Journal of Agricultural Science and Technology (2025) Vol. 27(5): 1199-1213

Screening of Sugar Beet Genotypes to Beet Curly Top Virus and Sugar Beet Cyst Nematode (*Heterodera schachtii*)

Roya Kazemi[1], Mehdi Saadati[1], Ali Mokhtasi[2], Majid Pedram[1], Mohammad Reza Atighi[1], and Masoud Shams-Bakhsh[1*]

ABSTRACT

The beet cyst nematode (BCN) (*Heterodera schachtii*) and beet curly top virus-severe (BCTV-Svr) (*Curtovirus betae*) are two important pathogens worldwide. In present study, the reaction of 14 genotypes to BCN and BCTV-Svr was separately assessed, using Jolgeh and Sanetta cultivars as susceptible and resistant controls, respectively, in completely randomized design experiments. Reactions were based on the cyst and egg counts and symptoms severity index. Experiments were performed in the greenhouse of Tarbiat Modares University, Tehran, Iran, and were repeated twice independently. Based on the results of initial experiments, the S1-960090, S1-940324, S1-960294, and S1-960284 genotypes resistant to the BCN, were selected for further investigation. Furthermore, the reaction of the four selected genotypes to BCN, BCTV-Svr, and the mixture of the two pathogens was evaluated by analyzing their growth, physiological, and biochemical characteristics, and virus accumulation. Resistant genotypes showed higher levels of defense-related enzymes such as catalase, guaiacol peroxidase, and polyphenol oxidase, whereas susceptible genotypes exhibited significant reductions in photosynthesis, greenness, and chlorophyll a, b, and carotenoid content compared to non-inoculated and resistant plants. This is the first study conducted to search for dual-resistance sources against two devastating pathogens that frequently occur in the sugar beet-growing regions of Iran. Based on the results of this research, genotypes S1-960090 and S1-940324 were identified as resistant to both pathogens and are recommended for breeding purposes.

Keywords: *Beta vulgaris*, *Curtovirus,* Defense-related enzymes, Dual-resistance sources, Peroxidase, Polyphenol oxidase.

INTRODUCTION

Sugar beet (*Beta vulgaris* subsp. *vulgaris*), a biennial plant from the family Chenopodiaceae, is the source of one-third of global sugar production. Sugar beet is widely grown worldwide and Iran ranked 13th among the countries cultivating this crop (FAO, 2020). Like other crops, sugar beet is attacked by several pests and pathogens. The beet curly top disease, rhizomania, the beet weariness caused by beet cyst nematode (BCN), root-knot disease caused by root-knot-nematode, and root rots are the main diseases of this crop in the world (Harveson *et al.,* 2009). *Heterodera schachtii* (BCN) is widely distributed in Iran and is one of the main constraints of sugar beet production. The nematode-infected plants show symptoms like shorter main roots, numerous sub-roots, and dwarf phenotype compared to non-infected plants (Harveson and Jackson, 2008). The beet curly top virus (BCTV) (*Curtovirus betae*) is the type species of the genus *Curtovirus* in the family *Geminiviridae*. This virus causes severe damage to plants, resulting in symptoms including leaf curling, stunted

[1] Department of Plant Pathology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Islamic Republic of Iran.
[2] Department of Agronomy, Faculty of Agriculture, Tarbiat Modares University, P. O. Box: 14115-336, Tehran, Islamic Republic of Iran.
*Corresponding author; e-mail: shamsbakhsh@modares.ac.ir

growth, and vein swelling. BCTV affects more than 300 different crops, weeds, and ornamental plants from 44 families. It is naturally transmitted persistently by the leafhoppers *Circulifer tenellus* and *C. haematocaps* (Gaur *et al.,* 2022).

Considering the significance and extent of BCN and beet curly top virus-severe (BCTV-Svr) distribution in Iran, it is advisable to utilize resistant varieties to minimize their harmful effects. In the previous study, 70 sugar beet genotypes were evaluated for resistance to *H. schachtii* under the greenhouse and field conditions in Iran, and three genotypes were resistant to this nematode (Motieeian *et al.,* 2016). Also, in another study, 50 sugar beet lines were evaluated for resistance to curly top disease caused by BCTV-Svr and beet curly top Iran virus (BCTIV) under greenhouse conditions, and five out of 50 lines were found to be resistant to both viruses (Montazeri *et al.,* 2016). Followingly, the reaction of 38 sugar beet genotypes was evaluated to inoculation with each BCTV-Svr and BCTIV using their infectious clones under the greenhouse condition. As the result, 10 and seven sugar beet genotypes were identified as resistant to BCTV-Svr and BCTIV, respectively. In the field experiment with natural infection of viruses, the selected genotypes were evaluated and six genotypes were identified as resistant to BCTV-Svr and BCTIV. (Saadati *et al.,* 2021). Moreover, different plant genotypes have been screened against BCTV-Svr such as coriander cultivars (*Coriandrum sativum* L.) (Saadati *et al.,* 2024) and common bean landrace (Larsen *et al.,* 2010). Several studies have been performed to evaluate the resistance of sugar beet genotypes to BCN or BCTV-Svr in Iran. Since both pathogens are widely distributed throughout the country, sugar beet plants could likely be infected with both diseases simultaneously. However, there has been no evaluation of sugar beet genotypes for resistance to co-inoculation with both pathogens. Therefore, this study aimed to identify potential sources of resistance in sugar beet cultivars that are resistant to simultaneous inoculation with both BCN and BCTV-Svr.

MATERIALS AND METHODS

Plant Growth and Experiment Condition

Number of 14 genotypes, plus one susceptible (Jolgeh), and one resistant (Sanetta) cultivar to BCN (Ghaemi *et al*., 2018) as the controls, were obtained from the Sugar Beet Seed Institute, Karaj, Iran. Plants were grown in an insect-free greenhouse located in the Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran, at 25°C±2 with a 16-hour light and 8-hour dark photoperiod. The initial screening was conducted with 14 genotypes and their reaction were separately assessed to BCN and BCTV-Svr based on the cyst and egg count and symptoms severity index, respectively. In further investigations, four genotypes were selected and assessed to BCN, BCTV-Svr, and their mixture. The treatments included not inoculated control (C) plants, inoculated with BCTV-Svr (V), inoculated with BCN (N), first inoculated with BCN and three days later with BCTV-Svr (NV), first inoculated with BCTV-Svr, and three days later inoculated with BCN (VN), and simultaneous inoculation with BCN and BCTV-Svr (N+V). All experiments were performed in a completely randomized design with seven replications each, and were independently repeated twice.

Nematode Inoculation and Nematode Counting

Soil samples infested with *Heterodera schachtii* were collected from farms around Miandoab Sugar Factory, located in northwest Iran. Cysts were collected using the Fenwick (1940) method and a 60-mesh sieve with 250-micrometer openings. A few cysts were washed in water several times to prepare for morphological identification. The cysts were lemon-shaped with

protuberant neck and vulval regions at the body end. The top cone was ambifenestrate, and the under-bridge was robust. Dark brown bullae were present underneath the vulval bridge. After morphological confirmation, freshly hatched second-stage juveniles were inoculated to canola (*Brassica napus* L.) seedlings for nematode multiplication (Nielsen *et al*., 2003). Canola plants were grown under greenhouse conditions under natural light during growing season at 25-30°C. Three to four months later, cysts were collected as described above and then transferred to plates containing 3 mM $ZnCl_2$ to promote the hatching of (Ghaemi *et al*., 2018). Around 300 freshly hatched second-stage juveniles were inoculated to each plant. To count the nematodes, six-week-old plants were harvested and the number of formed cysts was counted and compared across the examined accessions. To ensure accurate quantification, the plant roots were vigorously washed to ensure all adhered cysts were separated. Subsequently, the cysts were collected using the Fenwick method and a 60-mesh sieve from the soil surrounding the roots, along with the water run-off from the roots. The reproduction rate of BCN was assessed by counting the number of eggs in each cyst. To quantify the number of eggs per cyst, 10 cysts per plant accession were individually crushed in 1 mL of water, and the number of eggs was counted.

Virus Inoculation

Plant agro-inoculation was performed with an infectious clone of BCTV-Svr (GenBank accession No. X97203) kindly provided by Dr. S. A. A. Behjatnia from Shiraz University, Iran (Grimsley *et al.,* 1986). For the agro-inoculation of BCTV-Svr, *Agrobacterium tumefaciens* carrying binary vector constructs of the virus was cultured in a liquid yeast extract broth (YEB) medium. The cells were harvested by centrifugation and the pelleted cells were resuspended in distilled water containing 50 mM acetosyringone. Plant leaves at the three to five leaf stage were inoculated by infiltration of bacterial suspension kept at 25±2°C and monitored for viral symptoms (Saadati *et al*., 2023). Symptoms were scored using the manner previously described (Montazeri *et al.,* 2016). The scoring system ranged from 1 to 9, with 9 indicating the highest infection rate and displaying symptoms such as light and swollen veins on the underside of the leaves, the formation of small needle-like protrusions on the top of the leaves, and reduced plant growth.

Physiological Parameters

The chlorophyll measurement was performed according to the method described by Warren (2008). After extraction of chlorophylls by methanol, 200 µL of the sample was transferred into 96-well plates, and absorbance of chlorophyll a, chlorophyll b, and carotenoids was measured at 665, 652, and 470 nm, respectively, and were calculated by the formula given by Warren. (2008). The greenness was quantified by measuring leaf relative chlorophyll content using a SPAD device after calibration. For photosynthesis measurement the Li-Cor device (Li-3000, USA) was employed.

Biochemical Parameters

To measure the activity of enzymes, the whole plants were harvested two weeks after inoculation with either BCN or BCTV-Svr or a mixture of them. The pool of three plants was ground into a fine powder using liquid nitrogen and stored at -80°C until needed. All measurements were performed in five biological replicates and three technical replicates. Catalase activity was evaluated based on the rate of decomposition of H_2O_2 in the enzymatic reaction and measurement of adsorption at 240 nm with some modifications (Maehly

and Chance, 1954). For each sample, 200 mg of ground plant tissue was used to assess enzyme activity, and 50 mg of polyvinylpyrrolidone (PVP) was added to each sample. The powder was homogenized in 1 mL of extraction buffer by rough vortexing at full speed and centrifuged at 15,000 rpm for 15 minutes at 4°C. Afterward, 30 µL of supernatant was transferred to wells in 96-well plates, and then 155 µL of 50 mM potassium phosphate buffer (pH 7) was added to each well. To start the reaction, 30 µL of 20 mM of hydrogen peroxide was added to each well, and the absorbance was monitored using a microplate reader (Epoch BioTek, USA) for 3 minutes at 30 seconds intervals.

Evaluation of guaiacol peroxidase activity was performed based on the activity of guaiacol as a hydrogen donor with some modifications (Siguemoto and Gut, 2017). Extraction buffer was added to 200 mg fresh sample and homogenized in 1 mL extraction buffer by vortexing at full speed and centrifuged at 15,000 rpm for 15 minutes at 4°C. Afterward, 42 µL of supernatant was transferred to wells in 96-well plates, and 125 µL of 100 mM potassium phosphate buffer (pH 7) was added to each well. To start the reaction, 17 µL of 24 mM guaiacol and 17 µL of 12 mM H_2O_2 were added to each well, respectively. Absorption changes were measured at 30-second intervals at 470 nm for 3 min.

The enzymatic activity of polyphenol oxidase (PPO) was evaluated by the formation of quinones and quinone polymers which were colored (Siguemoto and Gut, 2017). To evaluate the PPO activity, 50 mg of PVP was added to 200 mg fresh samples and were homogenized by vortexing and centrifuged at 15,000 rpm for 15 minutes at 4°C. From the supernatant, 33 µL was transferred to each well, and 100 µL of 50 mM potassium phosphate buffer (pH 6.5) was added. After one min incubation at room temperature, the reaction was started by adding 67 µL of freshly prepared 50 mM catechol solution to each well and the absorption changes at 420 nm were measured using a micro-plate reader.

Virus Accumulation

The CTAB method was employed to extract DNA from leaf samples six weeks after inoculation (Doyle and Doyle, 1987). Then, the virus accumulation in inoculated plants was assessed by semi-quantitative polymerase chain reaction (semi-qPCR). Primers for semi-qPCR are shown in Table 1. To make sure the same amount of DNA was used for each PCR reaction, after DNA extraction, it was quantified using Nanodrop, and 100 ng of DNA was used for each reaction. The PCR program was as follows: initial denaturation at 95°C for 4 minutes followed by 18 cycles of denaturation at 95°C for 1 minute, annealing at 59°C for 45 seconds, and extension at 72°C for 1 minute. A final extension was performed at 72°C for 10 minutes. Finally, 4 µL of PCR product was run on the 1% agarose gel, and the intensity of bands was quantified using Image J software.

Table 1. Names, sequences, and the production size of primers used to quantify beet curly top virus-Severe accumulation.

Primer names	Sequences	Amplicon size (bp)	Reference
BCTV-Svr V1V/V1C.Forward	*5′-AGAAAATATACAAGAAATC-3′*	750 bp	Saadati *et al*., 2021
BCTV-Svr V1V/V1C.Reverse	*5′-TTAATAAAAATA ACATCTAC-3′*		
DNA18S1/S2.Forward	*5′-AACGGCTACCACATCCAAG- 3′*	500 bp	Faria *et al*., 2006
DNA18S1/S2.Reverse	*5′-ACG TCATTACTCCGATCCCG AA-3′*		

Statistical Analysis

All statistical analyses were performed with the SAS 9.2 software (SAS Institute Inc., Cary, NC). Analysis of Variance (ANOVA) of the data and tests of residual normality were performed using the GLM and UNIVARIATE procedures, respectively. The categorical and ordinal data were subjected to a rank transformation and analyzed using the nonparametric methods developed by Shah and Madden (2004) using the GLM procedure of SAS. Means were compared using the least significant difference (LSD) test at $P \leq 0.05$. Compound decomposition was performed in Minitab (Minitab® 18.1) software. Charts were drawn using Excel software.

RESULTS

The results of the analysis of variance of the effect of variety and the disease on physiological parameters indicated significant differences at a 1% probability level in chlorophyll a, b, total chlorophyll, carotenoid, greenness, catalase, guaiacol peroxidase, polyphenol oxidase, photosynthesis, root length, shoot, and root fresh and dry weight (Table S1). Furthermore, a combined analysis of variance was conducted on cyst and egg data from two experiments involving 14 sugar beet genotypes, along with a susceptible (Jolgeh) and a resistant (Sanetta) cultivar as control (Table S2) as well as two experiments of cyst and egg data for four selected genotypes along with Jolgeh as the susceptible cultivar (Table S3).

Initial Screening of Sugar Beet Genotypes and Cultivars

After eight weeks of plant inoculation by BCTV-Svr, symptoms were scored and it was shown that, among 14 assessed genotypes and cultivars, S1-960090 and S1-940324 genotypes exhibited the lowest and cultivar Hadiya; and S1-960284 and S1-960318 genotypes showed the highest score compared to cultivar Jolgeh as the susceptible host (Figure 1).

The number of cysts per plant six weeks after inoculation showed that genotypes S1-960284, S1-960294, S1-960295, S1-940324, and S1-960090 and Sanetta exhibited the lowest number of cysts per plant compared to cultivar Jolgeh as a susceptible host (Figure 2). The number of eggs per cyst was the lowest in genotypes S1-960284, S1-960294, S1-960295, and S1-960090,

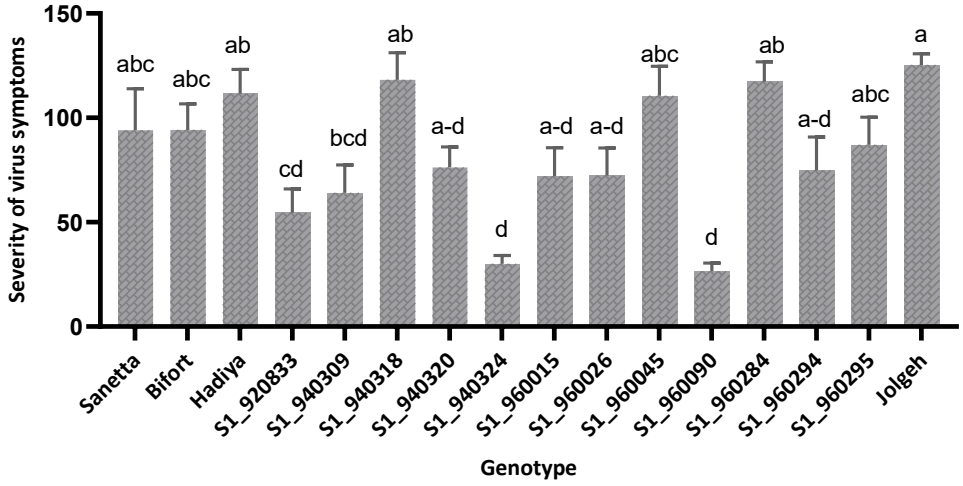

Figure 1. The mean of symptoms severity of genotypes and cultivars of sugar beet eight weeks post-inoculation by beet curly top virus-Severe.

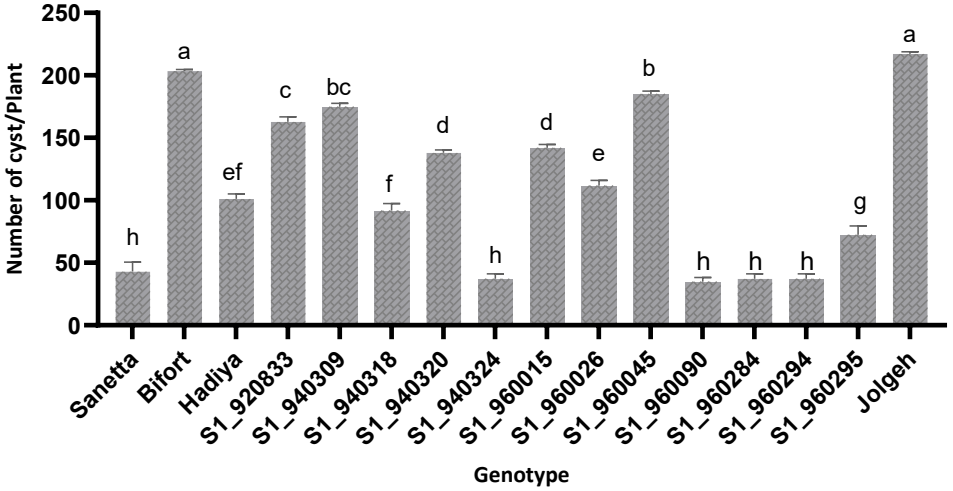

Figure 2. Number of cysts per plant of genotypes and cultivars of sugar beet six weeks post inoculation to beet cyst nematode in comparison with Jolgeh and Sanetta as susceptible and resistant cultivars, respectively. Different letters denote significant differences (LSD test, $P < 0.05$).

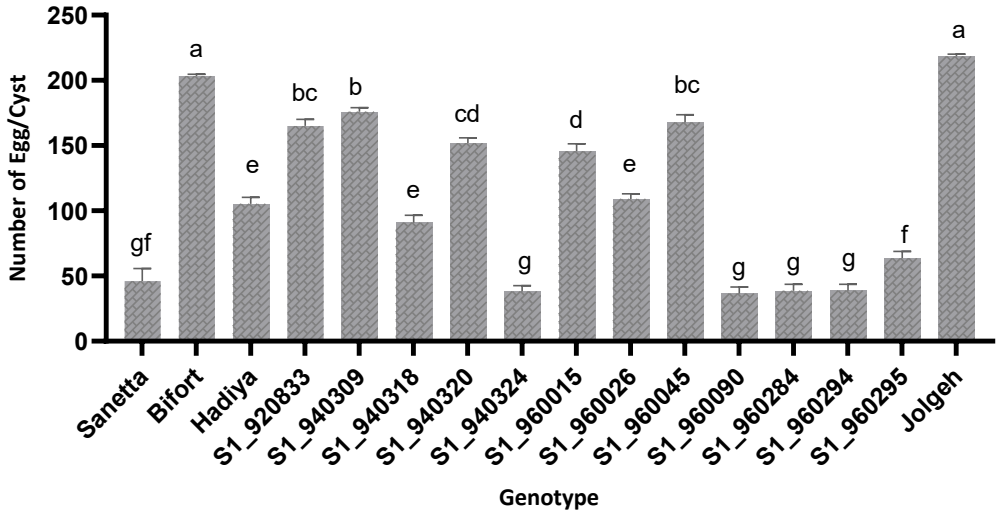

Figure 3. Number of eggs per cyst of genotypes and cultivars of sugar beet six weeks post-inoculation to beet cyst nematode in comparison with Jolgeh and Sanetta as susceptible and resistant cultivars, respectively. Different letters denote significant differences (LSD test, $P < 0.05$).

compared to the Jolgeh cultivar (Figure 3).

Reaction of Selected Genotypes

Based on the results obtained in the first screening, four genotypes, namely, S1-960090, S1-940324, S1-960284, and S1-960294 were selected for further analyses. All four genotypes showed resistance to inoculation with BCN, whereas, only S1-960090 and S1-940324 were resistant to BCTV-Svr and cultivar Jolgeh was also taken as the susceptible cultivar. Further treatments were considered for the selected cultivars to study their reaction to BCN and BCTV-Svr infection, including BCN (N), BCTV-Svr (V), inoculation first by BCN, and then with BCTV-Svr (NV), inoculation first by BCTV-Svr, and then with BCN

(VN) and simultaneous inoculation with BCN and BCTV-Svr (N+V).

The treatment N+V of Jolgeh cultivar exhibited the highest number of cysts per plant and the number of eggs per cyst as well as severity of virus symptoms, compared to other cultivars. In genotypes S1-960090 and S1-940324, treatments NV, VN, N+V resulted in smaller number of cysts and eggs compared to the treatment N, showing that the mixed infection induced more resistance than nematode alone. In the susceptible cultivar Jolgeh, the mixed infection (NV, VN, N+V), increased the number of cysts and eggs compared to the nematode alone, as well as same severity of virus symptoms and accumulation, similar to virus infection alone. Therefore, it seems that the interaction of the two pathogens increased the susceptibility in cultivar Jolgeh (Table 2).

Semi-Quantitative PCR

The accumulation of BCTV-Svr in the susceptible cultivar and the four selected genotypes was measured using semi-quantitative PCR six weeks after inoculation. The cultivar Jolgeh, as a susceptible host, showed the highest level of virus accumulation compared to the four selected genotypes (Figure 4-a). Simultaneous inoculation with BCN and BCTV-Svr exacerbated the symptoms and caused the highest virus accumulation compared to BCTV-Svr inoculation or their mixture, regardless of their priority (Figure 4-a, N+V). However, in the case of S1-940324 and S1-960090, mixed inoculations

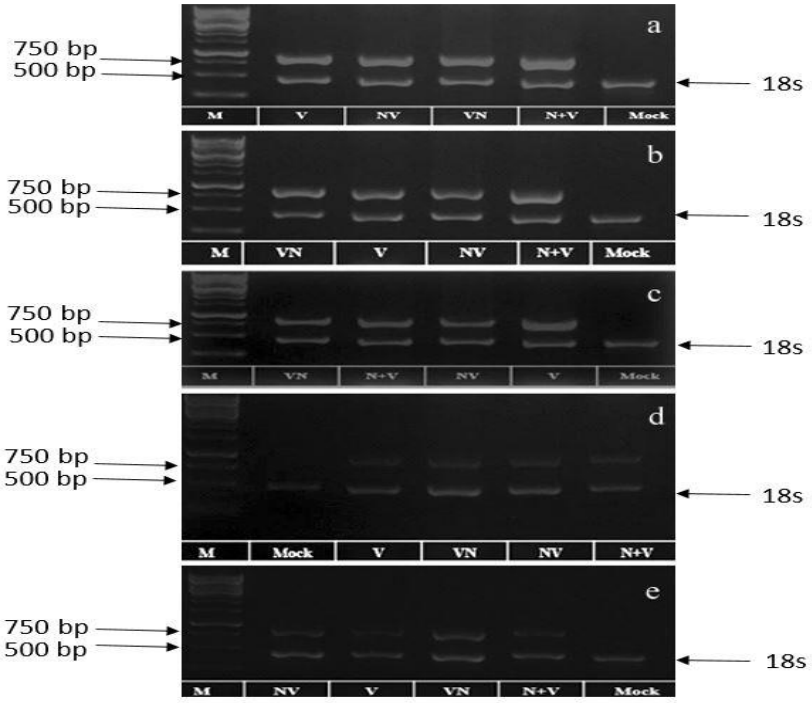

Figure 4. Semi qPCR for quantitation of virus accumulation in genotypes resistant to Beet Curly Rop Virus-Svr (BCTV-Svr). The band of 500 bp for 18S rDNA fragments and a specific 750 bp for BCTV-Svr were obtained for resistant genotypes (**a)** Cultivar Jolgeh, (**b)** Genotype S1-960294, (**c)** Genotype S1-940284, (**d)** Genotype S1-960090, and (**e)** Genotype S1-940324. M: Marker, Mock: Non-inoculated plants, V: Plants only infected with BCTV-Svr, NV: First BCN, and then BCTV-Svr VN: First BCTV-Svr, and then BCN, N+V: Simultaneous infection of BCN, and BCTV-Svr.

caused higher virus accumulations compared to virus alone treatment (Figure 4). In the case of the two other genotypes, S1-960284 and S1-960294, however, the level of BCTV-Svr accumulation in the mixed inoculation (N+V, VN, NV) was lower compared to the virus treatment alone (Tables 2 and 3, Figure 4).

Table 2. The results of the analysis of variance of the effect of genotype and the disease of the accumulation of virus, severity of virus symptoms of Beet Curly Rop Virus-Svr (BCTV-Svr), the number of cysts per plant, and the number of eggs per cyst in individual and mixed infection with BCTV-Svr and beet cyst nematode.

	df	Semi-quantitative	Severity of virus symptoms	Number of cyst/Plant	Number of egg/Cysts
Genotype	4	759.65**	348207.26**	26009.76**	26405.51**
Pathogen	3	11.17**	17181.76**	1133.35**	1367.90**
Genotype×Pathogen	12	58.84**	11352.12**	2151.23**	2247.88**
CV (%)		2.47	20.84	14.65	13.67

** Significant at %1 probability level.

Table 3. Comparison means of the accumulation of virus, severity of virus symptoms of Beet Curly Rop Virus-Svr (BCTV-Svr), and the number of cysts per plant, and eggs per cyst in individual and mixed infection with BCTV-Svr and Beet Cyst Nematode (BCN). [a]

Treatment		Semi-quantitative	Severity of virus symptoms	Number of cyst/Plant	Number of egg/Cyst
Genotype	Pathogen				
Jolgeh	N	-	-	100.6 cd	100.9 cd
	NPV	33.1 a	270.5 a	116.3 a	117.5 a
	NV	27.13 c	229.4 b	105.8 bc	104.7 bc
	V	23 e	211.5 bc	-	-
	VN	25.08 d	251.4 a	111.4 ab	110.9 ab
S1-940324	N	-	-	54.67 g	59.25 hi
	NPV	9.127 n	61.54 j	18 ij	15.92 lm
	NV	11.58 l	114.1 fg	29.17 h	23 kl
	V	6.821 o	33.89 k	-	-
	VN	12.29 k	92.79 gh	32.33 h	26.42 jk
S1-960090	N	-	-	54.67 g	58.75 hi
	NPV	6.682 o	38.21 k	11.67 j	10.83 m
	NV	10.54 m	64.07 ij	24.33 hi	24 jkl
	V	5.367 p	18.5 k	-	-
	VN	9.542 n	84.71 hi	18 ij	17.83 klm
S1-960284	N	-	-	62.25 fg	62.83 gh
	NPV	22.17 f	196.7 cd	91.67 de	91.5 de
	NV	16.26 i	169.4 e	54.67 g	50.08 i
	V	28.71 b	219.3 b	-	-
	VN	18.25 h	183.2 de	88.67 e	89.5 ef
S1-960294	N	-	-	57.83 g	62.42 gh
	NPV	10.65 m	98.14 gh	24.33 hi	32.5 j
	NV	15.39 j	161.6 e	82 e	81.5 f
	V	11.5 l	131.4 f	-	-
	VN	20.72 g	179.7 de	71.75 f	69.67 g

[a] Means with common letters in each column indicate no significant difference based on the LSD test at the 5% level. V: Plants only infected with BCTV-Svr, NV: First BCN, and then BCTV-Svr, VN: First BCTV-Svr, and then BCN, N+V: Simultaneous infection of BCN, and BCTV-Svr.

Chlorophyll, Greenness, Photosynthesis, and Carotenoid Content

In the susceptible cultivar Jolgeh, inoculation with BCTV-Svr, BCN, or a mixture of them, regardless of their priority, reduced the chlorophyll a and b contents. In the case of carotenoids, simultaneous inoculation with both pathogens showed a synergistic effect and substantially reduced carotenoid content of plants. The initial inoculation with BCN, then BCTV-Svr also caused a reduction of carotenoids but to a lesser extent. In the case of S1-940324, photosynthesis and chlorophyll a and b content were reduced upon inoculation with either pathogen or their mixture. In the case of carotenoid content, the BCN infection, NV and VN reduced the carotenoid content compared to non-infected plants. For the other three genotypes, substantial reduction of photosynthesis capacity, chlorophyll a, carotenoid contents, and overall greenness and photosynthesis were observed upon inoculation compared to non-infected plants, especially when they were infected by BCN solely. The decrease in carotenoid, greenness, and chlorophyll a and b in the susceptible cultivar Jolgeh was more pronounced than the resistant genotypes. Photosynthesis and greenness in resistant genotypes S1-960090 and S1-940324 were not significantly different in infected and non-infected plants (Tables 4 and 5).

Table 4. Average comparison of the photosynthesis and growth parameters in mixed infections of Beet Cyst Nematode (BCN) and Beet Curly Top Virus-Svr (BCTV-Svr) or individual infection by BCN or BCTV-Svr.

Treatment		Photosynthesis	Root length (cm)	Shoot fresh weight (g plant^{-1})	Root fresh weight (g plant^{-1})	Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})
Genotype	Pathogen						
Jolgeh	C	4.17 ab	29.13 a	6.5 abc	3.393 abcd	0.99 a	0.87 ab
	N	1.51 fghi	17 def	2.403 jkl	2.103 ghij	0.4033 kl	0.3633 ijk
	NPV	0.60 j	18.17 cd	1.023 n	1.09 k	0.2733 n	0.1527 m
	NV	1.31 hi	17.47 de	1.583 lmn	1.527 jk	0.39 klm	0.2167 lm
	V	1.41 ghi	15.6 efghi	1.9 klmn	2.503 efgh	0.45 jk	0.5933 efg
	VN	1.14 ij	10.17 no	1.213 mn	1.883 ij	0.3267 lmn	0.31 jkl
S1-940324	C	4.41 ab	19.7 c	7.073 ab	3.597 a	0.9967 a	0.8933 ab
	N	4.03 b	14.2 hijk	3.8 fghi	2.523 efgh	0.63 fgh	0.59 efg
	NPV	4.25 ab	12.13 lmn	5.1 de	3.283 abcd	0.72 def	0.6933 de
	NV	4.12 b	14.83 ghij	4.393 ef	2.943 cde	0.6167 ghi	0.6667 de
	V	4.33 ab	14.57 ghijk	6.5 abc	3.457 abcd	0.8133 cd	0.75 bcd
	VN	4.15 ab	14 ijkl	4.197 efg	2.993 bcde	0.6533 fgh	0.6733 de
S1-960090	C	4.83 a	24.13 b	7.397 ab	3.55 ab	1.073 a	0.8767 ab
	N	4.13 b	12.63 klm	4.007 efgh	2.6 efg	0.7 efg	0.6 ef
	NPV	4.41 ab	7.9 p	5.8 cd	3.333 abcd	0.88 bc	0.7233 cde
	NV	4.25 ab	11.37 mn	5.103 de	3 bcde	0.7567 de	0.6867 de
	V	4.56 ab	12.97 jklm	6.807 abc	3.493 abc	0.9767 ab	0.783 abcd
	VN	4.38 ab	10.33 no	4.997 de	3.047 abcde	0.7967 cde	0.6933 de
S1-960284	C	4.09 b	25.4 b	6.307 bc	3.353 abcd	0.9833 a	0.8667 abc
	N	2.14 def	16.17 efgh	2.603 jkl	2.147 ghi	0.41 kl	0.4033 hij
	NPV	1.9 defgh	17.03 def	1.557 lmn	1.557 jk	0.32 lmn	0.2267 klm
	NV	2.22 de	16.1 efgh	2.203 jklm	2.15 ghi	0.4467 jk	0.4333 hij
	V	1.5 efghi	9.233 op	1.203 mn	2.907 def	0.2967 mn	0.5867 efg
	VN	2.01 defg	16.47 defg	1.953 klmn	1.983 hij	0.38 klm	0.3867 hij
S1-960294	C	3.25 c	28.87 a	7.553 a	3.463 abcd	1.04 a	0.91 a
	N	2.57 cd	13.93 ijkl	3.203 ghij	2.5 efgh	0.58 hi	0.58 efg
	NPV	2.50 d	15.07 fghi	3.013 hijk	2.357 fghi	0.5267 ij	0.51 fgh
	NV	2.42 d	15.4 fghi	2.247 jklm	2.2 ghi	0.4367 jk	0.45 ghij
	V	2.47 d	16 efgh	2.823 ijk	3.297 abcd	0.4767 jk	0.7067 de
	VN	2.41 d	15.33 fghi	2.017 klmn	2.257 ghi	0.4133 kl	0.48 fghi

[a] Means with common letters in each column indicate no significant difference based on the LSD test at the 5% level. V: Plants only infected with BCTV-Svr, NV: First BCN, and then BCTV-Svr, VN: First BCTV-Svr, and then BCN, N+V: Simultaneous infection of BCN, and BCTV-Svr.

Table 5. Average comparison of the chlorophyll a, b, carotenoid, greenness, and enzymes, including Catalase (CAT), Guaiacol Peroxidase (GPX), and Polyphenol Oxidase (PPO) in mixed infections of Beet Cyst Nematode (BCN) and Beet Curly Top Virus-Svr (BCTV-Svr) or individual infection by BCN or BCTV-Svr.[a]

Treatment		Chlorophyll a (µg mL^{-1})	Chlorophyll b (µg mL^{-1})	Total Chlorophyll	Carotenoid (µg mL^{-1})	Greenness (SPAD)	CAT (U g^{-1} FW)	GPX (U g^{-1} FW)	PPO (U g^{-1} FW)
Genotype	Pathogen								
Jolgeh	C	7.96 abc	7.882 ab	15.85 ab	5.451 a	44.9 bc	0.614 k	0.717 lm	0.564 klm
	N	3.29 ij	3.584 jkl	6.88 klm	4.99 abc	19.38 hij	0.514 m	0.627 o	0.445 op
	NPV	1.71 k	1.809 m	3.523 n	1.91 jk	11.94 l	0.335 o	0.459 q	0.229 r
	NV	2.50 jk	2.584 lm	5.072 mn	4.03 bcde	15.46 kl	0.448 n	0.574 p	0.364 q
	V	3.60 hij	3.658 jkl	7.255 kl	5.12 ab	21.42 gh	0.564 l	0.667 n	0.5007 no
	VN	2.957 ijk	2.99 klm	5.948 lm	4.63 abcd	17.46 ijk	0.508 m	0.624 o	0.434 p
S1-940324	C	8.213 ab	8.106 a	16.32 ab	3.59 defg	50.2 a	0.6821 i	0.75 jk	0.659 ij
	N	5.08 efg	6.04 efg	11.12 fgh	2.57 ghijk	49.4 a	0.7849 f	0.865 f	0.7823 efg
	NPV	6.498 de	6.65 bcde	13.1 cdef	3.50 defg	50.02 a	0.8943 c	0.995 b	0.9011 abc
	NV	5.842 def	6.11 defg	11.95 fgh	3.01 efghij	49.6 a	0.8445 d	0.932 de	0.8431 cde
	V	7.007 abcd	7.38 abcd	14.4 bcd	3.56 defg	50.12 a	0.9012 c	1.00 b	0.9116 ab
	VN	5.806 def	6.08 defg	11.89 fgh	3.28 efgh	50 a	0.8643 d	0.955 cd	0.8669 bcd
S1-960090	C	8.423 a	8.14 a	16.56 a	3.16 efghi	50.48 a	0.7195 h	0.764 ij	0.6923 hi
	N	5.864 def	6.15 defg	12.02 fgh	1.83 k	50.12 a	0.818 e	0.8601 fg	0.8147 de
	NPV	6.559 cd	6.67 bcde	13.2 cdef	2.19 hijk	50.4 a	0.9683 b	1.00 b	0.9067 ab
	NV	5.865 def	6.40 cdef	12.25 def	1.95 jk	50.2 a	0.8983 c	0.91 e	0.8006 ef
	V	7.15 abcd	7.511 abc	14.66 abc	3.00 efghij	50.46 a	0.998 a	1.09 a	0.9586 a
	VN	6 def	6.19 defg	12.2 efg	2.024 ijk	50.38 a	0.918 c	0.9701 c	0.9185 ab
S1-960284	C	8.005 ab	8.091 a	16.1 ab	5.254 a	49.5 a	0.605 k	0.612 o	0.5527 lmn
	N	4.234 ghi	4.324 hij	8.557 ijk	3.6 cde	21.78 gh	0.715 h	0.73 kl	0.6972 hi
	NPV	2.986 ijk	3.161 jkl	6.147 lm	3.15 efghi	15.84 jk	0.655 j	0.662 n	0.6038 jkl
	NV	4.281 ghi	4.392 hij	8.673 ijk	4.006 bcde	22.58 fgh	0.68 i	0.7 m	0.6257 jk
	V	3.577 hij	3.689 ijkl	7.266 kl	1.92 jk	17.06 ijk	0.567 l	0.572 p	0.5112 mn
	VN	3.88 ghij	3.99 hijk	7.874 jkl	3.78 def	19.96 hi	0.6604 ij	0.67 n	0.6147 jkl
S1-960294	C	7.98 abc	7.899 ab	15.89 ab	3.66 defg	48 ab	0.612 k	0.733 kl	0.5759 kl
	N	4.99 fgh	5.107 fgh	10.1 ghi	2.68 fghijk	30.04 d	0.7604 fg	0.858 fg	0.7493 fgh
	NPV	4.89 fgh	4.99 ghi	9.887 hij	3.0 efghijk	28.4 de	0.7501 g	0.828 h	0.7243 gh
	NV	4.301 ghi	4.42 hij	8.721 ijk	1.89 jk	23.68 fg	0.651 j	0.788 i	0.6161 jk
	V	6.98 bcd	7.3 abcde	14.2 bcde	3.61 defg	42.12 c	0.7242 h	0.838 gh	0.691 hi
	VN	4.352 ghi	4.45 hij	8.805 ijk	2.23 hijk	26.12 ef	0.6755 ij	0.773 ij	0.6415 ij

[a] Means with common letters in each column indicate no significant difference based on the LSD test at the 5% level. V: plants only infected with BCTV-Svr, NV: first BCN, and then BCTV-Svr VN: first BCTV-Svr, and then BCN, N+V: simultaneous infection of BCN, and BCTV-Svr.

Growth Parameters

The root length in the susceptible cultivar, Jolgeh, was significantly decreased by all treatments. The same trend was also observed in all four selected genotypes. The measurements of other growth parameters, including root fresh and dry weight, and shoot fresh weight showed the same trend in the susceptible cultivar and all four selected genotypes. The decrease in photosynthesis, fresh and dry shoot weight, and fresh and dry root weight was more pronounced in the Susceptible cultivar, Jolgeh, compared to the resistant genotypes (Table 4).

Enzyme Measurements

A significant reduction of activity was observed in the susceptible cultivar, Jolgeh, for all the three examined enzymes. In the

case of resistant genotypes, in contrast, the general trend of accumulation of defense-related enzymes was observed upon inoculation with BCN, BCTV-Svr, or their mixture, regardless of their priority (Table 5).

DISCUSSION

Developing resistant cultivars against plant pathogens has been one of the long-lasting approaches to fend off their attack. For this, finding and screening resistance sources have caught the special attention for plant breeders and pathologists. In the present research, four out of 14 surveyed genotypes and cultivars showed higher resistance to both BCN and BCTV-Svr. The growth factors in resistant cultivars were affected by inoculation with both BCN and BCTV-Svr, or in their mixed inoculation, compared to non-infected plants, showing their negative effect on plant growth, as expected upon infection. The same observation was seen in the susceptible cultivar Jolgeh. Infection either by BCN or BCTV-Svr, or their mixture, also reduced photosynthesis, greenness, and content of chlorophyll a, b, and carotenoids in plants.

A similar finding was observed in other studies where nematode infection caused a significant reduction in plant growth (Briar *et al.,* 2016). These observations are attributed to the up-taking of nutrients by nematodes and the deprivation of plants from nutrients (Ahmed *et al.,* 2009).

Upon inoculation with nematodes, growth retardation is associated with yellowing (chlorosis) and reduced chlorophyll a, b, and carotenoid contents. Nematode infection leads to less photosynthesis capacity of the infected plants. This phenomenon has been shown in other studies where inoculation with nematodes reduces photosynthesis capacity of plants by decreasing chlorophyll a, b, and carotenoid contents (Vasil'eva *et al.,* 2003; Nafady *et al.,* 2022). Furthermore, several studies have revealed that virus infection destroys the chloroplasts structure, reduces the number of chloroplasts in a cell, increases the activity of chlorophyll decomposition enzymes, and decreases the chlorophyll content and photosynthetic rate (Zhang *et al.*, 2014; Wang *et al.*, 2020). The decrease in photosynthesis capacity of potato plants due to PVY infection results in reduced levels of chlorophyll a, b, and carotenoids, eventually leading to growth retardation (Anzlovar *et al.,* 1996). Likewise, BCTV-Svr caused vein discoloration in both resistant genotypes and susceptible cultivars compared to non-infected plants (Saadati *et al.,* 2021). Consistent with these studies, the present study demonstrated that simultaneous inoculation with BCN and BCTV-Svr, also reduced photosynthesis, greenness, chlorophyll a, b, and carotenoid content in both susceptible and the four selected resistant genotypes.

Resistance of plants to pathogens is usually associated with the accumulation and higher activity of defense-related enzymes (Santos and Franco, 2023). In the susceptible cultivar Jolgeh, levels of catalase, peroxidase, and polyphenol oxidase were lower following inoculation with BCN, BCTV-Svr, or their mixture, compared to non-infected control plants. In contrast, the selected resistant genotypes showed increased accumulation of all three enzymes across all treatments.

The suppression of production of defense-related enzymes upon inoculation with nematodes was shown before in susceptible plants, in agreement with our finding in the susceptible cultivar, Jolgeh. Susceptible tomato plants infected with *M. javanica* showed a lower level of catalase upon nematode infection (Sahebani and Gholamrezaee, 2022). It can be attributed to a lower level of H_2O_2 production in such plants. In citrus, *Penicillium digitatum* suppresses the production of H_2O_2 to promote the infection rate in susceptible citrus fruit, while *Penicillium expansum*, the non-host pathogen, induces H_2O_2 accumulation (Macarisin *et al.,* 2007). It has been shown that catalase activity is

increased by increasing the accumulation of H_2O_2 (Beffagna and Lutzu, 2007). In the interaction of susceptible and resistant cultivars of wheat with *Pyricularia oryzae,* higher production of antioxidant enzymes, like catalase and peroxidase, was noted in resistant cultivars compared to susceptible ones (Debona *et al.,* 2012). Accumulation of polyphenol oxidase in susceptible cultivar, Jolgeh, and four resistant genotypes showed the same trend as antioxidant activities. PPOs are involved in catalyzing the oxygen-dependent oxidation of phenols to quinones. The quinones act as antibiotics and toxic compounds to pathogens (Junior *et al.,* 2022). Quinones are also involved in signaling by activating leucine-rich-repeat receptor-like kinases (Laohavisit *et al.,* 2020). In the present study, the selected resistant genotypes showed a higher level of PPOs when infected with either pathogens or their mixtures. Plants use various strategies to fight against pathogens; biochemical compounds are one of the first weapons in their arsenal. They are involved in signaling or directly act as toxic compounds to pathogens. This capacity of plants to resist pathogens has been used in several studies where genes involved in producing these compounds are employed to induce resistance against the pathogens (Li and Steffens, 2002; Li *et al.*, 2020).

In this study, sugar beet genotypes were screened against two important pathogens including BCTV-Svr and BCN for the first time in Iran. Four selected genotypes were investigated for their biochemical mechanisms against BCTV-Svr, BCN, or their mixtures. The results showed that genotypes S1-960090 and S1-940324 exhibited promising resistance and could be used in breeding programs to develop resistant sugar beet cultivars against simultaneous BCN and BCTV-Svr infections.

ACKNOWLEDGEMENTS

We would like to thank Sugar Beet Seed Institute, Karaj, Iran, for providing the seeds of genotypes and cultivars used in this study. We also thank Tarbiat Modares University for providing the necessary facilities and financial support. We would like to thank Dr. Farahnaz Jahanshahi Afshar, Dr. Fatemeh Azad, and Mr. Hojat Moulai for their help.

REFERENCES

1. Ahmed, N., Abbasi, M. W., Shaukat, S. S. and Zaki, M. J. 2009. Physiological Changes in Leaves of Mungbean Plants Infected with *Meloidogyne javanica*. *Phytopathol. Mediterr.*, **48(2)**: 262-268.
2. Anzlovar, S., Kovac, M. and Ravnikar, M. 1996. Photosynthetic Pigments in Healthy and Virus-Infected Potato Plantlets (*Solanum tuberosum* L.) Grown *in Vitro*. *Phyton*, **36**: 221-230.
3. Beffagna, N. and Lutzu, I. 2007. Inhibition of Catalase Activity as an Early Response of *Arabidopsis thaliana* Cultured Cells to the Phytotoxin Fusicoccin. *J. Exp. Bot.*, **58**: 4183–4194.
4. Briar, S. S., Wichman, D. and Reddy, G. V. P. 2016. Plant-Parasitic Nematode Problems in Organic Agriculture. In: "*Organic Farming for Sustainable Agriculture*", (Ed.): Nandwani, D. Springer, PP. 107–122.
5. Debona, D., Rodrigues, F. Á., Rios, J. A. and Nascimento, K. J. T. 2012. Biochemical Changes in the Leaves of Wheat Plants Infected by *Pyricularia oryzae*. *Phytopathology*, **102**: 1121–1129.
6. Doyle, J. J. and Doyle, J. L. 1987. A Rapid DNA Isolation Procedure for Small Quantities of Fresh Leaf Tissue. *Phytochem. bull.*, **19**: 11-15.
7. FAO. 2020. World Food and Agriculture - Statistical Yearbook 2020. FAO, Rome.
8. Faria, J. C., Albino, M. M. C., Dias, B. B. A., Cançado, L. J., da Cunha, N. B., Silva, L. D. M. and Aragão, F. J. 2006. Partial

Resistance to *Bean Golden Mosaic Virus* in a Transgenic Common Bean (*Phaseolus vulgaris* L.) Line Expressing a Mutated Rep Gene. *Plant Sci.,* **171**: 565–571.

9. Fenwick, D. W. 1940. Methods for the recovery and counting of cysts of *Heterodera schachtii* from soil. *J. Helminth.,* **18**: 155–172.
10. Ghaemi, R., Pourjam, E., Safaie, N. Mahmoudi, S. B. and Mehrabi, R. 2018. Evaluation of Sugar Beet Cultivars Resistance to Beet Cyst Nematode under *in Vitro* Conditions. *J. Sugar Beet,* **34**: 65–74.
11. Grimsley, N., Hohn, B., Hohn, T. and Walden, R. 1986. "Agroinfection," an Alternative Route for Viral Infection of Plants by Using the Ti Plasmid. *Proc. Natl. Acad. Sci. USA,* **83**: 3282–3286.
12. Gaur, R. K., Sharma, P. and Czosnek, H. 2022. *Geminivirus: Detection, Diagnosis and Management*. Academic Press, Elsevier.
13. Harveson, R. M. and Jackson, T. A. 2008. *Sugar Beet Cyst Nematode*. Univ Nebraska, USA.
14. Harveson, R. M., Hanson, L. E., and Hein, G. L. 2009. *Compendium of Beet Diseases and Pests.* 2nd Edition, American Phytopathological Society, APS Press.
15. Junior, M. A. D., Edzang, R. W. N., Catto, A. L. and Raimundo, J. M. 2022. Quinones as an Efficient Molecular Scaffold in the Antibacterial/Antifungal or Antitumoral Arsenal. *Int. J. Mol. Sci.*, **23**: 1-16.
16. Laohavisit, A., Wakatake, T., Ishihama, N., Laohavisit, A., Wakatake, T., Ishihama, N., Mulvey, H., Takizawa, K., Suzuki, T. and Shirasu, K. 2020. Quinone Perception in Plants *via* Leucine-Rich-Repeat Receptor-Like Kinases. *Nature*, **587**: 92–97.
17. Larsen, R. C., Kurowski, C. J., and Miklas, P. N. 2010. Two Independent Quantitative Trait Loci Are Responsible for Novel Resistance to Beet Curly Top Virus in Common Bean Landrace G122. *Phytopathology,* **100**: 972-978.
18. Li, L. and Steffens, J. C. 2002. Overexpression of Polyphenol Oxidase in Transgenic Tomato Plants Results in Enhanced Bacterial Disease Resistance. *Planta*, **215**: 239–247.
19. Li, Q., Qin, X., Qi, J., Dou, W., Dunand, C., Chen, S. and He, Y. 2020. CsPrx25, a Class III Peroxidase in *Citrus sinensis*, Confers Resistance to Citrus Bacterial Canker through the Maintenance of ROS Homeostasis and Cell Wall Lignification. *Hortic*. *Res.,* **7**:192.
20. Macarisin, D., Cohen, L., Eick, A., Rafael, G., Belausov, E., Wisniewski, M. and Droby, S. 2007. *Penicillium digitatum* Suppresses Production of Hydrogen Peroxide in Host Tissue during Infection of Citrus Fruit. *Phytopathology*, **97(11)**: 1491–1500.
21. Maehly, A. C. and Chance, B. 1954. The Assay of Catalases and Peroxidases. *Methods Biochem. Anal*., **1**: 357–424.
22. Montazeri, R., Shams-bakhsh, M., Mahmoudi, S. B. and Rajabi, A. 2016. Evaluation of Sugar Beet Lines for Resistance to *Beet Curly Top Viruses*. *Euphytica*, **210**: 31–40.
23. Motieeian, L., Nasr Esfahani, M. and Olia, M. 2016. Screening of Sugar Beet Genotypes to Beet Cyst Nematode. *J. Sugar Beet*, **32(2)**: 107-121.
24. Nafady, N.A., Sultan, R., El-Zawahry, A. M., Mostafa, Y. S., Alamri, S., Mostafa, R. G., Hashem, M. and Hassan, E. A. 2022. Effective and Promising Strategy in Management of Tomato Root-Knot Nematodes by *Trichoderma harzianum* and Arbuscular Mycorrhizae. *Agronomy*, **12(2)**:315.
25. Nielsen, E. L., Baltensperger, D. D., Kerr, E. D. and Rife, C. L. 2003. Host Suitability of Rapeseed for *Heterodera schachtii*. *J. Nematol.,* **35(1)**: 35-38.
26. Saadati, M., Rajabi, A. and Shams-bakhsh, M. 2021. Identification of Resistant Sugar Beet (*Beta vulgaris* L.) Genotypes against Beet Curly Top Disease. *J. Agric. Sci. Technol.,* **23(2)**: 473–484
27. Saadati, M., Ayari, M. and Shams-bakhsh, M. 2023. The Effect of Beet Curly Top Virus on Growth and Phytochemical Constituents of Coriander (*Coriandrum sativum* L.). *S. Afr. J. Bot.*, **162**:804-812.
28. Saadati, M., Azarian, A., Ayari, M. and Shams-bakhsh, M. 2024. Screening of Coriander (*Coriandrum sativum* L.)

Genotypes to Beet Curly Top Virus-Severe and Beet Curly Top Iran Virus Infection. *Physiol. Mol. Plant Pathol.*, **129**: 102210.
29. Sahebani, N. and Gholamrezaee, N. 2022. The Ability of *Meloidogyne javanica* to Suppress Salicylic Acid-Induced Plant Defence Responses. *Nematology*, **24(5):** 499-508.
30. Santos, C. and Franco, O. L. 2023. Pathogenesis-Related Proteins (PRs) with Enzyme Activity Activating Plant Defense Responses. *Plants*, **12(11)**: 2226.
31. Shah, D. A. and Madden, L. V. 2004. Nonparametric Analysis of Ordinal Data in Designed Factorial Experiments. *Phytopathology*, **94(1)**: 33–43.
32. Siguemoto, E. S. and Gut, J. A. W. 2017. Validation of Spectrophotometric Microplate Methods for Polyphenol Oxidase and Peroxidase Activities Analysis in Fruits and Vegetables. *Food Sci. Technol.,* **37**: 148–153.
33. Vasil'eva, I. S., Vanyushkin, S. A., Zinov'eva, S. V., Udalova, Z. V., Bolychevtseva, Y. V. and Paseshnichenko, V. A. 2003. Photosynthetic Pigments of Tomato Plants under Conditions of Biotic Stress and Effects of Furostanol Glycosides. *Appl. Biochem. Microbiol.*, **39**: 606–612.
34. Wang, K. L., Deng, Q. Q., Chen, J. W. and Shen, W. K. 2020. Physiological and Molecular Mechanisms Governing the Effect of Virus-Free Chewing Cane Seedlings on Yield and Quality. *Sci. Rep.,* **10**: 10306. 67344-4
35. Warren, C. R. 2008. Rapid Measurement of Chlorophylls with a Microplate Reader. *J. Plant Nutr.,* **31**: 1321–1332.
36. Zhang, Y., Xie, Z., Wang, R., Kutcher, H. R., Wang, Y. and Guo, Z. 2014. Single and Mixed Viral Infection Reduced Growth and Photosynthetic Pigment Content, Damaged Chloroplast Ultrastructure and Enhanced Virus Accumulation in Oriental Lily (*Lilium auratum* cv. Sorbonne). *Philippine Agric. Sci.,* **97(2)**: 138–147.

غربالگری ژنوتیپ های چغندرقند به ویروس پیچیدگی بوته چغندرقند و نماتد سیستی چغندرقند (*Heterodera schachtii*)

رویا کاظمی، مهدی سعادتی، علی مختصی، مجید پدرام، محمدرضا عتیقی، و مسعود شمس بخش

چکیده

نماتد سیستی چغندر (Heterodera schachtii) (BCN) و ویروس پیچیدگی شدید بوته چغندرقند(Curtovirus betae) (BCTV-Svr) دو بیمارگر مهم مزارع چغندرقند در سراسر جهان هستند. در این مطالعه، واکنش 14 ژنوتیپ به طور جداگانه با استفاده از ارقام جلگه و سانتا به عنوان شاهد حساس و مقاوم در قالب طرح کاملاً تصادفی در مقابل آلودگی با BCN و BCTV-Svr مورد ارزیابی قرار گرفت. واکنش ها بر اساس شمارش سیست و تخم و شاخص شدت علائم بود. آزمایش ها در گلخانه دانشگاه تربیت مدرس، تهران، ایران انجام شد و دو بار به طور مستقل تکرار شد. بر اساس نتایج آزمایش های اولیه، ژنوتیپ های S1-960090، S1-940324، S1-960294 و S1-960284 مقاوم به BCN برای بررسی بیشتر انتخاب شدند. علاوه بر این، واکنش چهار ژنوتیپ منتخب به BCN، BCTV-Svr و ترکیب (مخلوط) دو بیمارگر با تجزیه و تحلیل رشد، ویژگی‌های فیزیولوژیکی، بیوشیمیایی و تجمع ویروس مورد ارزیابی قرار گرفت. ژنوتیپ های مقاوم سطوح بالاتری از آنزیم های مرتبط با دفاع مانند کاتالاز، گایاکول پراکسیداز و پلی فنل اکسیداز را نشان دادند، در حالی که

ژنوتیپ های حساس کاهش قابل توجهی در فتوسنتز، سبزی و محتوای کلروفیل a،b و کاروتنوئید در مقایسه با گیاهان غیر مایه‌زنی شده و مقاوم نشان دادند. این اولین مطالعه‌ای است که به منظور جستجوی منابع دوگانه مقاومت در برابر دو بیمارگر مهم که اغلب در مناطق چغندرقند کاری ایران رخ می دهند، انجام شده است. بر اساس نتایج این آزمایش، ژنوتیپ های S1-960090و S1-940324 مقاوم به هر دو بیمارگر شناسایی شدند و برای اهداف اصلاحی توصیه می شوند.