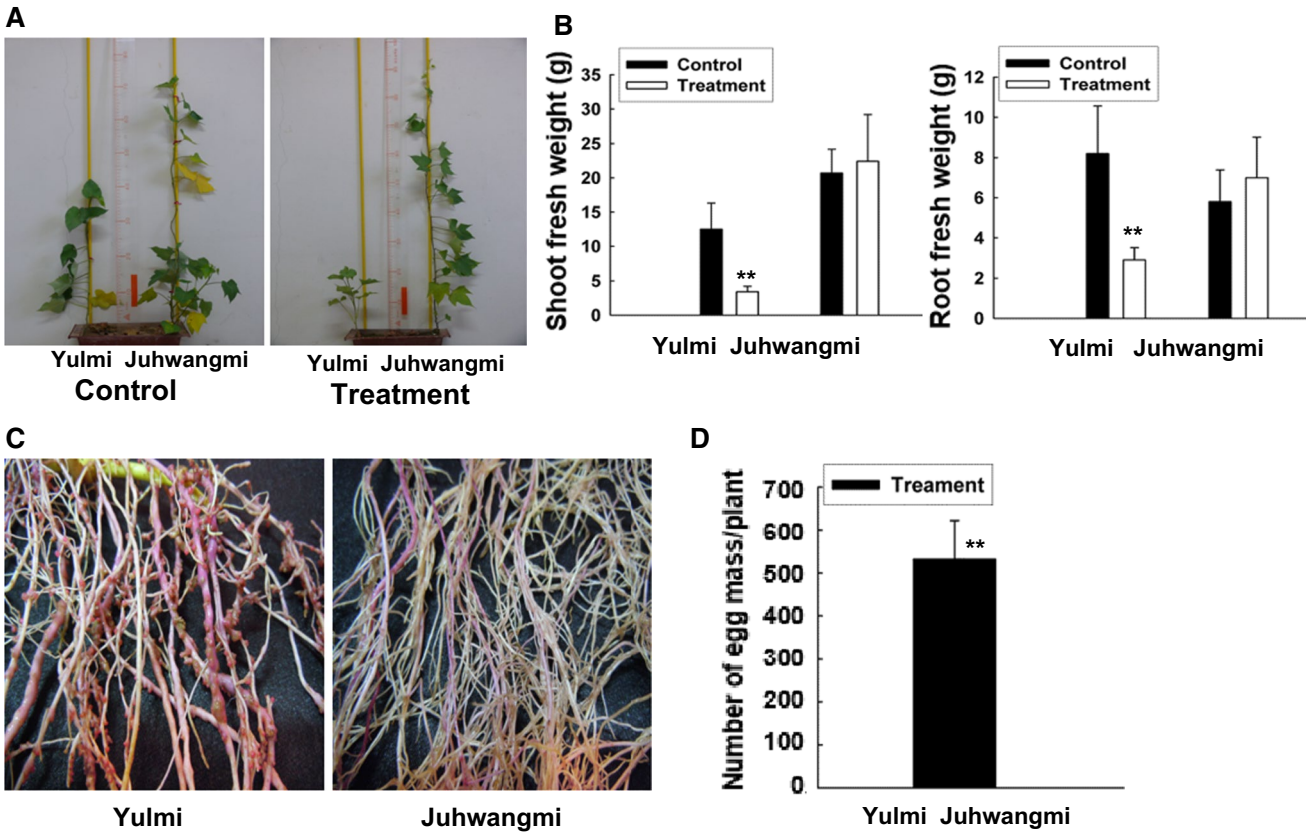

Fig. 1 The effect of the root-knot nematode *M. incognita* on the growth of Yulmi and Juhwangmi sweet potato plants conducted in growth chambers. **a** Growth inhibition of Yulmi and Juhwangmi cultivars by *M. incognita*. The plants were photographed 50 days after planting the cuttings in steam-sterilized sand (control) and non-sterilized sand (treatment) containing 1154 ± 176 *M. incognita* second-stage juveniles per 300 g of soil. **b** Fresh weight of shoots and roots. The fresh weights (g) were determined 50 days after the cuttings had been planted in steam-sterilized sand and non-sterilized sand. **c** Egg masses formed by *M. incognita*. Egg masses were photographed 50 days after the cuttings had been planted in non-sterilized sand containing *M. incognita*. **d** The quantification of egg masses formed by *M. incognita*. Egg masses were counted 50 days after the cuttings had been planted in non-sterilized sand containing *M. incognita*. Egg masses were not formed in the sweet potato cultivars cultured in sterilized soil. Data represent mean ± SD of ten replicates. The experimental results are the average of ten independent plants and four different biological replications

overrepresented among identified sweet potato proteins (Fig. 3 and Table 1). Other categories, such as "cellular component organization", "biological regulation", "multicellular organismal process", "multi-organism process" and "developmental process" were also represented among proteins sets, suggesting involvement of the corresponding biological functions in the general response of sweet potato to nematode infection.

Quantitative analysis of identified proteins involved in the response of the sweet potato fibrous roots to RKNs

To investigate the molecular change that occurs under RKN-infected conditions in the fibrous roots of sweet potato, the identified sweet potato 15 proteins were quantitatively analyzed (Fig. 4). The expression patterns of three protein spots, such as spot 1, spot 5, and spot 25, were increased in Yulmi under RKN infection compared with control conditions by 5.9, 5.4 and 2.6 folds, respectively, whereas spot 23 were increased in Juhwangmi by RKN infection compared with control conditions by 1.9-fold (Fig. 4a). However, 27 and 48 protein spots showed decreased expression patterns during RKN treatment in Yulmi only. The other protein spots showed induced expression patterns during RKN infection in Yulmi (spots 2, 3, 6, 10, 46, 51 and 54) or Juhwangmi (spot 4) (Fig. 4b). The protein spots were not detected in both cultivars during control conditions.

We also attempted to determine whether RKN infection results in a change of nematode proteins in fibrous roots of Yulmi, thus identified nematode five proteins were quantitatively analyzed (Fig. 5). The relative quantities of these RKN proteins were increased only during *M. incognita* infection in the fibrous root of Yulmi.

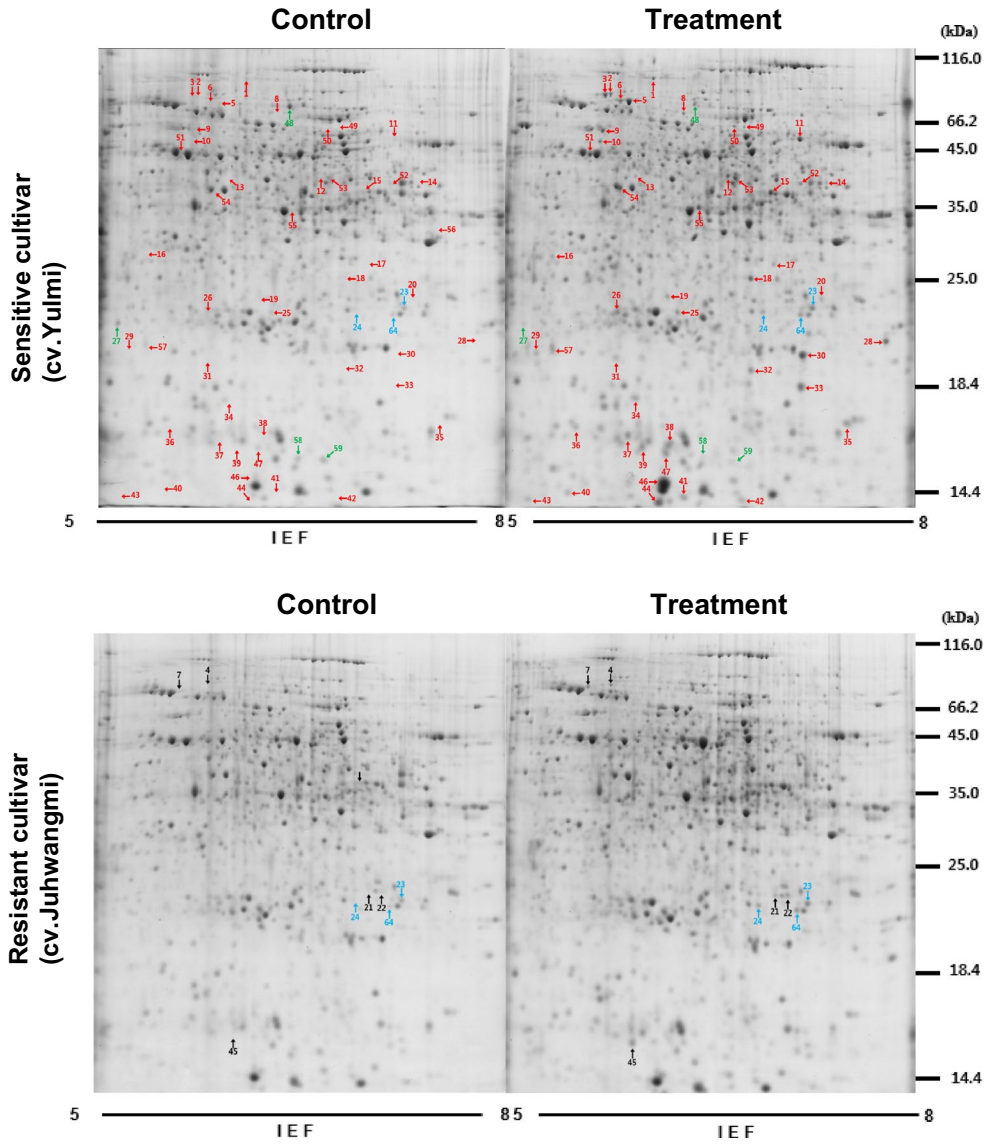

Fig. 2 High-resolution 2-DE of total protein extracted from fibrous roots of the RKN-sensitive Yulmi and RKN-resistant Juhwangmi sweet potato cultivars. The proteins were first separated on IPG strips (pH 5–8), followed by SDS-PAGE on 11.5% polyacrylamide gels. Gels were stained with colloidal CBB. Spot numbers indicate proteins that were differentially expressed in Yulmi or Juhwangmi plants. Red arrows indicate Yulmi-specific spots up-regulated during RKN infection; green arrows indicate Yulmi-specific spots up-regulated during RKN infection; black arrows indicate Juhwangmi-specific spots up-regulated during RKN infection; sky blue arrows indicate spots up-regulated in both cultivars during RKN infection. 10 gels per a biological replicate are assessed

Correlation between protein and transcript abundance in the sweet potato fibrous root during RKN treatment

To determine whether the expression levels of identified proteins correlated with the abundance of their transcriptional expression levels, we investigated quantitative real-time PCR analysis using gene-specific primers for several genes encoding proteins in the sweet potato fibrous root during RKN treatment (Fig. 6). Our results showed that consistent with proteomic data, transcript levels of HSP 70 (spot 2), APX (spot 25), and Actin (spot 54) up-regulated in fibrous roots of Yulmi during RKN infections.

Discussion

Despite considerable efforts invested in the investigation of the physiological responses of sweet potato to RKN infection, the biochemical and molecular mechanisms involved remain incompletely understood. In the present study, we

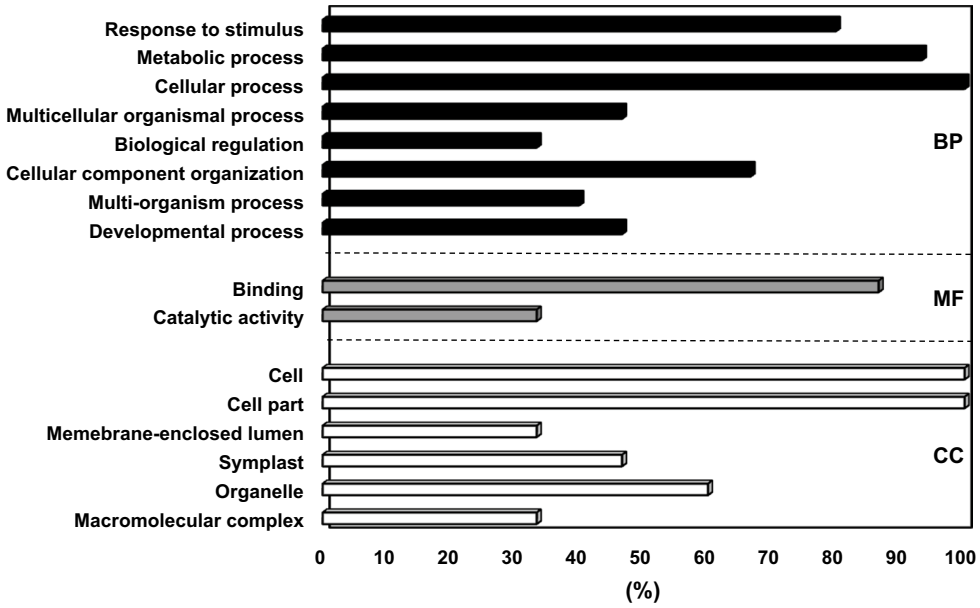

Fig. 3 Number of proteins (%) in the overrepresented GO categories of Yulmi and Juhwangmi sweet potato cultivars. The GO subcategories including biological process, molecular function, and cellular component are shown. *BP* biological process, *CC* cellular component, MF molecular function

evaluated the differences in fibrous root proteomic profiles of RKN-resistant and -sensitive sweet potato cultivars. The expression of numerous proteins was altered in the fibrous roots of each cultivar when *M. incognita* infected and fed on these sweet potato organs (Fig. 2). *M. incognita* not only triggers a defense response in the sweet potato root tissue but also redesigns the morphological features on the root region to form a gall and converts sweet potato cells into giant cells (GCs) for feeding (Fig. 1).

Differentially up-regulated proteins involved in the cell cycle and structure-related metabolism were detected in the fibrous roots of Yulmi plants (Fig. 3 and Table 1). Regulation of the cell cycle is of pivotal importance to plant growth and development, also during RKN infection (Inze and De Veylder 2006). The proteomic results in this study show that the response of the cell division cycle protein correlates with increased plant cell division that appears at the infection site and is caused by *M. incognita* infection and feeding (Fig. 1 and Table 1). Cells selected by *M. incognita* for feeding become multinucleate GCs. Engler et al. (1999) reported cell cycle activation in GCs. Ibrahim et al. (2011) also reported that the expression levels of cyclin in GCs are increased compared with other cyclin-dependent kinases by *Meloidogyne* infection. Our proteomic result exhibit changed expression of proteins involved in the cellular and cytoskeleton structure. We found that the expression of alpha-tubulin and actin proteins is increased in the fibrous roots of Yulmi sweet potato plants after *M. incognita* infection (Fig. 3 and Table 1). Therefore, we suggest that the up-regulation of the cell division cycle proteins, alpha-tubulin, and actin in RKN-infected sweet potato fibrous roots coincides with the cellular division and rearrangements of the cytoskeleton that appear during GC generation.

When a nematode invades a plant root, it must control or repress the plant defense response to successfully establish a constant feeding site (Bird 1996; Williamson and Gleason 2003). Our proteomic analysis data revealed changes in the protein expressions associated with response of the defense mechanism. Stress-responsive proteins induced in Yulmi or Juhwangmi plants after RKN infection. The number of RKN-induced proteins involved in defense responses was higher in Yulmi fibrous roots than in Juhwangmi fibrous roots (Fig. 3 and Table 1). Yulmi-specific RKN-responsive proteins included those encoding putative HSP70 proteins (spots 2, 3, 5, and 6), APX (spot 25), and sporamin A proteins (spot 27). Juhwangmi-specific RKN-responsive proteins were two HSP70 proteins (spots 4 and 7). It is known that HSPs are molecular chaperones responsible for protein folding, degradation, and translocation during cellular processes, and stabilizing cell membranes (Wang et al. 2004). In plants, expression analyses of spinach and *Arabidopsis* Hsp70 genes revealed that many Hsp70 s are responded to stress conditions, such as drought, cold and heat (Sung et al. 2001). However, the cellular functions of Hsp70 during nematode infestation are not fully understood. Interestingly, accumulation of some nematode HSP proteins was also reported during nematode infection (Him et al. 2009). Therefore, we suggest that HSPs likely play a defensive role

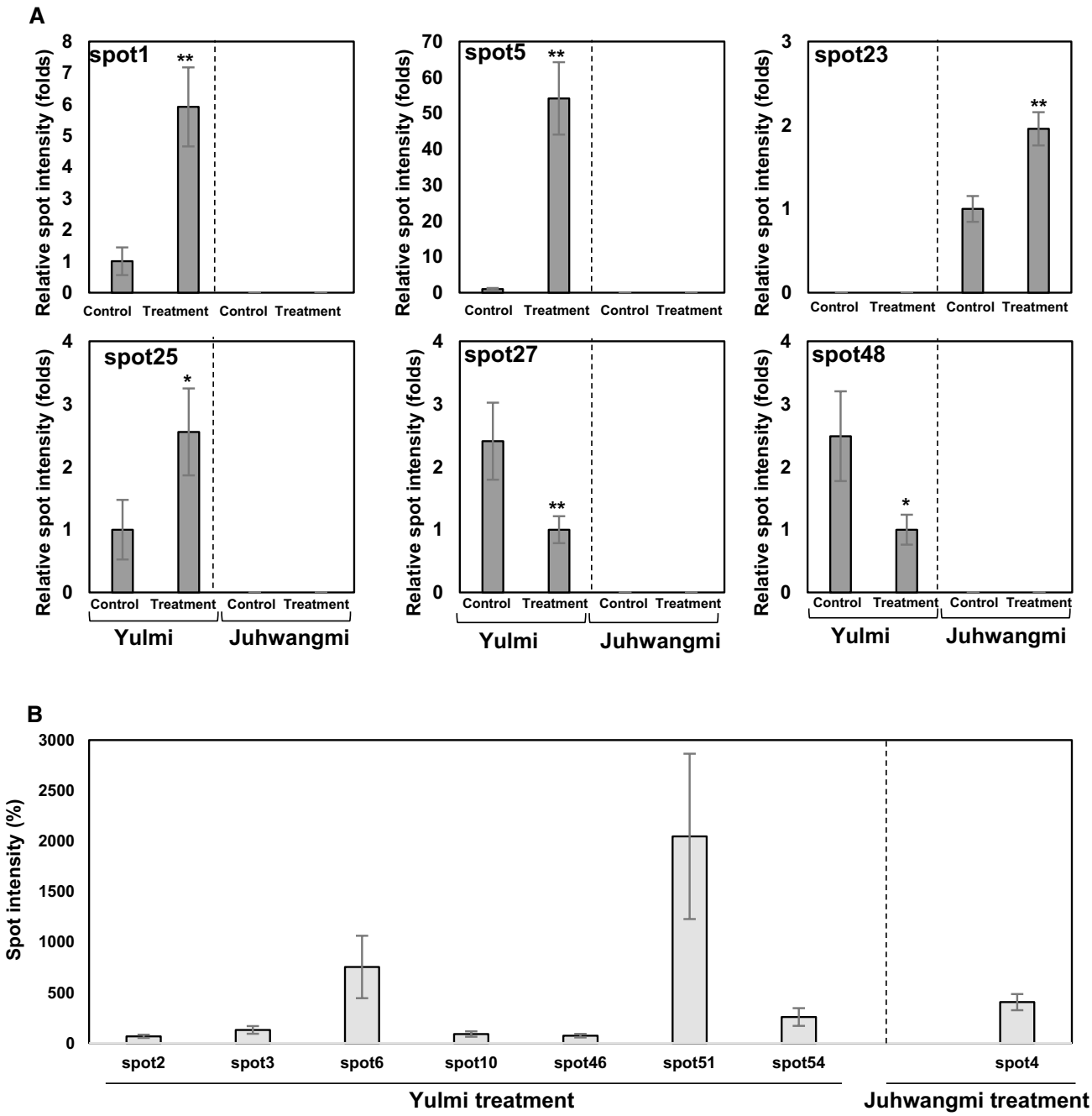

Fig. 4 Comparison of the expression levels of the identified sweet potato proteins from the fibrous roots of Yulmi and Juhwangmi cultivars in response to RKN infection. **a** Changes of relative spot intensity of six identified proteins under RKN treatments. **b** Spot intensities of induced eight identified proteins under RKN treatments in Yulmi or Juhwangmi. Data presented represent the average of five replicates. Statistical significance of differences between the control and treatment groups were determined by one-way ANOVA with LSD post hoc test ($P < 0.05$ and $P < 0.01$)

in the interaction of plant and RKN, affecting the plant's defense response and/or acting as protective molecules, e.g., chaperones. Sporamins, encoded by a multigene family, are the major storage glycoproteins of the sweet potato storage roots. They perform important functions, e.g., as serine protease inhibitors with a trypsin-inhibiting activity (Yeh et al. 1997). Proteinase inhibitors constitute important plant defense strategies and act as antifeedants against nematodes (Böckenhoff and Grundler 1994). Cai et al. (2003) reported that the trypsin-inhibiting activity is a significant factor that hampers the generation of cyst nematodes in the sporamin expressing hairy roots in the sugar beet. The expression

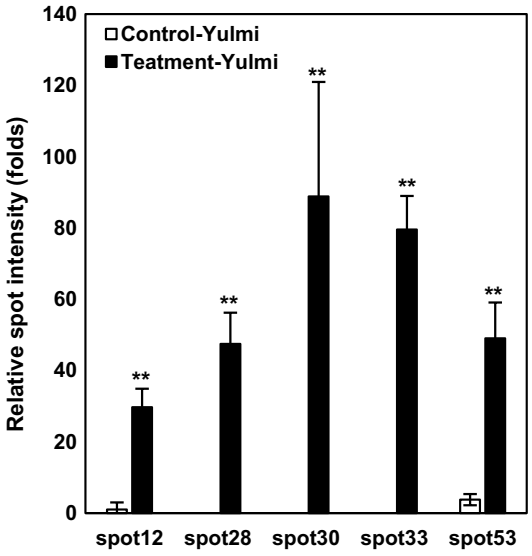

Fig. 5 Comparison of the expression levels of nematode proteins identified in the fibrous roots of Yulmi cultivar plants infected with RKN. Data presented represent the average of five replicates. Statistical significance of differences between the control and treatment groups were determined by one-way ANOVA with LSD post hoc test ($P < 0.05$ and $P < 0.01$)

of sporamin proteins reduced in Yulmi plant roots during RKN infection detected in this study adds to the growing body of evidence corroborating the biological function of these sporamins in nematode resistance. APXs are intracellular enzymes, with high affinity for ascorbate, an electron donor in the hydrogen peroxide (H_2O_2)-reducing reaction (Chen and Asada 1989). Recent studies have focused on the changes in gene expression and activity of APXs subjected to various stress conditions including pathogen infection in plants (Shigeoka et al. 2002). Simonetti et al. (2010) reported changes in APX activity in wheat roots in response to a nematode (*Heterodera avenae*) attack. Cytosolic APX isozymes were induced in the roots of both lines in response to nematode infection. In this study, APX protein was up-regulated in the RKN-infected Yulmi cultivar plants. Therefore, our results suggested that in the sweet potato fibrous roots, infected with RKN, a rise in APX accumulation appears, probably a ROS-regulating response triggered by the increasing presence of H_2O_2 and similar to that activated under abiotic stress conditions (Table 2).

The establishment of GCs, together with hypertrophy and hyperplasia of the root cortex cells that lead to the root-knots formation, is manifested by extensive expressional changes, and could be discerned in the differential protein expressions of in the roots of the nematode-sensitive Yulmi cultivar. A coordinated expression of several identified proteins occurs to orchestrate the manipulation and regulation of fundamental features of development in the plant cells during compatible host–nematode interactions (Jammes et al. 2005). The notion that regulation of the plant–nematode interaction contributes to nematode-induced stress-responsive proteins was confirmed by data from the fibrous roots of RKN-susceptible Yulmi plants (Table 1 and Fig. 4). GST (spot 28) and Prxs (spots 30 and 33) were highly expressed in these roots. In GCs at the feeding site, the endophytic parasitic nematodes must adapt to the host defense system and environment conditions. Melillo et al. (2006) reported that oxidative burst appears in a compatible interaction between *M. incognita* and tomato, in plant cells surrounding the

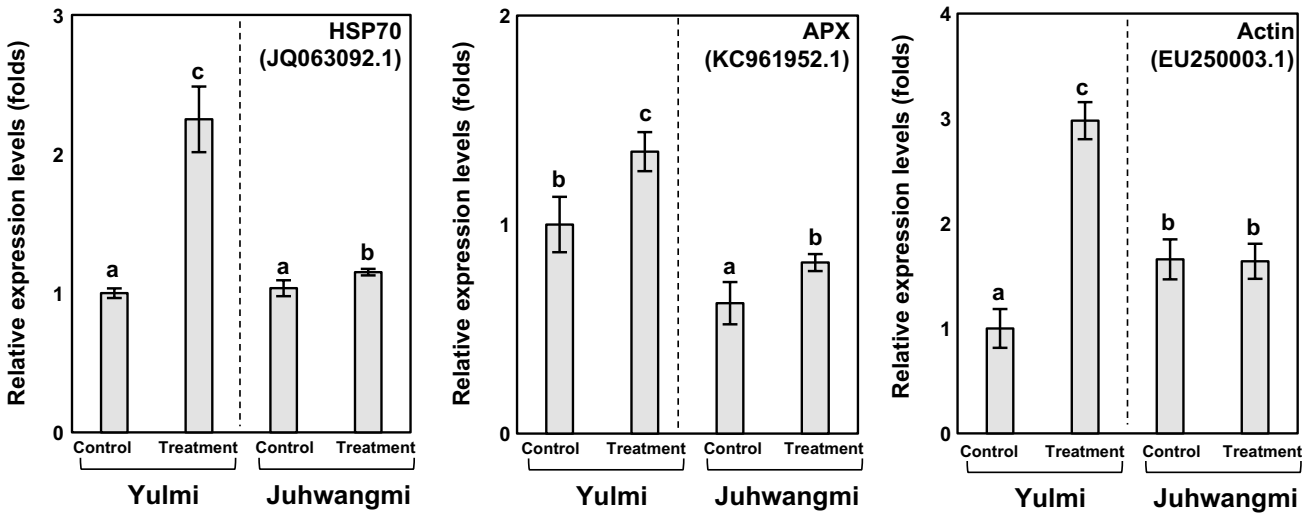

Fig. 6 Quantitative real-time PCR of genes encoding selected proteins that were responded in the fibrous roots of Yulmi and Juhwangmi cultivars in response to RKN infection. Alpha-tubulin served as an internal control. Data shown are the mean ± SE of three independent samples. Bars labeled with the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple range test

Table 2 Primer sequences used for expression analysis of sweet potato genes in this study

CDNA	Forward primer	Reverse primer	Product size (bp)
Heat shock protein 70 (JQ063092.1)	GGGAGGCAGATGAATTTGAA	TAGTCCACCTCCTCAATCTTGG	181
Ascorbate peroxidase (KC961952.1)	AGCGCTCTGGATTTGAAGGT	GCTTCAAATGAGCCTCTGCA	213
Actin 4 (EU250003.1)	TCCCCTAATGAGTGTGATGTGA	CTCATTCATGCCGGTATCGT	153
α-tubulin (BM878762.1)	CAACTACCAGCCACCAACTGT	CAAGATCCTCACGAGCTTCAC	212

migrating nematode, and in recently differentiated feeding sites. By comparison, at later stages of infection, in mature feeding cells, H_2O_2 concentrations are much lower, possibly as a result of an active regulation of plant defenses during nematode infection. Many ROS scavenging enzymes have been detected in RKNs and are thought to protect the parasite from ROS damages (Molinari and Miacola 1997). GST and Prxs are known to stress-related defense proteins, and detoxify ROS in response to a variety of stress conditions (Dubreuil et al. 2007). To overcome harmful effects of the oxidative burst, RKNs have acquired ROS removing or scavenging defense system including Prxs and GSTs (Molinari and Miacola 1997). Therefore, previous findings combined with our results suggest that significance of antioxidant mechanisms during plant–RKN interaction.

Conclusions

In summary, we identified changes in the abundances of important functional proteins in RKN-sensitive and -resistant sweet potato cultivars during nematode parasitism. Some of these host proteins participate in GC development at the feeding cell, required by *M. incognita* and for formation of gall structure. Our studies provide new insights into interaction with host–parasite in sweet potato, a major root crop plant. In the future, some of these proteins may be used to control PPN infection through molecular genetic engineering in plants to silence or overexpress the genes that promote or suppress formation of gall and GC structure.

Author contribution statement YHK, JJL, SWL, DWL, JSC conceived and designed the experiments. JH, JCW, YHJ, KJN and JJL performed the experiments. YHK, JJL, SWL, JCJ and DWL analyzed the data. JH, JCW, JWY, HWL and SCP contributed analysis/materials/reagents tools. YHK and JJL wrote the paper.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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