Academia Journal of Agricultural Research 4(5): 306-314, May 2016
DOI: 10.15413/ajar.2016.0705
ISSN: 2315-7739

Academia Publishing

Research Paper

Effect of Potassium levels on Suppressing Root-knot Nematode (*Meloidogyne incognita*) and Resistance Enzymes and Compounds Activities for Tomato (*Solanum lycopersicum L.*)

Accepted 26th April, 2016

Xiujuan Zhao[1], Wei Hu[1], Shuxiang Zhang[1]*, Qi Zhao[2] and Qian Wang[1]

[1]Institute of Agricultural Resource and Regional Planning, Chinese Academy of Agricultural Science, Beijing, 100081, China.
[2]Capital Normal University, Beijing, 100048, China.

*Corresponding author. E-mail: zhangshuxiang@caas.cn.

ABSTRACT

This study was carried out to investigate the effect of different potassium (K) levels on suppressing root-knot nematode (*Meloidogyne incognita*) and the mechanism for resistance enzymes and compounds. The superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and phenylalanine ammonialyase (PAL) activities and the total phenol, flavonoid and malondialdehyde (MDA) contents of two tomato cultivars (susceptible-HS and tolerant-HR) infected with *Meloidogyne incognita* were determined at four levels of K (0, 4, 8, 16 mM) respectively by hydroponics. The results showed that the disease index of root-knot nematode for the two tomato cultivars was significantly decreased with K application as compared to the control. The best treatment in both cultivars was 8 mM, in which the disease index was decreased by 46.2% in HS and 92.2% in HR. Meanwhile, the activities of SOD, CAT, POD, PAL, and content of total phenol and flavonoid with *M. incognita* in both cultivars were enhanced with increasing K from 0 to 8 mM but did not show increasing trends from 8 to 16 mM. In addition, K application clearly reduced the MDA content as compared to the control. It was suggested that the proper nutrient, K concentration could significantly reduce the disease index by inducing related enzyme activities and resistant matter.

Key words: Tomato, root-knot nematode, resistance enzyme activities, nutrient potassium, hydroponics, tomato cultivars.

INTRODUCTION

The tomato (*Solanum lycopersicum L.*) is one of the most widely grown vegetables in the world and is often severely attacked by the root-knot nematode, *Meloidogyne incognita* is a predominant and widely prevalent species (Sahebani and Hadavi, 2008; Kiewnick and Sikora, 2006; Chen et al., 2011).

The root-knot nematode is a serious problem in tomato production, causing considerable economic losses (Barker and Koenning, 1998; Koenning et al., 1999). Sikora and Fernandez (2005) reported over 30% yield losses in three vegetables (eggplant, tomato and melon) (Sikora et al., 2005).

Many attempts have been made to control root - knot nematodes (Khan et al., 2008; Naz et al., 2015) including antagonistic bacteria and fungi (Giannakou et al., 2004) chemical nematicides (organophosphates and carbamates), new genetic resistant cultivars (Randhawa et al., 2001; Sakhuja and Jain, 2001) and chemical substances induction, for example, by BABA or SA (Molinari and Loffredo, 2006; Sahebani et al., 2011; Khan et al., 2014; Molinari and Loffredo, 2006).

All of the mentioned methods have played an important role in tomato production. Chemical nematicides generally permit partial and short-term control of nematodes and can be harmful to human health and the environment

(Zuckerman and Esnard, 1994; Hussain et al., 2011; Kayani et al., 2012).

However, chemical substances induction, for example, plant nutrients as an induction agent, can preserve the environment and sustain productivity. Therefore, there are potential prospects for their increased use. These chemical substances can be used to induce resistance in many plants against pathogens including fungi, bacteria, viruses and nematodes (Kessmann et al., 1994; Cohen, 1994; Oostendorp and Seikora, 1990).

The plant defenses against pathogens can be induced by the activation of enzymes related to and accumulation of plant defense metabolites, which are the most important mechanisms of chemical inducers in plants (Mitchell and Walters, 2004; Venter et al., 2014).

Potassium (K) is one of the macronutrients needed for plant growth (Zhishen et al., 1999).

Sufficient K supply to plants can reduce some types of diseases, for example, septoria leaf blotch and powdery mildew in winter wheat and barley (Mitchell and Walters, 2004; Zörb et al., 2014).

It was suggested that K_2PO_4 might induce systematic resistances (Venter et al., 2014; Orober et al., 2002), of which changes in protective enzyme activity are an important indicator. However, the impact and mechanism of K in tomato infection with nematodes is still unclear to date.

We hypothesized that K could induce resistance to nematode infection by regulating antioxidant defense activities and consequently reducing membrane oxidative damage. To test this hypothesis, a *M. incognita* inoculation experiment was conducted to investigate the influence of K on resistance using two tomato cultivars with opposite resistance to root-knot nematode: a high-resistance cultivar (HR) and a high-susceptibility cultivar (HS).

MATERIALS AND METHODS

Plant cultivation

The greenhouse experiment was conducted in March, 2009 at the Chinese Academy of Agricultural Sciences, Beijing. The air temperature within the greenhouse during the experimental period varied between 20 and 30°C. Two contrasting tomato cultivars were selected based on their resistance to *M. incognita*: cv. 'precocious-2' (susceptible, HS) and '06h-42' (resistant, HR).

Tomato seeds were sown in irrigated medium. Three weeks later, four seedlings were transferred to a 3 L container with a continuously ventilated hydroponic system including 1.5 mM calcium (Ca^{2+}), 1.0 mM magnesium (Mg^{2+}), 1.0 mM sulfate (SO_4^{2-}), 0.67 mM phosphate ($H_2PO_4^-$), and 39 µM iron (Fe), 23 µM boron (B), 9 µM manganese (Mn), 0.3 µM molybdenum (Mo), 0.95 µM copper (Cu), and 3.5 µM zinc (Zn). Nitrogen was supplied at 7.6 mM as a mixture of KNO_3 and $Ca(NO_3)_2$. Four levels of K were applied at 0 (K_0), 4 (K_1), 8 (K_2), and 16 (K_3) mM, and $Ca(NO_3)_2$ was used to regulate the nitrate concentration (Coïc and Lesaint, 1975).

There were four (4) tomato plants in each container and 8 repetitions for each treatment. The culture solution was renewed every three days and the pH maintained at 5.5to 6.0 with diluted sodium hydroxide and hydrochloric acid.

Plant sample collection and pretreatment

After the plants had grown with different K levels for 30 days, the top leaves (3 to 4 from the topmost) were picked and freeze-dried (Free Dry System/Freezone®4.5, Labconco, Missouri, USA) to analyze the activities of enzymes and the content of MDA, phenolics and flavonoids.

Nematode inoculum preparation and inoculation procedures

The inoculum of root-knot nematode, *M. incognita,* isolated from naturally infected tomato, was obtained from pure culture raised from a single egg mass and maintained on tomato roots (*S. lycopersicum L.*, nematode susceptible variety Super Marmande) in a greenhouse. The Meloidogyne was identified on the basis of the perineal pattern system of matured females. Infected plants were uprooted and the entire root system dipped in water and washed gently to remove adhering soil. Egg masses of *M. incognita* were picked using forceps. Egg masses were rinsed with sterile water, placed in 0.5% sodium hypochlorite to dissolve the gelatinous matrix, agitated for 4 min and rinsed with sterile water on a sieve with 26 mm pores. The eggs were incubated for five days using the modified Baermann funnel method to obtain the second-stage juveniles (J2). The nematode inoculum was used for *in vitro* and pot experiments.

Root-knot nematode, *M. incognita* Chitwood, was isolated from infected roots of a susceptible tomato (*Lycopersicon esculentum* Mill, donations from Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing, China). Nematode eggs were obtained from galled roots according to Hussey (1973) and mixed with an artificial medium (50% commercial Plantimax® + 50% carbonized rice husk) to a final concentration of 60 eggs/ml.

The inocula of the nematode suspension were moved into a hydroponic system, reserving approximately 2000 nematode eggs per plant after transfer to plastic containers. The disease index of different treatments was evaluated after 30 days of inoculation by root-knot nematode (Evans et al., 1993). The calculation of disease index was as follows: In the 0 level, there was no root node; In the 1st level there was a small number of root nodes, which accounted for 1 to 25% of the whole root; In the 2nd level there was medium number of root nodes, which accounted for 26 to 50% of the entire root; In the 3rd level there was 51 to 75%; and in the 4th level

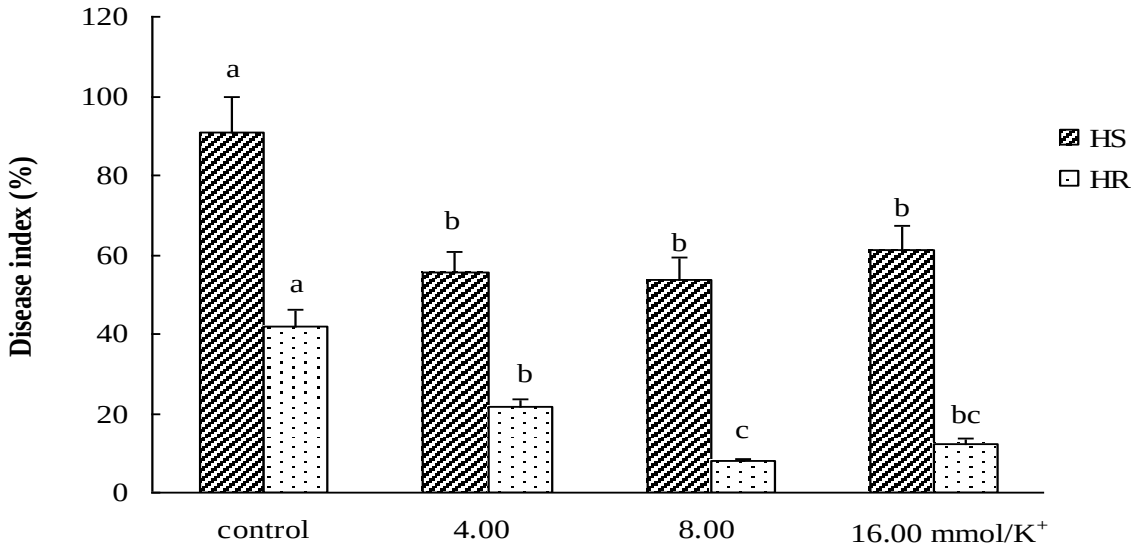

Figure 1. Effect of different levels of K on the root-not nematode disease index of tomato with *Meloidogyne incognita* (values represent means of 10 replicates (±SE), (HS, high-susceptibility cultivar and HR, high-resistance cultivar).

it was more than 3. Disease index was calculated using the formula:

$$\text{Disease index} = \frac{4A + 3B + 2C + 1D + 0E}{4N} \times 100\%$$

A to E means the number of the corresponding level of plants; N=A+B+C+D+E.

Determination of lipid peroxidation and antioxidant enzyme, phenylalanine ammonia-lyase activities

Lipid peroxidation was determined by estimating the malondialdehyde (MDA) content. First, frozen top leaves (0.5 g) were ground with 5 ml phosphate buffer (pH 7.8) into a fine powder in a mortar by liquid nitrogen. Then, the homogenate was centrifuged at 4°C 10,000 × g for 20 min and the supernatant removed for MDA determination.

Frozen or fresh top leaves (0.5 g) were prepared in the same way as for measuring MDA content. The peroxidase (POD) activity was analyzed through measuring of the oxidation of guaiacol. The assay mixture contained 50 mM sodium phosphate (pH6.0), 28 μL guaiacol and 19 μl 30% H_2O_2. The absorbance was recorded five times at 470 nm in 30 s. The variation of absorbance per min per gram fresh weight (U/g · min FW) represents enzyme activity.

Determination of the content of total phenol and flavonoid

The topmost leaves (3 to 4) were freeze-dried (Free Dry System/Freezone®4.5, Labconco, Missouri, USA) and homogenized. The ground powders of the samples were then extracted twice by shaking at 25°C for 24 h with solvents (1:10, w/v) of various polarities, including distilled water, methanol (80%), ethanol (80%) and acetone (80%). The solvent extracts obtained with the same solvent were then combined. After filtering through Whatman No. 1 filter paper (Whatman, Maidstone, UK), the extract underwent vacuum concentration and freeze desiccation.

The total phenol content of the samples was measured according to a modification of Singleton et al. (1999) procedures and the total flavonoid determined by the modified colorimetric method of Zhishen et al. (1999) and catechin (Sigma-Aldrich Co.) used as a standard.

Statistical analyses

The data in the tables and figures are expressed as the means of 3 replicates ± S.E. Data were statistically analyzed by ANOVA using SAS version 9.0 (Statistical Analysis System Institute Inc., Cary, NC, USA). The statistical significance between mean values was compared using Duncan's multiple range test ($P<0.05$).

Results

Effect of K levels on disease indexes of tomato root-knot Nematode infection

Figure 1 shows the effects of different levels of K on the disease index of tomato root-knot nematode. The disease index of root-knot nematode was significantly reduced

Table 1. Dynamic changes in SOD after inoculated for two tomato cultivars grown at different levels of K for 25 days (U/g · FW).

Factor and level	Sampling time				
	5th day	10th day	15th day	20th day	25th day
K_0	134.5c	241.8c	176.4c	162.5c	153.4b
K_1	167.5b	327.1b	289.4b	214.2b	174.3b
K_2	184.2a	401.2a	371.5a	341.1a	241.5a
K_3	158.9b	337.5b	291.4b	204.3b	167.1b
HR	174.5a	486.6a	340.1a	272.8a	188.3a
HS	151.9b	167.2b	224.3b	188.3b	179.8a
Inoculation	162.4a	491.3a	405.1a	286.9a	198.9a
Non-inoculation	164.1a	162.5b	159.2b	174.1b	169.2b
Interaction (F- value)					
K level × cultivar	22.12	20.51*	39.78**	8.79*	14.84*

Note: a, b means significant differences; $^{**}P<0.01$, and $^{*}P<0.05$.

Table 2. Dynamic changes in POD after inoculated for two tomato cultivars grown at different levels of K for 25 days (U/g · min FW).

Factor and level	Sampling time				
	5th day	10th day	15th day	20th day	25th day
K_0	51.5c	89.4c	72.4c	68.7c	62.4b
K_1	69.8b	129.5b	120.1b	103.6b	96.7a
K_2	81.5a	151.4a	144.8a	138.3a	105.5a
K_3	80.4a	140.6a	131.9b	104.6b	98.2a
HR	87.5a	184.9a	130.7a	123.4a	108.1a
HS	54.1b	70.5b	103.9b	84.2b	73.3b
Inoculation	74.5a	180a	157.4a	134a	111.9a
Non-inoculation	67.1a	75.4b	77.2b	73.6b	69.5b
Interaction (F- value)					
K level × cultivar	9.44	18.71*	1.48	5.41*	4.88*

compared to the control after K applications from 4 to 16 mM. The greatest reduction of root-knot nematode infection rate was at 8 mM, which led to reductions of 46.2% in HS and 92.2% in HR, respectively. Treatment with 16 mM K resulted in 38.8% reduction in HS and 87.6% in HR. Under the same treatment, HR showed a better effect on suppressing the rate of *M. incognita* than HS. These data showed that the disease index of tomato root-knot nematode can be decreased by the application of appropriate concentrations of K.

Effect of different potassium levels on protective enzyme activities of tomato

As shown in Tables 1 to 3 in both cultivars, the protective enzyme activities of SOD, POD and CAT after K treatments were higher than in the respective controls from the 5th to the 25th day after inoculation with *M. incognita*, which revealed that potassium could increase the self-protective ability of the tomato against *M. incognita.* The enzyme activities of both cultivars were enhanced significantly with increasing K levels from 0 to 8 mM but decreased from 8 to 16 mM. The SOD and CAT activities with K_2 were significantly higher than with K_1 and K_3 from the 5th to the 25th day, whereas the POD activity with K_2 was only higher than with K_1 and K_3 at the 15th and 20th days.

Significant differences were observed in different periods of growth, and the activities of SOD, POD and CAT in the control (0 mM) increased slowly from the 0th to the 10th day and declined rapidly from the 10th to the 25th day compared to the K treatments. However, the enzyme activities with the K treatments declined slowly for K_1 (4 mM) and K_3 (16 mM) until the 15th day and for K_2 (8 mM) until the 20th day. The highest enzyme activities for K_2 (8 mM) treatment were significantly greater than the others. In addition, the enzyme activities significantly increased in the interaction of potassium + cultivars as compared to all other treatments

Table 3. Dynamic changes in CAT after inoculated for two tomato cultivars grown at different levels of K for 25 days (U/g · min FW).

Factor and level	Sampling time				
	5th day	10th day	15th day	20th day	25th day
K_0	77.5c	125.6c	100.4c	90.8c	84.6c
K_1	89.5b	161.8b	158.4b	119.5b	107.1b
K_2	120.4a	210.4a	197.2a	181.5a	131.4a
K_3	92.6b	170.5b	166.7b	123.4b	115.9b
HR	108.5a	250.7a	176.2a	154.1a	128.9a
HS	81.5b	83.4b	135.1b	103.5b	90.6b
Inoculation	90.7a	231.6a	218.5a	159.4a	124.9a
Non-inoculation	99.3a	102.5b	92.8b	98.2b	94.6b
Interaction (F- value)					
K level × cultivar	5.74*	14.22	35.38*	21.50*	10.53*

Table 4. Dynamic changes in total phenolic content after inoculate for two tomato cultivars grown at different levels of K for 25 days (mg/g FW).

Factor and level	Sampling time				
	5th day	10th day	15th day	20th day	25th day
K_0	5.11c	11.23c	7.62c	6.81c	5.62c
K_1	6.85b	14.18b	12.34b	8.73b	8.06b
K_2	8.31a	17.48a	15.62a	14.29a	10.32a
K_3	6.51b	13.77b	11.98b	8.15b	7.67b
HR	7.32a	20.62a	12.61a	11.62a	9.66a
HS	6.07b	7.71b	11.17a	7.19b	6.17b
Inoculation	7.21a	21.99a	17.66a	12.28a	9.51a
Non-inoculation	6.18a	6.34b	6.12b	6.53b	6.32b
Interaction (F- value)					
K level × cultivar	0.034	0.125	0.035	0.124	5.13*

(P<0.05). The results indicated that SOD, POD and CAT activities were responsive to K levels and tomato cultivars (Tables 1 to 3).

The SOD, POD, and CAT activities in HR were notably higher (P＜0.05) than in HS in the experiment and the maximum enzyme activities in HR were significantly higher than in HS (Tables 1 to 3).

Effects of different K treatments on content of resistance substance (total phenol, flavonoid) in tomato

The total phenolic and flavonoid content after K treatments for both cultivars showed significant increases compared to the control from the 5th to the 25th day after inoculation of *M. incognita* Chitwood.

The total phenolic and flavonoid contents of both cultivars were significantly enhanced with increasing K application from 0 to 8 mM and decreased from 8 to 16 mM. The total phenolic content with K_2 was significantly higher than with K_1 and K_3 from the 5th to the 25th day, and the flavonoid content with K_2 was higher than with K_1 and K_3 except on the 15th day.

The total phenolic and flavonoid contents of the control (0 mM) declined rapidly from the 10th to the 25th day compared to the K treatments and the resistance substance after the application of K_1 (4 mM) and K_3 (16 mM) began to descend slowly until the 15th day and after the application of K_2 (8 mM) until the 20th day. The maximum total phenolic and flavonoid contents of K_2 (8 mM) were significantly greater than for the other treatments.

The total phenolic and flavonoid contents reached their maximum on the 10th day for HR and on the 15th for HS, and the maximum total phenolic and flavonoid contents of HR were both significantly higher than for HS (Tables 4 and 5). Inoculation could clearly increase the total phenolic and flavonoid content from the 10th to the 25th day as compared to non-inoculation (Tables 4 and 5).

Table 5. Dynamic changes in flavonoid content after inoculated for two tomato cultivars grown at different levels of K for 25 days (OD325/g FW).

Factor and level	Sampling time				
	5th day	10th day	15th day	20th day	25th day
K_0	24.5c	52.1c	33.6c	30.5c	27.8c
K_1	37.5b	80.2b	77.3ab	59.7b	55.3a
K_2	54.6a	98.6a	85.7a	80.6a	62.4a
K_3	40.1b	77.9b	68.2b	52.8b	43.9b
HR	48.2a	112.3a	69.8a	71.3a	60.1a
HS	30.1b	42.1b	62.6a	40.5b	34.6b
Inoculation	41.3a	113.8a	90.9a	73.2a	54.4a
Non-inoculation	37.05a	40.6b	41.5b	38.6b	40.2b
Interaction (F- value)					
K level × cultivar	0.042	0.847	0.345	0.124	0.061

Table 6. Dynamic changes in PAL after inoculated for two tomato cultivars grown at different levels of K for 25 days (U/mg · min FW).

Factor and level	Sampling time				
	5th day	10th day	15th day	20th day	25th day
K_0	24.1c	64.2c	36.7c	33.5c	28.9c
K_1	40.6b	84.3ab	76.2ab	55.8b	50.7a
K_2	55.1a	91.3a	82.6a	74.5a	59.3a
K_3	38.7b	79.6b	71.4b	49.1b	40.6b
HR	46.8a	116.9a	60.8a	66.8a	59.1a
HS	32.4b	42.8b	59.9a	39.6b	30.7b
Inoculation	40.7a	120.3a	78.9a	68.8a	49.1a
Non-inoculation	38.6a	39.4b	41.8b	37.6b	40.6a
Interaction (F- value)					
K level × cultivar	7.54*	15.32*	2.668*	3.689*	8.794*

Effect of K levels on related PAL activity to resistance in tomato

In the hydroponics culture, from the 5th to the 25th day after inoculation with *M. incognita* Chitwood, the enzyme activity of PAL after K treatments of both cultivars significantly increased in comparison to the control. The enzyme activities of both cultivars were also enhanced significantly with increasing potassium from 0 to 8 mM but clearly decreased from 8 to 16 mM. The PAL activity for K_2 was significantly higher than for K_1 and K_3 from the 5th to the 20th day.

The PAL activity of the control declined slowly after the K application treatments from the 10th to the 25th day. The PAL activity of HR reached its maximum on the 10th day, while the PAL activity of HS reached its maximum on the 15th, and the maximum PAL enzyme activity of HR was significantly higher than for HS (Table 6).

Inoculation could clearly increase the activity of the PAL enzyme from the 10th day compared to non-inoculation. In addition, the interaction of potassium × cultivars was significantly higher than the effect of potassium or cultivars (P<0.05). The results indicated that PAL activities responded to different potassium levels and tomato cultivars (Table 6).

Effect of different Potassium levels on MDA content

K application clearly reduced the MDA content compared to the control. The MDA content with no K increased stably from the 5th to the 25th day and reached its maximum at the 10th day and decreased from the 15th to the 25th day. Our results showed that the MDA content in the HR cultivar (XXY) was significantly lower than in HS except on the 10th day. The concentration of MDA in HR increased from the 5th to the 10th day and then declined from the 10th to the 25th

Table 7. Dynamic changes in MDA content after inoculated for two tomato cultivars grown at different levels of K for 25 days (μmol/g FW).

Factor and level	Sampling time				
	5th day	10th day	15th day	20th day	25th day
K_0	3.08[a]	3.37[a]	3.83[a]	4.12[a]	4.23[a]
K_1	2.41[b]	2.91[b]	2.56[b]	2.48[b]	2.33[b]
K_2	1.99[c]	2.73[b]	2.44[b]	2.18[c]	2.11[b]
K_3	2.54[b]	2.82[b]	2.59[b]	2.37[b]	2.28[b]
HR	2.18[b]	2.85[a]	2.47[b]	2.19[b]	1.97[b]
HS	2.89[a]	3.06[a]	3.24[a]	3.38[a]	3.5[a]
Inoculation	2.47[a]	3.4[a]	3.05[a]	2.86[a]	2.79[a]
Non-inoculation	2.6[a]	2.51[b]	2.66[b]	2.71[b]	2.68[b]
Interaction (F- value)					
K level × cultivar	1.47*	0.84	2.41*	3.41*	4.17*

day, but it increased slowly in the HS from the 5th to the 25th day. Tomato plants with 8 mM potassium showed the lowest MDA at both the 5th and 20th day. The data showed that the cultivars × potassium interaction was significantly lower than for potassium or cultivars individually (P<0.05) (Table 7).

Discussion

Effect of potassium (K) levels on suppression of tomato root-knot nematode

This study showed that the disease index of tomato root-knot nematode can be significantly depressed by (46.2 to 92.2%) with an appropriate concentration of K (8 mM) in culture solution. The results showed increased protection in tomato against infestation by root-knot nematode after treatment with potassium. Upon treatment of barley leaves with 10 to 25 mM potassium phosphate, the powdery mildew infection rates reduced by 16%, and when the K concentration was increased to 35 to 50 mM, the infection rates of powdery mildew decreased by 45% (Oostendorp and Seikora, 1990). Venter et al. (2014) indicated that the treatment of both susceptible (Scheepers) and resistant (Tugela DN) wheat cultivars with potassium phosphate (25 mM potassium phosphate (K_3PO_4) showed reduced severity of symptoms with decreased numbers of aphids feeding on plants in comparison with untreated plants.

These results indicate that different K levels can be applied to different crops for various diseases. Insufficient or excessive K will affect the secondary metabolism of crop and lead to physiological inhibition.

In the hydroponics culture experiments in this, from the 5th to the 25th day after inoculation with *M. incognita* Chitwood, the protective enzyme activities of SOD, POD and CAT after K treatment (from 0 to 8 mM) were higher than in the control. The results revealed that K could increase the activity protective enzymes against infection of *M. incognita*. Similar results regarding POD activity was reported in barley (Mitchell and Walters, 2004), suggesting that potassium phosphate led to significant increases in peroxidase activity in leaves. The antioxidant enzymes are the most efficient protective mechanism against pathogens, including catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), and others (Halliwell and Gutteridge, 1985; Allen, 1995).

Many studies have shown that protective enzyme activities were improved after inoculation with noxious pathogens or micro-organisms in susceptible or resistant cultivars (Singh et al., 2003; Wu, 1996). SOD activity increased in incompatible host-pathogen interactions, as reported for potato and *Phytophthora infestans*, pearl millet and *Sclerospora graminicola*, and tobacco and downy mildew (Kang, 2008).

Peroxidases are part of an antioxidant system and also catalyze the oxidation of phenolic compounds (Bhaskar et al., 2001). Peroxidases play a critical role in several metabolic processes such as the oxidation of phenols, suberization, and the reinforcement of cell walls in plants. The enzyme activity results suggest that potassium induces similar or higher levels of regulation compared to infestation with root-knot nematode in SOD, CAT, and POD to ensure the plant's ability to overcome the threat of an invading pest.

Effect of K levels on PAL enzyme activities, the content of resistance substances and MDA

The results showed that the phenol and flavonoid contents increased with increasing K concentration, which is consistent with other research (Bhaskar et al., 2001).

The changes in PAL activities in the experiments were consistent with the results for phenols and flavonoids and the PAL enzymes are among the key enzymes for the synthesis of phytoalexins, phenolic compounds and lignin metabolism (Franke et al., 2002). Mitchell and Walters

(2004) reported that the enzyme activities of PAL increased further when the second leaves of potassium phosphate-treated plants were inoculated with powdery mildew. Phenolic compounds are also natural antifungal compounds that reduce pathogen attacks and make the plants less susceptible to pathogen attack (Southerton and Deverall, 1990). Based on the maintenance of high levels of protective enzymes, it has been suggested that the synthesis of phenols can significantly improve plant disease resistance and pest control (Gawande et al., 2002; Siranidou et al., 2002).

The phenols and flavonoids are key metabolites for phenolic compounds in plants. The data in the paper showed that increasing K (0, 4, 8 mM) respectively can clearly reduce the MDA content, protect membrane peroxidation and maintain cell integrity in the tomato, while excess K (16 mM) showed the reverse effects. The MDA content can reflect the degree of lipid peroxidation resulting from the pathogen. Lipid peroxidation and polymerization reactions occur in membrane proteins, leading to cell damage and death (Peng and Kuc, 1992).

Generally, lipid peroxidation and cell damage are induced in plants after pathogen infection, causing cell membrane damage and producing large amounts of MDA. In the results, K also increased PAL activity and phenol and flavonoid contents and decreased the MDA content after infection compared with the control, suggesting an important role of K in removing ROS induced by root-knot nematode infection.

Conclusions

The root-knot nematode infection rates (disease indexes) of tomato were significantly reduced when potassium was applied. The lowest index in both cultivars was found for the K_2 treatment with 46.2 and 92.2% in HS and HR, respectively. For both cultivars, with increasing K concentration up to 8 mM, the PAL, SOD, CAT and POD activities and the total phenol and flavonoid contents were gradually enhanced and the MDA content reduced. However, a higher K concentration (16 mM) showed the opposite effects. The results showed that manipulating K concentrations in soil has potential prospects for suppressing root-knot nematode.

ACKNOWLEDGEMENTS

This work was supported by the project “Modulation technology for water and nutrients in the soil environment for plant growth (2013AA102901)”. The author wishes to thank Dr. Gao from the Institute of Vegetables and Flowers, CAAS for providing tomato materials.

REFERENCES

Allen RD (1995). Dissection of oxidative stress tolerance using transgenic plants. Plant Physiol. 107(4): 1049-1054.

Barker KR, Koenning SR (1998). Developing sustainable systems for nematode management. Annu. Rev. Phytopathol. 36: 165-205.

Bhaskar CV, Pao GR, Reddy KB (2001). Effect of nitrogen and potassium nutrition on sheath rot incidence and phenol content in rice (*Oryza sativa* L). India. J. Plant Physiol. 16(3): 254-257.

Chen ZD, Wang PS, Jiu YL, Zhou Y (2011). Tomato and cucumber root knot nematode disease prevention in the south. Acta Hort (Sinica). 38: 2566.

Cohen Y (1994). 3-Aminobutyric acid induces systemic resistance against peronospore tabacina. Physiol. Mol. Plant Pathol. 44(4): 273-288.

Coïc Y, Lesaint C (1975). The water and mineral nutrition of plants in advanced horticulture. The technique document of SCPA. 23: 1-22.

Evans KD, Trudgill DL, Webster JM (1993). Plant parasitic nematodes in temperate agriculture. UK: Cambridge.

Franke R, Hemm MR, Denault JW, Ruegger MO, Humphreys JM, Chapple C (2002). Changes in secondary metabolism and deposition of an unusual lignin in the ref8 mutant of Arabidopsis. Plant J. 30(1): 47-59.

Gawande VL, Patil JV, Naik PM (2002). Plant biochemical defense against powdery mildew (Erysiphe polygoni D C) disease in mungbean (vigna radiate (L.)Wilczek). J. Plant Biol. 29: 337-341.

Giannakou IO, Karpouzas DG, Prophetou-Athanasiadou D (2004). A novel non-chemical nematicide for the control of root-knot nematodes. Appl. Soil Ecol. 26(1): 69-79.

Halliwell B, Gutteridge JMC (1985). Free radicals in biology and medicine, 3rd ed. New York: Oxford University Press.

Hussain MA, Mukhtar TARIQ, Kayani MZ (2011). Efficacy evaluation of Azadirachta indica, Calotropis procera, Datura stramonium and Tagetes erecta against root-knot nematodes Meloidogyne incognita. Pak. J. Bot. 43: 197-204.

Kang NJ (2008). Inhibition of powdery mildew development and activation of antioxidant enzymes by induction of oxidative stress with foliar application of a mixture of riboflavin and methionine in cucumber. Sci Hort. 118(3): 181-118.

Kayani MZ, Mukhtar T, M, Hussain A (2012). Evaluation of nematicidal effects of cannabis sativa L. and zanthoxylum alatum Roxb. Against root-knot nematodes, Meloidogyne incognita. Crop Prot. 39: 52-56.

Kessmann H, Staub T, Hofmann C, Maetzke T, Herzog J, Ward E, Uknes S, Ryals J (1994). Induction of systemic acquired disease resistance in plants by chemicals. Ann. Rev. Phytopathol. 32: 439-459.

Khan MR, Haque Z, Kausar N (2014). Management of the root-knot nematode Meloidogyne graminicolainfesting rice in the nursery and crop field by integrating seed priming and soil application treatments of pesticides. Crop Protect. 63: 15-25.

Khan Z, Kim SG, Jeon YH, Khan HU, Son SH, Kim YH (2008). A plant growth promoting rhizobacterium, Paenibacillus polymyxastrain GBR-1, suppresses root-knot nematode. Bioresour. Technol. 99(8): 3016-3023.

Kiewnick S, Sikora RA (2006). Biological control of the root-knot nematode Meloidogyne incognita by Paecilomyces lilacinus strain 251. Biol. Cont. 38(2): 179-187.

Koenning SR, Overstreet C, Noling JW, Donald PA, Becker JO, Fortnum BA (1999). Survey of crop losses in response to phytoparasitic nematodes in the United States for 1994. J. Nematol. 31(4S): 587-618.

Mitchell AF, Walters DR (2004). Potassium phosphate induces systemic protection in barley to powdery mildew infection. Pest Manag. Sci. 60(2): 126-134.

Molinari S, Loffredo E (2006). The role of salicylic acid in defense response of tomato to root-knot nematodes. Physiol Mol Plant Pathol. 68(1-3): 69-78.

Naz I, Saifullah, Palomares-Rius JE, Khan SM, Ali S, Ahmad M, Ali A, Khan A (2015). Control of southern root knot nematode Meloidogyne incognita (Kofoid and White) Chitwood on tomato using green manure of *Fumaria parviflora Lam* (Fumariaceae). Crop Protect. 67: 121-128.

Oostendorp M, Seikora RA (1990). In vitro interrelationship between rhizospher bacteria and Heterodera schachtii. Rev Nematol. 13(3): 269-274.

Orober M, Siegrist J, Buchenauer H (2002). Mechanisms of phosphate-induced disease resistance in cucumber. Eur. J. Plant Pathol. 108(4): 345-353.

Peng M, Kuc J (1992). Peroxidase-generated hydrogen peroxide as a source of antifungal activity in vitro and on tobacco leaf disks. Phytopathology. 82: 696-699

Randhawa N, Sakhuja PK, Singh I (2001). Management of root-knot nematode Melodogyne incognita in tomato with organic amendments. Pl Dis Res. 16: 274-276.

Sahebani N, Hadavi N (2008). Biological control of the root-knot nematode Meloidogyne javanica by Trichoderma harzianum. Soil Biol. Biochem, 40(8): 2016-2020.

Sahebani N, Hadavi NS, Zade FO (2011). The effects of β-amino-butyric acid on resistance of cucumber against root-knot nematode, *Meloidogyne javanica*. Acta Physiol. Plant. 33(2): 443-450.

Sakhuja PK, Jain RK (2001). Nematode diseases of vegetable crops and their management. In:Thind TS, Kalyani P, editors. Diseases of fruits and vegetables and their management. India: Ludhiana.

Sikora RA, Fernández E. Nematodes parasites of vegetables. In: Liuc M, Sikora RA, Bridge J, editors (2005). Plant parasitic nematodes in subtropical and tropical agriculture. Wallingford: CAB International. pp. 319-392.

Singh UP, Bahadur A, Singh DP, Sarma BK (2003). Non-pathogenic powdery mildews induce resistance in pea (*Pisumsativum*) against Erysiphe pisi. J. Phytopathol. 151(7-8): 419-424.

Singleton VL, Orthofer R, Lamuela-Raventos RM (1999). Analysis of total phenols and other oxidation substrates and antioxidant by means of Folin-Ciocalteu reagent. Methods Enzymol. 299: 153-178.

Siranidou E, Kang Z,Buchenauer H (2002). Studies on symptom development, phenolic compounds and morphological defence responses in wheat cultivars differing in resistance to Fusarium Head blight. J. Phytopathol. 150(4-5): 200-208.

Southerton SG, Deverall BJ (1990). Changes in phenylalanine ammonia-lyase and peroxidase activities in wheat cultivars expressing resistance to the leaf-rust fungus. Plant Pathol. 39(2): 223-230.

Venter E, Mansoor CV, Sibisi P, Botha A (2014). Potassium phosphate induces tolerance against the Russian wheat aphid (Diuraphis noxia, Homoptera: Aphididae) in wheat. Crop Protect. 61: 43-50.

Wu YX (1996). A preliminary study on the induction of cellular defense system and resistance of hybrid rice to bacterial blight. J. Plant. Pathol. 26: 127-131.

Zhishen J, Mengcheng T, Jianming W (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64(4): 555-559.

Zörb C, Senbayram M, Peiter E (2014). Potassium in agriculture - status and perspectives. J. Plant Physiol. 171(9): 656-669.

Zuckerman BM, Esnard J (1994). Biological Control of Plant Nematodes-Current Status and Hypotheses [Japanese]. Jpn. J. Nematol. 24(1): 1-13.

Cite this article as:

Zhao X, Hu W, Zhang S, Zhao Q, Wang Q (2016). Effect of Potassium levels on Suppressing Root-knot Nematode (Meloidogyne incognita) and Resistance Enzymes and Compounds Activities for Tomato (Solanum lycopersicum L.). Acad. J. Agric. Res. 4(5): 306-314.