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GENOTYPIC AND BIOCHEMICAL VARIATION IN THE RESPONSE OF BARLEY TO THE ROOT-KNOT NEMATODE (*MELOIDOGYNE JAVANICA*) AT SEEDLING STAGE

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ABSTRACT

The present study was conducted to evaluate 28 commercial cultivars and two promising breeding lines of barley in terms of resistance to *Meloidogyne javanica* nematode and to investigate the synthesis level of peroxidase, superoxide dismutase, catalase and polyphenol oxidase enzymes in different days after inoculation in greenhouse condition. Based on the results of evaluation, Jolge and Nimrouz cultivars were highly resistant, Rihan and Zarjow were very susceptible. The rest of the cultivars were ranked between these groups in resistant, moderately resistant and moderately susceptible groups. Comparison of the activity of antioxidant enzymes in highly resistance cultivars (Jolge and Nimrouz) and very susceptible cultivars (Rihan and Zarjow) roots showed that the activity of peroxidase, superoxide dismutase and polyphenol oxidase increased in the root of the highly resistant cultivars and decreased or remains unchanged in very susceptible cultivars. Nevertheless, catalase showed a decreasing trend after inoculation by nematodes in highly resistance cultivars, and in general; its level in resistant cultivars was less than susceptible cultivars. Therefore, changes in the activity of these enzymes can be attributed to different levels of resistance among these cultivars.

Keywords: Barley cultivars, Enzymes, *Meloidogyne javanica*, Resistance, Susceptible.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the most important agricultural crops and ranked as fourth most important cereal crops after wheat, corn and rice in terms of global production (Czembor *et al.*, 2007). Internal parasitic nematodes are very important plant parasitic nematodes in agriculture that impose a lot of damage annually to agricultural crops in different regions of the world (Abad *et al.*, 2003). The most important plant parasitic nematodes belong to the genus *Meloidogyne* (Moens *et al.*, 2009). This group of nematodes secrete substances that cause hypertrophy and hyperplasia conditions in the root parenchyma after entering the host roots. *M. javanica* is considered the most important species in the *Meloidogyne* genus (Dhandaydham *et al.*, 2008; Caillaud *et al.*, 2008). One of the best management stratagies in an integrated pest management program is using nematode resistant cultivars (Suzuki *et al.*, 2012). In some reports, barley is introduced as a resistant crop to the RKNs that can be sown in contaminated lands to reduce the population of RKNs (Karajeh *et al.*, 2011; Harris and Maramorosch, 2013). Various interactions have been studied between the host plants and RKNs. EL-Mesalamy (2013) examined resistance of some field crops such as wheat, barley and broad bean to RKN, *M. javanica*. They demonstrated a significant reduction in root and shoot fresh weight in all tested plants and slight resistance was mentioned for barley cultivars. In another study, the resistance of barley and wheat cultivars to RKNs, *Meloidogyne* spp. were examined and it was concluded that all barley cultivars were resistant to *M. arenaria* but susceptible or moderately susceptible to *M. javanica* (Ibrahim *et al.*, 1988). The resistance of some plant cultivars to RKNs has been the

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subject of several studies (Opperman *et al.*, 1988; Kaloshian *et al.*, 1989; Hussain *et al.*, 2016; Kayani and Mukhtar, 2018).

Nematode damages, as well as other stressors can also stimulate the production of free intracellular radicals of oxygen, including superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl (Sairam and Tyagi, 2004). These compounds incur a lot of damages via oxidation of lipids, proteins, and nucleic acids into cells (Blokhina *et al.*, 2003). Several proteins are produced in the resistant host after the invasion of pathogens that interfere in resistance mechanisms (Mehta *et al.*, 2008). PROX, PPO, CAT, and SOD have a very important role in response to various stressors that would collect produced oxygen free radicals at stress time and by various oxidations take part in defense mechanisms of the plant (Mittler, 2002). In other words, plants infection by a pathogen alters the activity of oxidizing and hydrolyzing enzymes (Mohammadi and Kazemi, 2002). Several reports exist regarding the association between increased activities of oxidizing enzymes, especially PROX (Rani *et al.*, 2008; Arun *et al.*, 2010; Mahdy and Sally, 2011; Anjum *et al.*, 2012). Usually, the activities of these enzymes in the infected resistant tissues are more than infected tissues of susceptible cultivars (Sankar *et al.*, 2017). The main objective of the current research was to find the sources of RKN resistance among commercial cultivars and promising breeding lines of barley. Furthermore, to determine the relationship between the levels of resistance with the antioxidant enzymes synthesis. Such studies have been conducted on other plants including tomatoes (Singh and Khurma, 2007), cucumbers (Aboulipour *et al.*, 2011), cowpea (Oliveira *et al.*, 2012), Okra (Mukhtar *et al.*, 2014) and Sweet Potato (Karuri *et al.*, 2017), but so far, no comprehensive study has been conducted on barley varieties for resistance to root-knot nematodes in Iran. The main objective of this study was to investigate resistance levels of 28 commercial cultivars and two promising breeding lines of barley to *Meloidogyne javanica* through the study of growth and pathogenicity factors. In addition, after identifying susceptible and resistant varieties, the activity of enzymes related to resistance was investigated, to determine which of these enzymes and to what extent they have contributed to the of resistance phenotype.

MATERIALS AND METHODS

Plant materials: In this experiment, 28 commercial cultivars and 2 promising breeding lines of barley were used to evaluate their reaction to *M. javanica* (that is improved by morphometric and molecular methods). Details of these cultivars and lines are presented in (Table 1).

Nematodes population: In order to prepare the initial population of the nematode (*M. javanica*), sampling was done from infected samples in greenhouses in the Kashmar city from Khorasan Razavi province, northern East Iran. Samples were placed in special plastic bags, then along with ice, they were transferred to the laboratory and stored at 4°C.

Morphological and Molecular identification: Nematodes were first extracted from soil samples by using the Jenkins (1964) method and fixed by using the method of De Grisse (1969). Permanent slides were taken to evaluate the morphological and morphometric characteristics of the samples. The samples were identified using the Taylor and Netscher (1974) modified method. The slices of the cuticular network of the adult female body were prepared based on the method suggested by Hartman and Sasser (1985), and the second instar larvae were identified using Jepson (1987) method. The morphometric characterization of nematodes was conducted by using the Olympus CX41 microscope equipped with a drawing tube, also necessary morphological characteristics factors to identify the species were measured. Then species were identified by valid keys.

To confirm the accuracy of identified species, molecular analysis was performed based on the Ghaderi *et al.* (2014) method. A single live nematode from pure culture (population of *M. javanica* was reared with single egg-mass inoculation on young tomato seedling for pure culture) was selected and transferred to a small drop of AE buffer (10 mM Tris-Cl, 0.5 mM EDTA; pH 9.0) on a clean slide and crushed by using a clean slide cover. The suspension was collected from the slide by adding 20 µl AE buffer. DNA samples were kept at −20°C until being used as PCR templates. The optimal thermo-cycling conditions include three phases as follows: initial denaturation at 95 °C for 4 min; 35 amplification cycles (94 °C for 30 s; 52 °C for 40 s; 72 °C for 80 s); and final extension at 72 °C for 10 min. PCR products were cleaned and send for sequencing (Table 2).

Table 1. Barley Cultivars and lines used in this study.

No.	Cultivar/line	Pedigree	Growth habit	Year of release
1	Makouee	Star	Winter	1990
2	Bahman	WA 2196-68/NY6005-18, F1//Scotia I	Winter	2008
3	Yousouf	Lignee 527/Chn-01//Gustoe/4/Rhn-08/3/Deir Alla 106//Dl71/Strain 205	Spring	2009
4	Nosrart	Karoon/Kavir	Facultative	2008
5	Nik	Lignee 527/NK1272//JLB 70-63	Spring	2011
6	Behrokh	Novosadski-444	Spring	2013
7	Fajre-30	Lignee131/ Gerbel//Alger-Ceres	Facultative	2008
8	Rihan	Rihane	Spring	1994
9	Nimrouz	Trompillo	Spring	2008
10	Sahra	LB.Iran/Una8271//Gloria"S"/Com"S"	Spring	2003
11	Jonoub	Gloria "S "/Copal "S "	Spring	1997
12	Zahak	Poa/Hjo/Ojina	Spring	2012
13	Walfajre	CI-108985	Facultative	1985
14	Kavir	Arivat	Facultative	1979
15	Zarjow	Landrace (Hamedan)	Facultative	1949
16	Aras	Arumir	Spring	1988
17	Loot	Congona/Borr	Spring	2012
18	Rihan-03	Rihan-03	Spring	-
19	Karoon	Strain 205	Spring	1980
20	Dasht	Probestdwarf	Facultative	1993
21	Torkman	Rihane "S "-04	Spring	1993
22	Torsh	Landrace (Khorasan)	Spring	-
23	Afzal	Chah Afzal (Landrace Yazd)	Facultative	1996
24	Govharan	Rhn-03//L.527/NK1272	Spring	2016
25	Khatam	LB.Iran/Una 8271//Gloria"s"/Com"s"/3/Kavir	Facultative	2016
26	Mehr	Roho/Mazorka//Trompi	Facultative	-
27	CB-91-8	Makouee/C.C89//Rihane"s"/3/Roho/Mazurka	Winter	-
28	CB-91-10	Torsh/Lgia	Winter	-
29	Jolge	Makouee//Zarjow/80-5151	Winter	2015
30	Mahtab	Berake-54	Winter	-

Table 2. Sequence of 18s primer.

Primer name	5'-3'	Gene region	Reference
1813F (forward)	CTGCGTGAGAGGTGAAAT	18s	Holterman *et al*., 2008
2646R (reverse)	GCTACCTTGTTACGACTTTT	18s	Holterman *et al.*, 2008

Mass propagation of nematode: Susceptible tomato cultivar (Mobil) was planted in sterilized (autoclaved for one hour at 121°C and 2.1 atmospheric pressure) medium of farm soil, peat and sand (1:1:1) for proliferation of RKNs. Mass and purified cultivation were obtained according to Fassuliotis (1979) method. In order to extract inoculum, Hussey and Barker (1973) method was used. Holes were made near the root of each plant and 2000±10 juveniles were inoculated when the barleys were in 3-4 leaf period. Plants were placed at a temperature of 25 to 30°C in the greenhouse.

INOCULATION EXPERIMENTS AND STATISTICAL ANALYSES

The experiment was performed in a completely randomized design with three replications for each barley genotypes. The same conditions were also used for control treatments. For the measurement of parameters, 60 days after inoculation, plants with minimal damage to their roots were taken out of the pot and after washing the roots, growth factors like root and shoot length and their fresh/dry weight (Kayani *et al*., 2018; Mukhtar, 2018) were measured.

Nematode induced factors such as the number of larvae per 100 grams of soil around the roots, egg number per gram root, and the number of galls and bags in per gram root (Whitehead and Hamming, 1965; De Grisse, 1969; Taylor and Sasser, 1978) were performed. The final population of nematodes in the soil was obtained by De Grisse (1969) method. Whitehead and Hamming (1965) method was used in centrifugal flotation and the nematode reproduction factor was conducted by dividing the final population to the initial population. Indexing ratings to the gall per gram of roots was according to Taylor and Sasser method (1978).

Determination of antioxidants enzymes: Total protein was extracted based on the method described by Kar and Mishra (1976). To achieve this, root tissues were sampled every day and directly used for the extraction of protein. For each sample, 200 mg of tissue was ground in liquid nitrogen using a mortar and pestle. Then 3 ml of 100 mM sodium phosphate buffer (pH 6.8) was added and properly mixed. Then the samples were centrifuged at 17000×g for 15 min at 4°C. The supernatant (enzyme extract) was transferred to new microtube on ice until assayed. Protein concentration was measured using Bradford method, in which bovine serum albumin (BSA) was used as a standard protein (Bradford, 1976).

Superoxide Dismutase (SOD): Total SOD activity was measured using the method described by Yu and Rengel (1999). Illumination of riboflavin in the presence of O_2 and electron donor (like methionine) produces superoxide anion (O^{2-}). The O^{2-} reacts with NBT and reduction of NBT with O^{2-} produces a blue coloured formazan that can be measured at the wavelength of 560 nm. The principle of SOD activity assay was based on the inhibition of NBT photochemical reduction. Percentage of inhibition was calculated using the formula described by Abrahim *et al.*, (2012). In SOD activity assay, the reaction mixture consisted of 100 µl EDTA (1 mM), 100 µl HEPES (500 mM), 100 µl Na_2CO_3 (500 mM, pH 10.4), 100 µl methionine (130 mM), 100 µl Triton X-100 (0.25% w/v), 10 µl NBT (7.5 mM), 50 µl enzyme extract in 430 µl sodium phosphate buffer (100 mM, pH 6.8) and 10 µl riboflavin (200 µM) as starter of reaction. After incubation under fluorescent light for 10 min, A560 was measured for each sample. One SOD unit is known as the amount of enzyme that inhibited the rate of NBT reduction by 50%. The SOD activity was expressed as U SOD mg^{-1} protein (Beauchamp and Fridovich, 1971).

Peroxidase (PROX): PROX activity was measured using guaiacol as electron donor substrate. The reaction contained 955 µl potassium phosphate buffer (50 mM, pH = 6.8), 15 µl guaiacol (900 mM), 20 µl H_2O_2 (400 mM) and 10 µl enzyme extract. The reaction was initiated by adding H_2O_2 and guaiacol oxidation was measured at 470 nm using spectrophotometer. Enzyme activity, expressed as µmol min^{-1} mg^{-1} protein of oxidized guaiacol, was calculated using the extinction coefficient of 26.6 mM^{-1} cm^{-1} for guaiacol (Lin and Kao, 1999).

Catalase (CAT): In order to measure CAT activity, the method described by Aebi (1984) was used. The reaction mixture contained 20 µl enzyme extracts, 960 µl phosphate buffer (50 mM, pH 6.8) and 20 µl H_2O_2 (100 mM). It was recorded spectrophotometrically at 240 nm, expressed as µmol min^{-1} mg^{-1} protein and calculated by means of the extinction coefficient of 36 mM^{-1} cm^{-1} for H_2O_2.

Polyphenol Oxidase (PPO): For PPO assay, catechol solution (0.3 ml) and phosphate buffer (2.5 ml) were placed in a cuvette and measured using spectrophotometer at 495 nm. The enzyme extracts (200 µl) were added to the solution obtained from the last phase and the change in absorbance was recorded for every 30 s up to 5 min. One unit of catechol oxidase or lactase is defined as the amount of enzyme that transforms one µmole of dihydrophenol to one µmole of quinine/minute (Esterbauer *et al.*, 1977).

Data were analyzed by ANOVA and mean comparisons were made using LSD test at probability level of $P<0.05$ using SPSS software version 23.

Hierarchical cluster analysis of resistance of 28 commercial barley cultivars and two promising breeding lines against root-knot nematode were made with SPSS based on their field performances.

RESULTS

Barley cultivars demonstrated different responses ($P<0.05$) to *M. javanica* (Table 3). The population of *M. javanica* multiplied on all the cultivars as indicated by the number of galls, reproduction factors, change in plant length and fresh/dry weight compared to control (Table 4). Presence of galls and egg-masses on the root of all barley cultivars showed that none of them was immune to RKN infection. The root galls among thirty cultivars were variable in number and size.

Table 3. Analysis variance of nematodes induced and growth parameters.

Sources changes	DF	Mean squares				
		Nember of gall in per gram root	RF	Change plant fresh weight (%)	Change plant dry weight (%)	Change plant length (%)
Cultivar	29	1197.11 **	4.92 **	680.74 **	1140.63 **	79.72 **
Error	60	103.65	1.12	0.607	1.75	4.27

**significant at P<0.01.

Assessment of root galls, RF and growth factors: Cluster analysis was based on nematode induced and growth factors (Table 4) and cultivars were divided into 6 categories by SPSS software (Figure 1). Rihan cultivar was ranked in a very susceptible category because it demonstrated the highest susceptibility compared to other 29 cultivars, with the highest number of gall (103.48) as well as the highest amount of reproduction factors (7.45). Moreover, fresh/dry weight and plant height in this cultivar showed the highest percentage of change compared to control (70.51, 80.31, and 28.31%, respectively) (Table 4). Zarjow was placed in the second category in terms of susceptibility to *M. javanica* than the rest of the cultivars. On the contrary, Jolge and Nimrouz cultivars, showed the highest resistance to *M. javanica* and the disease could not affect them a lot. Jolge had the lowest number of gall (19.06) and reproduction factors (1.53) when compared with other cultivars, and its fresh/dry weight and height were not significantly different from its control (2.12, 4.76 and 2.14%, respectively) (Table 4). Jolge and Nimrouz can be described as the most resistant cultivars that are classified in the highly resistant category. A large number of cultivars showed median behaviour, such that they are ranked as resistant and moderately resistant categories including 18 cultivars. Slightly susceptible category including six cultivars and disease had a negative effect on their growth compared to the previous category. These cultivars had a higher number of gall and reproduction factor of nematode (Figure 1).

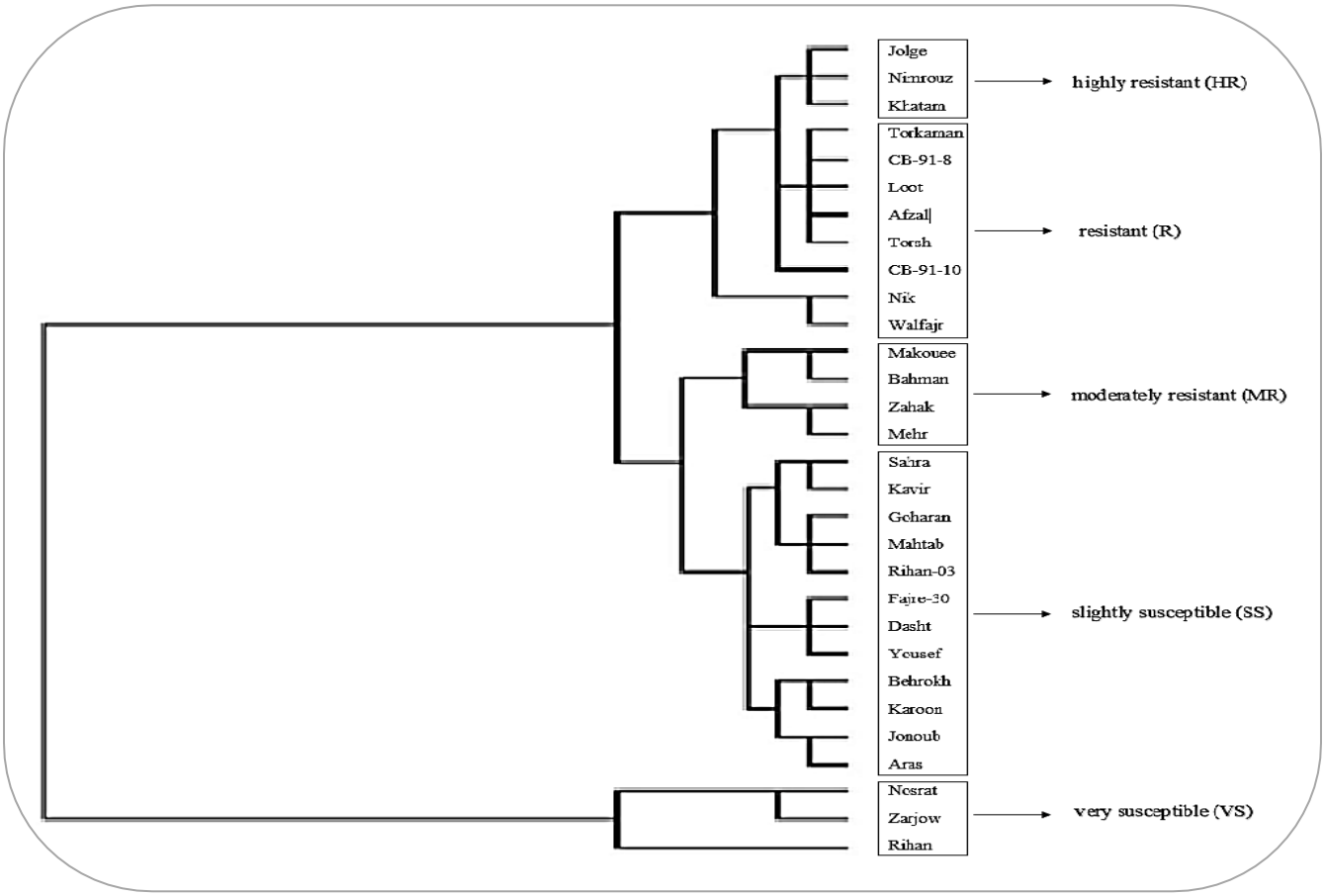

Figure 1. Tree diagram for comparing the Interaction of nematode induced and growth factors on barley cultivars and lines.

Table 4. Influence of *M. javanica* on barley cultivars and lines under greenhouse conditions.

No.	Cultivar	nematodes induced parameters		growth parameters**		
		Number of gall in per gram root	RF*	Change plant fresh weight (%)	Change plant dry weight (%)	Change plant length (%)
1	Makouee	64.58 ± 8.08^{bcde}	2.38 ± 0.13^{efghi}	20.61 ± 1.63^{hi}	49.27 ± 0.71^{e}	2.97 ± 0.99^{hi}
2	Bahman	61.12 ± 6.01^{bcdef}	2.03 ± 0.24^{fghi}	26.17 ± 1.03^{f}	57.17 ± 0.40^{d}	2.38 ± 1.03^{hi}
3	Yousef	69.87 ± 12.61^{bc}	2.30 ± 0.32^{efghi}	41.46 ± 0.72^{c}	23.93 ± 1.72^{k}	6.56 ± 2.84^{cdefg}
4	Nosrart	75.50 ± 16.62^{b}	2.69 ± 0.97^{efghi}	42.47 ± 0.58^{c}	65.20 ± 0.33^{c}	9.05 ± 0.82^{c}
5	Nik	30.15 ± 2.7^{jklm}	2.34 ± 0.45^{efghi}	37.51 ± 0.63^{d}	17.82 ± 1.03^{mn}	5.42 ± 0.72^{defghi}
6	Behrokh	73.44 ± 10.11^{b}	3.15 ± 0.59^{defghi}	24.57 ± 1.30^{g}	37.15 ± 0.78^{g}	4.35 ± 1.45^{fghi}
7	Fajre-30	63.59 ± 9.78^{bcdef}	2.42 ± 0.62^{efghi}	33.07 ± 0.66^{e}	10.11 ± 0.87^{pq}	3.60 ± 0.78^{ghi}
8	Rihan	103.48 ± 7.47^{a}	7.45 ± 1.30^{a}	70.51 ± 1.01^{a}	80.31 ± 0.38^{a}	28.31 ± 0.79^{a}
9	Nimrouz	21.70 ± 6.11^{lm}	1.75 ± 0.41^{hi}	8.13 ± 0.18^{o}	7.91 ± 1.47^{r}	2.25 ± 0.00^{i}
10	Sahra	68.49 ± 13.03^{bcd}	4.48 ± 1.59^{bcd}	12.47 ± 0.41^{m}	16.07 ± 1.56^{no}	4.97 ± 3.76^{defghi}
11	Jonoub	62.23 ± 10.55^{bcdef}	3.65 ± 0.82^{cdef}	33.14 ± 0.82^{e}	33.16 ± 0.19^{h}	5.73 ± 3.93^{cdefgh}
12	Zahak	55.31 ± 11.42^{cdefg}	5.08 ± 1.90^{bc}	12.09 ± 0.22^{m}	43.01 ± 0.56^{f}	3.95 ± 3.53^{fghi}
13	Walfajr	32.01 ± 8.05^{jklm}	3.60 ± 1.29^{cdefg}	33.49 ± 0.87^{e}	28.06 ± 2.24^{j}	7.96 ± 2.28^{cde}
14	Kavir	67.16 ± 10.08^{bcd}	3.80 ± 1.90^{bcde}	16.99 ± 0.35^{k}	14.77 ± 3.14^{o}	2.92 ± 0.72^{hi}
15	Zarjow	76.09 ± 6.44^{b}	5.43 ± 1.64^{b}	56.02 ± 0.93^{b}	70.45 ± 0.49^{b}	17.26 ± 2.73^{b}
16	Aras	49.69 ± 14.92^{efghi}	2.13 ± 0.12^{efghi}	36.44 ± 0.90^{d}	30.96 ± 1.44^{i}	4.58 ± 0.72^{efghi}
17	Loot	32.13 ± 4.39^{jklm}	2.51 ± 1.62^{efghi}	14.62 ± 0.40^{l}	16.36 ± 0.39^{no}	3.62 ± 0.63^{fghi}
18	Rihan-03	53.32 ± 6.33^{defgh}	1.92 ± 0.94^{ghi}	19.99 ± 0.34^{ij}	25.39 ± 0.75^{k}	3.62 ± 1.66^{fghi}
19	Karoon	65.44 ± 13.97^{bcd}	3.40 ± 1.29^{cdefgh}	21.36 ± 0.11^{h}	33.77 ± 0.58^{h}	8.19 ± 1.01^{cd}
20	Dasht	61.79 ± 12.59^{bcdef}	2.12 ± 0.62^{efghi}	34.03 ± 0.50^{e}	17.91 ± 0.26^{mn}	8.16 ± 4.08^{cd}
21	Torkaman	38.02 ± 11.60^{hijk}	2.83 ± 1.64^{defghi}	15.42 ± 0.95^{l}	16.31 ± 1.36^{no}	5.73 ± 0.90^{cdefgh}
22	Torsh	29.84 ± 5.49^{jklm}	2.42 ± 0.56^{efghi}	10.73 ± 0.56^{n}	21.18 ± 2.04^{l}	3.92 ± 3.70^{fghi}
23	Afzal	35.25 ± 4.61^{ijkl}	3.60 ± 0.52^{cdefg}	14.51 ± 0.98^{l}	11.40 ± 0.70^{p}	3.29 ± 0.81^{ghi}
24	Goharan	49.82 ± 1.02^{efghi}	3.82 ± 0.99^{bcde}	17.47 ± 1.17^{k}	11.41 ± 0.95^{p}	4.17 ± 0.66^{fghi}
25	Khatam	25.51 ± 4.13^{klm}	1.75 ± 0.50^{hi}	8.49 ± 0.17^{o}	9.03 ± 2.24^{qr}	3.29 ± 2.15^{ghi}
26	Mehr	43.81 ± 4.36^{ghij}	2.22 ± 1.23^{efghi}	10.53 ± 0.66^{n}	41.52 ± 0.28^{f}	5.40 ± 3.70^{defghi}
27	CB-91-8	40.81 ± 10.14^{ghijk}	2.43 ± 0.80^{efghi}	17.14 ± 0.61^{k}	19.30 ± 1.14^{lm}	8.99 ± 0.92^{c}
28	CB-91-10	25.86 ± 9.66^{klm}	3.37 ± 1.46^{cdefgh}	19.21 ± 0.83^{j}	27.84 ± 1.78^{j}	8.05 ± 1.00^{cd}
29	Jolge	19.06 ± 5.44^{m}	1.53 ± 0.37^{i}	2.12 ± 0.76^{p}	4.76 ± 1.76^{s}	2.14 ± 0.74^{i}
30	Mahtab	48.26 ± 12.97^{fghi}	2.38 ± 0.70^{efghi}	26.35 ± 0.65^{f}	16.35 ± 1.68^{no}	6.99 ± 0.93^{cdef}

*Final population/initial population.

**Growth parameters = Changes in growth parameters relative to the control in term of percentage.

Enzymes assay: After evaluating the resistance of the cultivars to the RKN, Jolge and Nimrouz cultivars as highly resistant and Zarjow and Rihan as susceptible to very susceptible cultivars were selected, then they were considered for enzyme assay. CAT, PROX, SOD, and PPO studied revealed that there is a significant difference among susceptible and resistant genotypes due to enzyme secretion in the roots (Table 5).

Table 5. Analysis variance of resistance enzyme activity.

Sources changes	DF	Mean squares			
		Peroxidase	Polyphenol oxidase	Superoxide dismutase	Catalase
Cultivar	3	41123.40**	0.0093**	13.551**	0.726**
Time	3	9096.12**	0.0035**	3.838**	0.662**
Cultivar × Time	9	7193.28**	0.0021**	2.178**	0.055**
Error	32	2.20	0.0000067	0.0092	0.00022

**significant at P<0.01.

Peroxidase activity: In the resistant cultivars (Jolge and Nimrouz), activity of PROX enzyme increased significantly after inoculation with nematode and peaked seven days after inoculation, but in susceptible cultivars, PROX activity decreased after inoculation, such that in Rihan cultivar, the enzyme activity increased on the third day after inoculation (82.56), and decreased on the fifth (49.95) and seventh days (41.35). In Zarjow, the amount of enzyme decreased in each stage (107.24, 89.95, 83.21 and 76.86, respectively) (Figure 2).

Polyphenol oxidase activity: The highest amount of PPO enzyme was observed in Jolge 7 days after inoculation (0.205). PPO enzyme in resistant cultivars (Jolge and Nimrouz) decreased on the third day after inoculation and increased on the fifth and seventh days, while in the susceptible cultivars of Rihan and Zarjow, this enzyme decreased along with a slight gradient (Figure 2).

Superoxide dismutase activity: Enzyme activity was similar to PPO, such that an increasing trend in this enzyme was observed in Jolge and Nimrouz cultivars, while a decreasing trend for enzyme was seen in Zarjow and Rihan cultivars (Figure 2).

Catalase activity: Enzyme activity showed a different behaviour compared to the other studied enzymes. On the first day after inoculation with nematode, the amount of CAT enzyme in Jolge and Nimrouz, was the lowest compared to Rihan and Zarjow. After this time, the amount of enzyme decreased in all cultivars on the third, fifth and seventh days after inoculation, but this decline was higher in resistant cultivars (Figure 2).

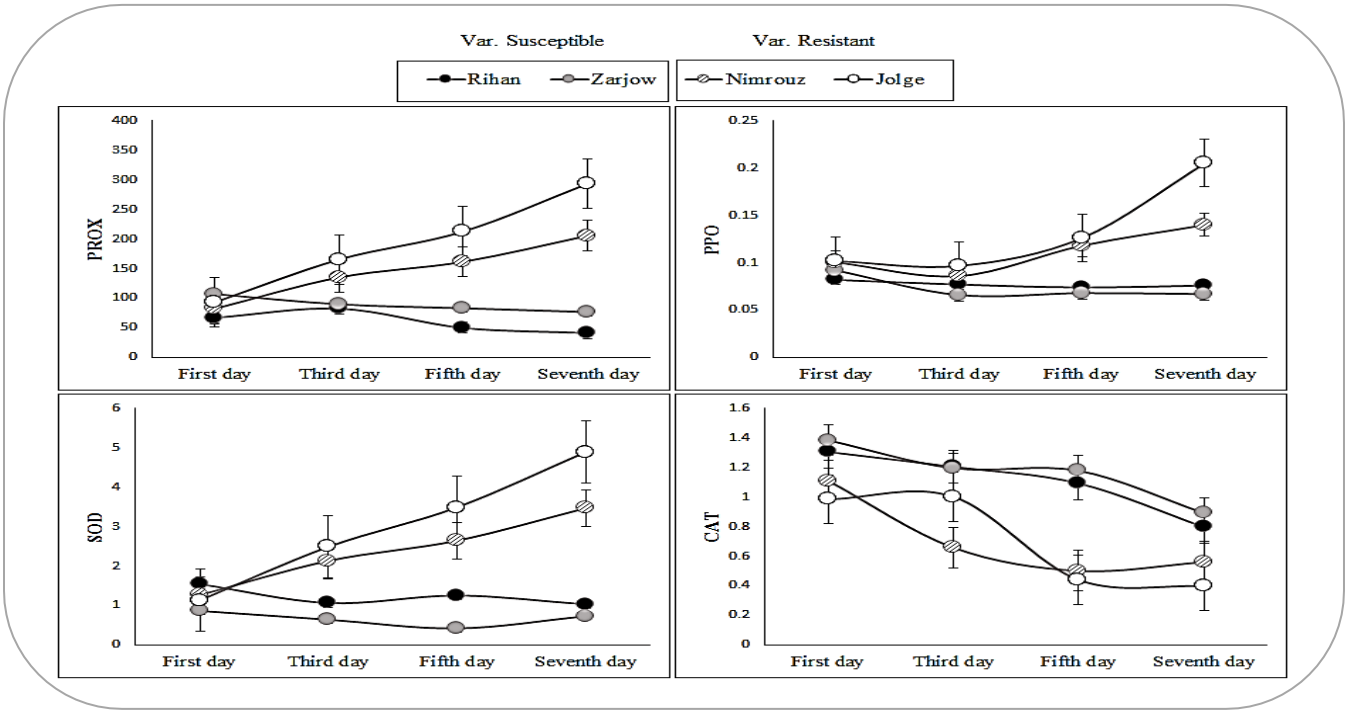

Figure 2. Enzyme activity (Catalase, Peroxidase, Polyphenol Oxidase and Superoxide Dismutase) in resistant and susceptible barley cultivars inoculated with *M. javanica*.

DISCUSSION

All cultivars showed variability in their response to *M. javanica* infestation. None of the cultivars and lines was immune against nematode and the disease influenced on all cultivars and lines; however, different levels of resistance were observed among cultivars and lines. There were significant differences in infection levels between cultivars, such that Jolge and Nimrouz cultivars showed the highest resistance to *M. javanica* and Rihan and Zarjow showed the highest sensitivity to the nematode. The number of nematodes in the root system of Jolge decreased dramatically over the course of the experiment compared with Rihan, suggesting that the nematodes cannot reproduce in roots of the highly resistant cultivars or might have migrated out of the tissues. Jolge with characteristics such as winter growth, premature breeding, the height of 90 cm, and resistance to lying and grain seed, powdery mildew (*Blumeria graminis*) and yellow rust (*Puccinia striiformis*) is considered an appropriate cultivar in most parts of Iran. Nimroz and Khatam cultivars were also highly resistant. Nimroz cultivar in previous studies showed resistance to drought, salinity, *Septoria tritici, Ustilago hordei, Ustilago tritici*. In addition, khatam cultivar showed tolerance to salinity and resistance to spike losses and lie down and semi-susceptible to yellow rust, powdery mildew and leaf spots. Results of this study revealed that this cultivar is also resistant to *M. javanica.* On the other hand, Rihan cultivar is semi-resistance to lying and grain seed, powdery mildew (*B. graminis*) and yellow rust (*P. striiformis*), and is appropriate for warm temperate regions, where conditions are provided for the growth of root-knot nematodes. Zarjow and Nosrat cultivars that arrange in susceptible groups witch Zarjow cultivar also susceptible to lie down and tolerance to chill and spike losses and resistance to salinity. Nosrat is semi-resistance to lie down, chill and drought. In addition, Nosrat is resistance to yellow rust and powdery mildew. According to the results of this study, if there was a root-knot nematode population in the cultivated field (with Rihan cultivar), the amount of damage to the product will be high. Ibrahim *et al.* (1988) compared the resistance of five barley cultivars against *M. javanica* and reported that all tested cultivars have relative susceptibility to this nematode and these findings are consistent with the results of this study. Johnson and Motsinger (1989) by examining the damage of *M. javanica*, *M. incognita*, *M. arenaria* on seven cultivars of wheat, five oats, and four barley cultivars, reported susceptibility in all tested plant species except two cultivars of Brooks and Florida 051 that were resistant to *M. arenaria* and *M. javanica* species. In comparing resistance study of 14 barley cultivars, seven wheat and four oat to RKN (*M. javanica*) in the initial infection, 1000 eggs and larvae, one of the cultivars of wheat and two barley cultivars were introduced as susceptible to nematode (Karajeh *et al.*, 2011). Before this, no study has been conducted on the level of oxidative enzymes secretion against RKN nematodes in barley cultivars in Iran. Overall, plant diseases are causing change in resistance enzymes activity and there are several reports based on the relative resistance and increase in antioxidant enzyme activity (Ngadze *et al.*, 2012; Arun *et al.*, 2010; Moghbeli *et al.*, 2017). In this study, the increase of SOD, PROX, PPO and suppression of CAT activity in Jolge and Nimrouz was higher than that in Rihan and Zarjow. The increased trend of PROX activity was a response of resistance that barley showed to *M. javanica*. PROX plays a special role in the construction of cell wall lignin that might increase the structural rigidity of plant tissues and halt nematode penetration (Quiroga *et al.*, 2000; Anjum *et al.*, 2012). Increase in the amount of resistance enzymes has been confirmed against different plant pathogens in several studies (Potpour *et al.*, 2000; Kuvalekar *et al.*, 2011; Anjum *et al.*, 2012). These changes are represented in infected tissues especially in resistant cultivars (Dixit *et al.*, 2017) that was quite consistent with the findings of this study. In addition, evaluation in the frequency of resistance enzymes to fungal disease (*Fusarium oxysporum* F. SP. *Radices-cucumerinum*) overlapped with our results (Moghbeli *et al.*, 2017). In our study, after nematode inoculation, PROX activity increased in resistant cultivars according to Nayak and Pandey (2016) and Dixit *et al.* (2017) studies. Aemmr *et al.* (2014) stated that SOD activity decreased when PROX activity increased in resistant cultivars, while in the present study, SOD activity increased in resistant cultivars and had a direct correlation with PROX activity. These results are in line with Guida *et al.* (1992) who reported that increase in SOD and PROX activity seems to be the result of an adaptive response, which provides the plant with protection against biotic and abiotic stress. CAT activity decreased in resistant and susceptible cultivars, but this reduction was more pronounced in resistant cultivars than susceptible cultivars. The findings of the current study were in agreement with those reported by Wynn *et al.*, (2013). Chen *et al.* (1993) reported that

Salicylic acid caused as inhibition in CAT activity in many plants, and improved plant systemic acquired resistance (SAR). CAT inhibition was reported to enhance the cellular level of H_2O_2, which was recognized in HR, as a trigger for hypersensitive cell death as well as a strong antimicrobial molecule (Arun *et al.*, 2010). Similar to PROX and SOD, PPO activity increased after nematode inoculation in resistant cultivars and decreased or did not change in susceptible cultivars. Rani *et al.* (2008) and Wynn *et al.*, (2013) also obtained similar results with our study. Our results indicated that measuring oxidative enzymes can be used as a biochemical marker to predict the level of resistance of barley genotypes to *M. javanica*. According to other reports and the results of our study, it cannot be indicated that barley is fully resistant to RKN, and by introducing resistant cultivars of this plant, can help to reduce nematode damage.

CONCLUSIONS

Today's, one of the most important and controversial issues is to introduce resistant or tolerant varieties to disease and tensions because, resistant varieties can help in growth production in agriculture without environmental damages. According to other reports and the results of our study, it cannot be indicated that barley is immune to RKN, and by introducing resistant cultivars of this plant, can help to reduce nematode damage. As In this study results showed that Jolge and Nimrooz varieties are most resistance to the disease and suitable for future genomical and biotechnical investigations. In addition, results indicated that measuring oxidative enzymes can be used as a biochemical marker to predict the level of resistance of barley genotypes to *M. javanica*.

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