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Research Article

Influence of salicylic acid nano-formulation on expression of peroxidase (113-114) genes and peroxidase and phenylalanine ammonia lyase in wheat cultivar susceptible to *Heterodera filipjevi*

Najmeh Ghazalbash[1], Naser Panjehkeh[1*], Zahra Tanhamaafi[2], Seyed Kazem Sabbagh[3], Mohammad Salari[1] and Mohsen Esmaeilzadeh Moghaddam[4]

1. Department of Plant Protection, Faculty of Agriculture, University of Zabol, Zabol, Iran.
2. Iranian Research Institute of Plant Protection, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran.
3. Department of Biology, Campus of Science, Yazd University, Yazd, Iran.
4. Seed and Plant Improvement Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran.

Abstract: The effects of salicylic acid (SA) nano-formulation on expression of peroxidase (113-114) genes and peroxidase and phenylalanine ammonia lyase (PAL) were investigated in wheat cultivar (Bezostaya) susceptible to *Heterodera filipjevi*. The wheat roots and leaves were randomly divided into control group and groups exposed to 62.5, 125 and 250µg/ml SA. A spectrophotometric analysis was carried out using root extracts from infected plants at 4, 7 and 11days post inoculation with nematode (DAI) for peroxidase and PAL. The expression of peroxidase (113-114) genes was evaluated by Real time PCR analysis. Peroxidase activity was significantly increased in treatments exposed to 250µg/ml of nanosalicylic acid at 11 DAI. Phenylalanine ammonia lyase activity was induced in the treatments exposed to 250 and 125µg/ml nanosalicylic acid compared to the control at 4 and 7 DAI, respectively. Phenylalanine ammonia lyase activity was also increased in the treatments exposed to 62.5 and 250µg/ml of nanosalicylic acid compared to the control at 7 DAI. The expression level of peroxidase 113-114 in wheat leaves was significantly raised at 4 DAI when 62.5µg/ml of nanosalicylic acid was used. There was also a significant difference between expression levels of peroxidase 113-114 genes at applications of 125 and 250µg/ml of SA in comparison with the control at 4 and 7 DAI, a significant decrease was revealed in the gene expression in treatments exposed to 62.5, 125 and 250µg/ml of nanosalicylic acid compared to the control at 11 DAI. It was concluded that higher concentrations of nanosalicylic acid have a potential effect on peroxidase and PAL activities in wheat infected by *H. filipjevi*. High concentration of nanosalicylic acid has inhibitory effects on the expression level of peroxidase gene.

Keywords: Cereal Cyst Nematode, PAL, Peroxidase, SA

Introduction

Cereals such as wheat, barley and oats are among the major staple crops with economic importance worldwide (Kaur *et al*., 2014). Wheat *Triticum aestivum* L. is one of the most important cultivated crops in Iran and is cultivated in all parts of the country under both irrigated and rain-fed conditions. The area under wheat cultivation was 5.7 million ha comprising 39% and 60% irrigated and non-irrigated, respectively in 2015 (Anonymous, 2016) and data have shown that 0.8 million ton

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* **Corresponding author**, e-mail: naserpanjehkeh@gmail.com
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(~8% of Iran's wheat production in 2014-2015) of wheat grain might be produced per year by allocating one million ha of the unused lands from the medium suitability class to rainfed wheat cropping (Mesgaran 2017). Cereal cyst nematodes, CCNs are globally known as the parasites of cereals and grasses, they limit the production of small grain cereals by invading the cereal host plants (Smiley *et al*., 2017). The nematodes form specific large multinucleate cells resulted from breakdown of cell walls and fusion of adjacent protoplasts called syncytia close to the vascular system of the root (Zhang *et al*., 2017). The crop losses caused by this group of nematodes have been reported from 20% in Pakistan. 50% both in Australia and Turkey up to 90% in Saudi Arabia (Riley *et al*., 2009). The effect of *H. filipjevi* on wheat and barley were evaluated under microplot and field conditions in different regions of Iran in recent years, viz Markazi, Khuzestan and Isfahan Provinces (Hajihassani *et al*., 2010; Ahmadi *et al*., 2013; Karimipour Fard *et al*., 2018). A more recent investigation proved the reduction of grain yield due to *H. filipjevi* from 20.4% up to 24.8% in three wheat cultivars in field conditions, meanwhile the reproduction factor was significantly reduced in plots by nematicide application (Karimipour Fard *et al*., 2018). Nematode management is mostly performed through application of a combination of agricultural, chemical, biological methods and resistant cultivars (Timper 2014).

Plants have evolved various defense mechanisms to resist infection by pathogens (War *et al.*, 2012). The phytohormone salicylic acid is an important signal molecule produced by a wide range of prokaryotic and eukaryotic organisms including plants (Lareen *et al*., 2016). Plant disease resistance is associated with high expression of defense genes and the accumulation of salicylic acid in the inoculated leaves. Plants often respond to the environmental stresses with increasing SA level (Loake and Grant, 2007; Oka and Cohen, 2001). Salicylic acid also regulates the activities of different enzymes such as peroxidase, polyphenol oxidase, superoxide dismutase and PAL which are the major components of induced plant defense against biotic and abiotic stresses. These enzymes play a significant role in the defense of plants against pathogens by catalyzing cell wall lignification. The pathways and regulation of SA biosynthesis in plants may be more complicated than was thought earlier (Van Loon *et al*., 2006; War *et al*., 2011 b).

Studies have shown that Plant-parasitic nematodes cause considerable damages to the agricultural crops worldwide and may kill a plant and reduce its productivity through feeding on the plant (Zinovieva, 2014; Ladner *et al*., 2008; Bird *et al*. 2015). There are also several reports indicating that SA is a key hormone in the plant defense which plays an important role in the local and systemic defense responses against various pathogens (Loake and Grant, 2007; Rabe *et al*., 2013).

Salicylic acid is a part of the Mi-1-mediated defense response to the root-knot nematode in tomato. Spraying SA may reduce root galling and increase plant growth in tomato infected by root knot nematode, *Meloidogyne javanica* (Almaghrabi *et al*., 2013). Phytochemical research have shown that β-aminobutyric acid reduces the number of *H. avenae* and *H. latipons* cysts on wheat and barley as plant resistance inducer and acetylsalicylic acid can reduce reproduction of *H. glycines* on the susceptible soybean cultivar (Oka and Cohen 2001). Recent studies have demonstrated that PAL activity has increased in response to different concentrations of SA at 5 days post inoculation (Ketabchi *et al*., 2014). Another known function of peroxidases is generating reactive oxygen species (ROS) in SA defense response pathways (Almagro *et al*., 2009). Experimental evidences also implicate class III peroxidases in plant defense response to pathogen/pest attacks, such as bacteria (Ahola-Iivarinen *et al*., 2009), fungi (Chassot *et al*., 2007), viruses (Periago *et al*., 2006) insects (Little *et al*., 2007) and cyst nematodes (Delibes *et al*., 2009) which play pivotal roles in SA defense response pathway by generating ROS (Jing *et al.*, 2011). In contrast, several evidences indicated that exposure to SA for 2 days after inoculation may reduce PAL activity (Ketabchi *et al.*, 2014).

A large amount of data has focused on the relation between SA and plants infected by nematodes, but the results are still conflicting in many aspects. The aim of this research was to investigate the effects of salicylic acid nano-formulation on expression of peroxidase (113, 114) and PAL in wheat plants susceptible to *H. filipjevi*.

Materials and Methods

Nematode preparation

Infested soil with *H. filipjevi* was collected from a wheat field in Hamadan province. The cysts were extracted by Fenwick-can technique (Fenwick, 1940). Cysts were picked using forceps, and surface sterilized in 0.5 percent NaOCl for 10 minutes and were rinsed several times in distilled water. The cysts were kept in the refrigerator at 4 °C and then were transferred to room temperature (10-15 °C) to enhance hatching. The freshly hatched nematode juveniles (J2) were collected in a glass beaker. Later, the nematode number per milliliter of nematode suspension was calibrated by taking average count (three aliquots) under the stereomicroscope.

Preparation of wheat genotypes

The soil (in small tube) in which wheat were grown was inoculated with the second stage juveniles (J2) at a population of 500 J2 per plant according to Dababat (2012) with some modification. Wheat cultivar (Bezostaya) susceptible to H. *filipjevi* was used in this experiment. Seeds were germinated on wet paper and sown in standard small tubes (16 cm in height × 2.5cm in diameter) filled with a sterilized mixture of sand, field soil, and organic material (70:29:1 v/v) which were maintained at 25 ± 2 °C in a growth chamber with a 16-hr photoperiod of artificial light and relative humidity of 70% (± 5). After 7 days, *H. filipjevi* J2 were added into the soil around the plant crown. The experiment was carried out as a completely randomized design with nine treatments and three replicates

Chemicals

Salicylic acid was obtained from Pasargad Novin Company (Iran) and its nano-formulations were fabricated with a surfactant-assisted ball milling process followed by a centrifugal separation. Standard solutions of different concentrations of formulated SA consisted of 62.5, 125 and 250µg/ml. Then the plants were sprayed using different concentration of nano-formulations of salicylic acid 5 days after seed germination.

Plant harvest

The plants were harvested at 63 days post inoculation and their roots were washed under slow running tap water for 1-2 min to remove the soil particles. The roots were sprayed with a strong water jet to dislodge white females and brown cysts that were collected on the lower sieve and counted under a stereomicroscope. The cysts were extracted from soil by Fenwick Can method technique (Fenwick, 1940). The data were analysed using MSTATC Software data statistical analysis and the means were grouped based on the least significant difference test.

Peroxidase activity assay

Peroxidase activity was assayed using the infected wheat roots at 4, 7 and 10 days post inoculation with *H. filipjevi* (Andres *et al.*, 2001; Delibes *et al.*, 2008; Simonetti *et al.*, 2010). Peroxidase activity was measured using Malik and Singh (1980) method. 0.5 Grams of infected plant root was extracted in 3ml of 0.1 M phosphate buffer at pH 7.0 by grinding root pieces in a pre-chilled mortar. The homogenate was then centrifuged at 18000g for 15min at 5 °C and the supernatant as enzyme source was stored on ice till the assay was carried out. The amount of 3ml of the buffer solution, 0.05ml guaiacol solution,

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0.1ml enzyme extract and 0.03ml hydrogen peroxide solution were pipetted out in a cuvette. The mixture was well shaken and placed in the spectrophotometer. The time required for the mixture optical density to be increased by 0.1 (Δt) at 436nm was recorded and used in the calculations. The enzyme specific activity units were calculated using the following formula:

g-1 f wt = [500/Δt] x [1/1000] x [TV/VU] x [1/f wt], where;

Δt = time change in minute; TV = total volume of the extract (ml); VU = volume used (ml); f wt = weight of the fresh root tissue (g).

Assay of Phenylalanine ammonia-lyase activity

Phenylalanine ammonia lyase activity was assayed using the infected wheat rootsat 4, 7 and 11 days post inoculation. Phenylalanine ammonia lyase was quantitatively determined at 290nm using a spectrophotometer following the Peltonen and Karjalainen (1995) method. Reaction mixture containing 2.5ml of 0.2% L-phenylalanine in 50mM Tris-HCl (pH 8.5), and 0.5ml of enzyme extract was incubated for 1 h at 40 °C after which, the optical density was recorded at 290nm. One unit of enzyme is defined as the amount of protein that catalyzed the appearance of 1µmol cinnamic acid/min at 30 °C.

Quantitative Real Time-PCR Analysis

To measure the expression of peroxidase genes (POX 113-114) in nematode infected wheat leaves at 4, 7 and 1١ days post inoculation, total RNA was isolated using the TRIZOL reagent according to the manufacturer's instructions (Gene all, South Korea). Traditionally, RNA is quantified by measuring UV absorbance using a spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Through measuring the optical density of RNA solution at wavelengths of 260 and 280 nm, it is possible to determine the concentration of solution as well as the presence of contaminants such as DNA, proteins and salts in the sample. A pure RNA sample has a 260/280 ratio range of 1.80 to 2.00. Total RNA was reverse transcribed into cDNA using a transcriptor first strand cDNA synthesis kit (Applied Biosystems), and quantitative real time PCR was carried outby using a LightCycler-FastStart DNA master SYBR Green I Kit (Applied Biosystems) and LightCycler apparatus (Roche Diagnostics).

The RT-PCR for peroxidase genes (POX 113-114) was carried out using the specific primers (Table 1). Actin gene was used to normalize the relative expression for genes of interest and calculated by $2^{\Delta\Delta CT}$ method and SYBR Green kit. The presence of the expected PCR products after quantitative real-time RT-PCR reactions was confirmed by an agarose gel electrophoresis.

Table 1 Specific primers for amplifying Peroxidase113, POX114 and Actin genes.

Genes	Primers	bp
Peroxidase 113	ATTGACAAACGAGTTACTGCCTACTAG (forward)	27
	GATTTGCTGCTGCTCGTACA (reverse)	20
Peroxidase 114	CGGTGACACCAACATCAACACTG (forward)	23
	CAGGAGCCCTTTCTGTGACAG (reverse)	21
Actin	GAAGCTGCAGGTATCCATGAGACC (forward)	24
	AGGCAGTGATCTCCTTGCTCATC (reverse)	23

Statistical analysis

The data was expressed as mean ± standard deviation (SD). One-way Analysis of Variance (ANOVA) and Turkey's post hoc-test were used to determine significant difference between groups ($P < 0.05$) in SPSS 20 software.

Results

According to Fig. 1, there was no significant difference in peroxidase activity of wheat exposed to 62.5µg/ml of nanosalicylic acid compared to control (without salicylic acid) and no difference was detected between

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peroxidase activity of wheat exposed to 250µg/ml of nanosalicylic acid in comparison with 125µg/ml of nanosalicylic acid at 4 and 7 days post inoculation (DAI) eitherl. However, peroxidase activity was significantly increased in the treatments exposed to 125 and 250µg/ml of nanosalicylic acid compared to 62.5µg/ml of nanosalicylic acid and control at 4 and 7 DAI ($P < 0.05$). There was also no statistically significant difference between the peroxidase activity of nanosalicylic acid at 62.5 and 125µg/ml compared with control at 11 DAI and no difference was found between peroxidase activity at 250 and 125µg/ml of nanosalicylic acid in comparison with 62.5µg/ml at 11 DAI. Although, peroxidase activity was increased considerably in treatments exposed to 250µg/ml of nanosalicylic acid at 11 DAI ($P < 0.05$).

As shown in Fig. 2, Phenylalanine ammonia lyase activity increased in the treatment exposed to 250µg/ml of nanosalicylic acid compared to 62.5 and 125µg/ml of nanosalicylic acid and control at 4 DAI ($P < 0.05$). No significant difference was detected between PAL activity of wheat exposed to 62.5 and 125µg/ml of nanosalicylic acid in comparison with control at 4 DAI. However, there was a significant increase in PAL activity in treatments of 125µg/ml of nanosalicylic acid compared to the other levels and control at 7 DAI ($P < 0.05$). The PAL activity substantially increased in the treatments exposed to 62.5 and 250µg/ml of nanosalicylic acid compared to control at 7 DAI ($P < 0.05$) and no significant difference was observed between these treatments at 7 DAI. No significant difference was observed in the PAL activity for different concentrations of nanosalicylic acid in comparison with control at 11 DAI.

Figures 3 and 4 show expression levels of peroxidase 113 and peroxidase 114 genes in wheat exposed to 62.5, 125 and 250µg/ml of nanosalicylic acid and at 4, 7 and 11 DAI, respectively. According to figures 3 and 4 nanosalicylic acid at 62.5µg/ml raised significantly the expression level of peroxidase 113-114 in wheat leaves at 4 DAI and there was also slight increase in their expression level compared to the control at 7 DAI. Although there was a significant decrease between genes expression exposed to 62.5µg/ml of nanosalicylic acid compared to the control at 11 DAI.

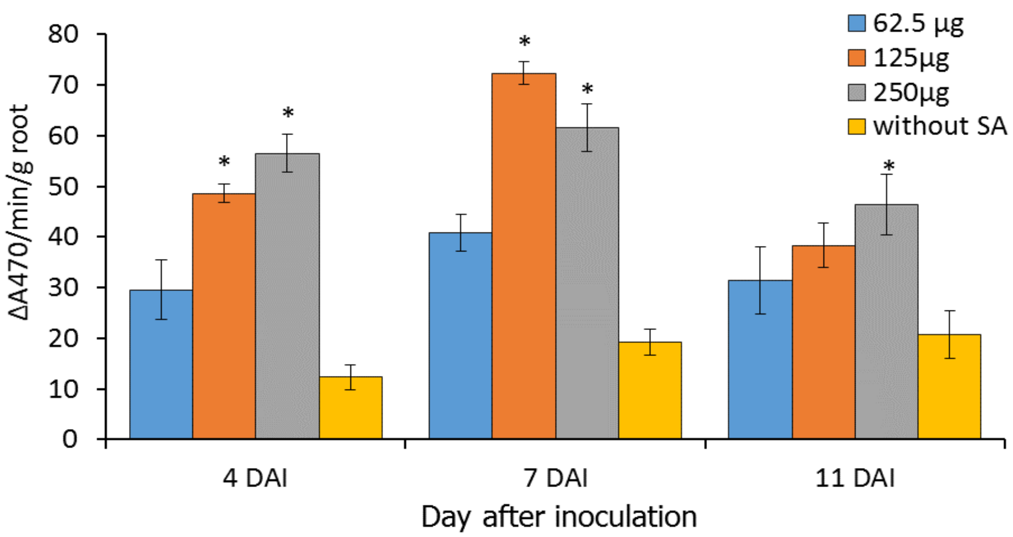

Figure 1 Effect of different concentrations of nanosalicylic acid on peroxidase activity in wheat cultivar Bezostaya susceptible to *Heterodera filipjevi* at 4, 7 and 11 days post inoculation. (*): Values are statistically significant at $P < 0.05$ compared to control group.

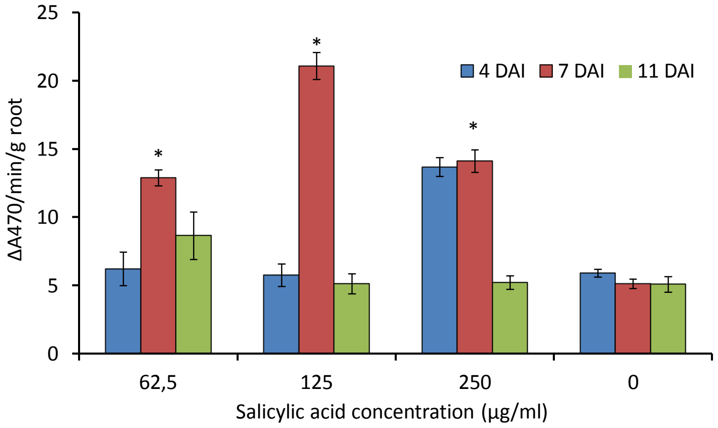

Figure 2 PAL activity of wheat cultivar Bezostaya susceptible to *Heterodera filipjevi* treated with different concentration of nano-salicylic acid at 4, 7 and 11 days after inoculation (DAI). (*): Values are statistically significant at P < 0.05 compared to control group.

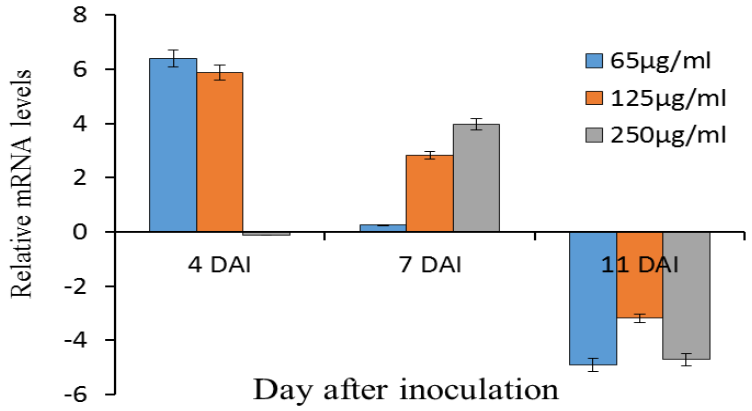

Figure 3 Effect of different concentrations of nanosalicylic acid on expression of peroxidase 113 gene on wheat cultivar Bezostaya susceptible to *Heterodera filipjevi* at 4, 7 and 11 days after inoculation (DAI).

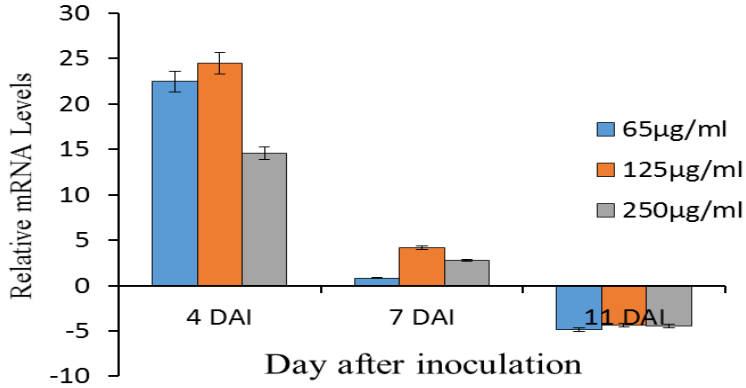

Figure 4 Effect of different concentrations of nanosalicylic acid on expression of peroxidase 114 gene on wheat cultivar Bezostaya susceptible to *Heterodera filipjevi* at 4, 7 and 11 days after inoculation (DAI).

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There was also significant increase between expression level of peroxidase 113-114 genes at 125µg/ml in comparison with control at 4 and 7 DAI and a significant reduction was observed in the expression level of peroxidase 113-114 exposed to 125µg/ml of nanosalicylic acid at 11 DAI.

Little reduction was found in the expression level of peroxidase 113 gene in 250µg/ml of nanosalicylic acid concentration in comparison with control at 4 DAI. Although, peroxidase 114 gene expression level significantly increased in the treatments with 250µg/ml of nanosalicylic acid at 4 DAI. There was also great increase in expression level of peroxidase 113-114 genes exposed to 250µg/ml of nanosalicylic acid compared with the control at 7 DAI. However, the expression levels of peroxidase 113-114 genes eased and considerably decreased in the treatment with 250µg/ml of nanosalicylic acid compared to control at 11 DAI.

According to table 2, there was significant decrease between the cyst number of wheat susceptible to nematode exposed to 62.5, 125 and 250µg/ml different concentrations of nano-salicylic acid compared to control (treated with nematode only).

Table 2 Analysis of variance for the cyst number in wheat cultivar Bezostaya susceptible to *Heterodera filipjevi* in response to different concentrations of nano-salicylic acid (SA).

variable	Number of samples	Sum	Mean	SD	SE	df
control	4.00	49.00	12.25	2.50	0.93	3
62.5µg/ml SA	4.00	17.00	4.25*	0.50	0.93	3
125µg/ml SA	4.00	16.00	4.00*	0.82	0.93	3
250µg/ml SA	4.00	32.00	8.00*	2.58	0.93	3

Coefficient of Variation = 26.10%.
*indicate significant differences at P < 0.05 compared to control.

Discussion

The results obtained from the greenhouse investigation proved that the different concentrations of salicylic acid cause a reduction in the cyst number of *H. filipjevi* in the susceptible wheat cultivar. In line with our investigation several reports indicate that SA induces plant immune system which can respond to various stresses and infections especially nematode infections (Asselbergh *et al.*, 2008;. Rejeb *et al*., 2014*).*

We have also shown that Spraying wheat leaves with nanoSA at high concentrations increases PAL activity at 4 DAI. However, different concentrations (62.5, 125 and 250µg/ml) of nanoSA had stronger effects on PAL activity at 7 DAI. Although, PAL activity did not significantly change in different concentrations of nanosalicylic acid at 11 DAI, demonstrating that high concentration of nanosalicylic acid may have inducible effects on PAL activity at 4 and 7 DAI whereby PAL activity was greatest in wheat roots sprayed with 125µg/ml of nanosalicylic acid at 7 DAI.

In agreement with our findings, there are some reports indicating that peroxidase and PAL are the essential enzymes involved in plant defense against stressors (War *et al*., 2011 a, b; Usha and Jyothsna, 2010) and that SA has been recognized to stimulate these enzymes in plants (Hu *et al*., 2009; Lu, 2009; Idrees *et al*., 2011). Previous study has demonstrated that the applied treatments of SA induce the activities of the PAL enzyme (Danaee *et al*., 2013). These enzyme activities could be correlated with nematode (*H. avenae*) infection in wheat whereby SA can boost the activity of peroxidase and PAL via reducing nematode penetration, final nematode population and its reproduction (Pokhare *et al*., 2012; Oka *et al*., 1997). Recent data have shown that wheat plants treated by SA had a significant increment in hydrogen peroxide and which seems to be related to increased superoxide dismutase and decreased catalase activities which SA may also generate oxidative stress/reactive oxygen species (ROS) in plants (Horváth *et al*., 2007;

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Makandar *et al*., 2012). In contrast to our findings, there are reports showing that salicylic acid has suppressing effect and can reduce the activity of peroxidase after 6, 10 and 20 days of application (Maksimov *et al*., 2014). Our results revealed that exposure of wheat leaves to lower concentrations (62.5 and 125µg/ml) of nano-salicylic acid enhances the peroxidase 113 and 114 genes expression levels at 4 and 7 DAI.

A few studies indicate that three of 13 class III peroxidases are significantly upregulated at 24 h after cereal cyst nematode infection, and their changes in relative expression were more than two-fold (Kong *et al*., 2015). In addition, There is an analytical research reporting that Genes encoding cytosolic forms of ascorbate peroxidase were induced in roots of both introgression wheat/Aegilops ventricosa H-93-8 line, carrying the Cre2 gene, and its parental line H-10-15 as susceptible control in response to nematode infection. (Simonetti *et al*., 2010).

The induction of the peroxidase activity in susceptible wheat roots could be correlated with physical and chemical barriers such as the cell wall, waxes, hairs, and secondary metabolites against pathogen which typically exhibit increased lignification, an oxidative burst, generation of cytotoxic compounds and induction of defense-related genes (Klotz *et al*., 1998). Genetic studies indicate that class III peroxidases activity in wheat roots carrying resistance genes may have greater inhibitory effects on cyst nematode infection (Andres *et al*., 2001; Montes *et al*., 2004). The induction of PAL activity in susceptible wheat roots could be correlated with production of SA via phenylpropanoid pathway (Wildermuth *et al*., 2001) and treating with nano-salicylic acid increases the PAL activity. PAL plays a central role in plant defense against invading pathogens such as bacteria, fungi, and viruses, exhibiting increases after infection by nematodes (Cui *et al*., 2001; Logemann *et al*., 2000; Vasyukova *et al*., 2009). The induction in the peroxidase expression level in susceptible wheat roots is dependent on the ROS production level which salicylic acid plays a key role in maintenance of the ROS levels and is necessary for peroxidase activity (Xu *et al*., 2017). Although, previous research has shown that systemic increase of H2O2 can increase peroxidase expression levels at 7 DAI in leaves of the susceptible line infected by the *H. avenae* that would occur as a result of lower Ascorbate peroxidase activity in roots of this line (Simonetti *et al*., 2010). Also several hypotheses have been formulated to explain why the function of salicylic acid in peroxidase expression has not been clearly understood. Our findings indicated that higher nanosalicylic acid concentrations have potential effects on peroxidase and PAL activities in wheat infected by *H. filipjev*i which may be involved in defense mechanisms in plants. We also demonstrated that lower nanosalicylic acid concentrations may induce peroxidase 113-114 expression level in wheat leaves infected by nematode and that high concentration of nano salicylic acid had inhibitory effects on peroxidase gene expression level. In conclusion, the results suggest that SA may play a putative role as a possible signaling component in the case of the infection of plants with nematodes using a model system consisting of wheat.

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تأثیر فرمولاسیون نانوسالیسیلیک اسید بر بیان ژن‌های پراکسیداز (۱۱۳-۱۱۴) و پراکسیداز و فنیل‌آلانین آمونیالیاز در گندم رقم حساس به *Heterodera filipjevi*

نجمه غزلباش[۱]، ناصر پنجه‌که[۱*]، زهرا تنهامعافی[۲]، سیدکاظم صباغ[۳]، محمد سالاری[۱] و محسن اسماعیل‌زاده[۴]

۱- گروه گیاه‌پزشکی، دانشکده کشاورزی، دانشگاه زابل، زابل، ایران.
۲- مؤسسه تحقیقات گیاه‌پزشکی کشور، سازمان تحقیقات، آموزش و ترویج کشاورزی، تهران، ایران.
۳- گروه زیست‌شناسی، پردیس علوم، دانشگاه یزد، یزد، ایران.
۴- مؤسسه تحقیقات اصلاح و تهیه نهال و بذر، سازمان تحقیقات، آموزش و ترویج کشاورزی، کرج، ایران.
پست الکترونیکی نویسنده مسئول مکاتبه: naserpanjehkeh@gmail.com
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چکیده: اثر فرمولاسیون نانوسالیسیلیک اسید بر بیان ژن‌های پراکسیداز (۱۱۳-۱۱۴) و آنزیم‌های پراکسیداز و فنیل‌آلانین آمونیالیاز (PAL) در گیاهان گندم رقم حساس به *Heterodera filipjevi* مورد بررسی قرار گرفت. ریشه و برگ گندم B ezostaya به‌طور تصادفی به گروه شاهد و گروه‌های در معرض به‌ترتیب با ۶۲٫۵، ۱۲۵ و ۲۵۰ میکروگرم در میلی لیتر نانوسالیسیلیک اسید تقسیم شدند. آنالیز اسپکتروفتومتری عصاره‌های ریشه‌ی گیاهان آلوده، ۴ ، ۷ و ۱۱ روز پس از تلقیح نماتد (DAI) برای ارزیابی آنزیم‌های پراکسیداز و PAL و آنالیز بیان ژن‌های پراکسیداز (۱۱۳-۱۱۴) با استفاده از Realtime-PCR انجام شد. فعالیت پراکسیداز به‌طور معنی‌داری در تیمارهای تحت تأثیر ۲۵۰ میکروگرم در میلی‌لیتر نانوسالیسیلیک اسید پس از ۱۱ روز اافزایش داشت. فعالیت PAL تحت تأثیر ۲۵۰ و ۱۲۵ میکروگرم در میلی‌لیتر نانوسالیسیلیک اسید در مقایسه با کنترل در چهار و هفت DAI افزایش یافت. فعالیت PAL نیز در نمونه‌های در معرض ۶۲٫۵ و ۲۵۰ میکروگرم در میلی‌لیتر نانوسالیسیلیک اسید نسبت به کنترل در هفت DAI تشدید شد..دوز ۶۲٫۵ میکروگرم در میلی‌لیتر نانوسالیسیلیک اسید باعث افزایش سطح بیان پراکسیداز ۱۱۳-۱۱۴ در برگ گندم در چهار DAI و هم‌چنین افزایش سطح بیان ژن‌های پراکسیداز ۱۱۳-۱۱۴ در سطح ۱۲۵ و ۲۵۰ میکروگرم در میلی‌لیتر در مقایسه با کنترل در چهار و هفت DAI شد. بین بیان ژن‌ها در معرض ۶۲٫۵، ۱۲۵ و ۲۵۰ میکروگرم در میلی‌لیتر نانوسالیسیلیک اسید در مقایسه با کنترل در یازده DAI کاهش معنی‌داری وجود داشت. نتایج نشان داد که غلظت‌های بالای نانوسالیسیلیک اسید تأثیر بالقوه‌ای بر فعالیت آنزیم‌های پراکسیداز و PAL در گندم آلوده به *H. filipjevi* دارد و غلظت بالای نانوسالیسیلیک اسید اثر مهاری بر سطح بیان ژن پراکسیداز دارا می‌باشد.

واژگان کلیدی: نماتد سیستی غلات، گندم، پراکسیداز، سالیسیلیک اسید

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