J. Agr. Sci. Tech. (2021) Vol. 23(2): 473-484

Identification of Resistant Sugar Beet (*Beta vulgaris* L.) Genotypes against Beet Curly Top Disease

M. Saadati[1], A. Rajabi[2], and M. Shams-Bakhsh[1*]

ABSTRACT

The Severe strain of *Beet Curly Top Virus* (BCTV-Svr) and *Beet Curly Top Iran Virus* (BCTIV) are considered as the main causal agents of sugar beet curly top disease in Iran and mixed infections of BCTV-Svr and BCTIV usually occur in nature. As the use of resistant cultivars is the safer and stable tool for management of the disease, the objective of current work was to identify sugar beet genotypes resistant to both agents. To this end, the reaction of thirty-eight sugar beet genotypes to infection by each of BCTV-Svr and BCTIV was separately evaluated using their infectious clones under the greenhouse condition. Incubation period, recovery, and disease severity index were considered for selection of resistant genotypes. As a result, ten and seven sugar beet genotypes resistant to, respectively, BCTV-Svr and BCTIV were selected. To evaluate the resistant genotypes, the experiments were repeated under greenhouse condition. In the field experiment with natural infection of viruses, the resistant genotypes were assessed and six sugar beet genotypes (S1 91019, S1 91022, S1 91023, S1 91028, S1 91029, and S1-91041) resistant to BCTV-Svr and BCTIV were identified; which could be used in future breeding programs.

Keywords: Agroinoculation, BCTIV, BCTV-Svr, Infectious clone, Virus management.

INTRODUCTION

Sugar beet (*Beta vulgaris* L.) of the family Chenopodiaceae is a biennial crop. Like other plants, sugar beets may suffer from several important pests and pathogens. Cyst nematode, root rots, rhizomania and Curly Top Disease (CTD) are considered among the major problems in sugar beet fields in Iran that can cause great reductions in yield.

Based on their host range, genome organization, genome-wide pairwise sequence identities, and type of insect vectors; members of the family *Geminiviridae* are classified into nine recognized genera and two unassigned species: *Curtovirus*, *Becurtovirus*, *Mastervirus*, *Begomovirus*, *Eragrovirus*, *Capulavirus*, *Grablovirus*, *Mastrevirus*, and *Topocuvirus* (Varsani *et al.*, 2017). CTD in Iran was first reported from Zarghan and Marvdasht sugar beet fields (Gibson, 1967). At present, *Beet Curly Top Virus-Severe* (BCTV-Svr) and *Beet Curly Top Iran Virus* (BCTIV) are known as the main causal agents of beet CTD in Iran (Bolok Yazdi *et al.*, 2008; Briddon *et al.*, 1998; Varsani *et al.*, 2014b). In 2016, *turnip curly top virus* and turnip leaf roll virus were reported in sugar beet as well as six other field crops (Kamali *et al.*, 2016). The symptoms of CTD not only are related to virus isolate, environmental conditions, susceptibility, and age of host plant, but also depend on its vector (Bennett, 1971; Duffus and Skoyen, 1977; Wintermantel and Kaffka, 2006). The typical symptoms of CTD in sugar beet at the early stage is vein clearing in young leaves (Bennett, 1971). As the disease

[1] Department Plant Pathology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Islamic Republic of Iran.
[2] Sugar Beet Seed Research Institute (SBSI), P. O. Box: 31585-4114, Karaj, Islamic Republic of Iran.
*****Corresponding author; e-mail: shamsbakhsh@modares.ac.ir

[DOR: 20.1001.1.16807073.2021.23.2.12.7]

progresses, leaves become crinkled, roll upward and inward, and, consequently, the plants are stunted (Sutic *et al.*, 1999).

Climate changes that have been happening in the last decades (Chakraborty, 2005; Stern and Stern, 2007), have also induced some epidemics, especially of thermophilic plant diseases that are transmitted by vectors (Chakraborty, 2005). Since these viruses are transmitted by leafhoppers and the best way to control such diseases is the use of resistant lines, seeking resistant sources for better management of the disease should be taken into consideration.

From earlier studies, it is evident that resistance to CTD in sugar beet can be controlled by several genes with low inheritance and also the use of these genotypes for breeding purposes would be difficult (Panella, 2005). In this regard, Panella and Strausbaugh (2011) evaluated resistance in thirty wild beets (*Beta vulgaris* subsp. *maritima* (L.) Arcang) to BCTV-Svr in field conditions infested with leafhoppers and recorded that none of the genotypes had noticeable resistance. In another similar experiment, it was reported that 19 out of 26 wild beets had similar resistance to BCTV-Svr as did the resistant control (Panella and Strausbaugh, 2011). Recently, five sugar beet genotypes have been reported that are at par with resistant check (Strausbaugh and Fenwick, 2018).

To evaluate the resistance in plants, Disease Severity Index (DSI) at 4^{th} and 8^{th} weeks post- inoculation, recovery (the difference in DSI between 4^{th} and 8^{th} weeks) and latent periods were measured in 50 accessions that were inoculated with viruliferous leafhoppers (*Circulifer haematoceps* M.&R.) at the two-leaf stage. The results indicated that four cultivars and one accession had the lowest DSI, higher recovery and longer latent periods (Salehi *et al.*, 2006). Similarly, 5 out of fifty sugar beet lines were identified resistant to both viruses of the CTD in Iran (Montazeri *et al*., 2016).

Owing to the fact that two *geminiviruses* (BCTV-Svr and BCTIV) are the main causal agents of CTD in sugar beet field in Iran, it is necessary to evaluate the reaction of sugar beet genotypes against both viruses. Therefore, the present study was carried out with this aim under greenhouse and field conditions; as a result, six genotypes resistant to BCTV-Svr and BCTIV were identified.

MATERIALS AND METHODS

Greenhouse Experiments

Thirty-eight genotypes of sugar beet (*Beta vulgaris* L.) procured from Sugar Beet Seed Institute (SBSI), Karaj, Iran, were screened in terms of their resistance to two species of *beet curly top virus*, BCTV-Svr and BCTIV, in four experiments. Plants were maintained in an insect-proof greenhouse at 25-28°C. The first experiment was carried out to evaluate possible resistance in all genotypes to BCTV-Svr and the genotypes showed resistant reaction were selected for further evaluation. In the second experiment, all the genotypes were tested in terms of their resistance to BCTIV infection alone and the resistant genotypes were selected. The third and fourth experiments were conducted in greenhouse and the resistant genotypes selected in the earlier experiments were evaluated for their reaction to BCTV-Svr and BCTIV.

Field Experiment

A final experiment was conducted under field condition where all the 14 resistant genotypes selected in the greenhouse experiments were evaluated for resistance to the natural infection with both virus species. The field experiment was conducted for two years, however, there was no natural infection during second year, and thus data was not analyzed. In all experiments under greenhouse conditions, two known lines i.e. one line as Resistant Control (F-20718, RC) and the other one as Susceptible Control (SBSI-2, SC) were used, whereas in the field

[DOR: 20.1001.1.16807073.2021.23.2.12.7]

experiments. In addition to these controls, additional Resistant Control (F-20364, RC) was also used.

The phenotypic characteristics used for determination of resistant genotypes included the incubation period, Disease Severity Index (DSI) and recovery. For incubation period, the times from inoculation to first appearance of symptoms, and for recovery, comparisons of symptoms between two time lapses i.e. weeks 4 and 8 for BCTV-Svr and weeks 6 and 12 for BCTIV were assayed. To assess the DSI, each inoculated plant was graded using a 1-9 disease severity scale (Montazeri *et al.*, 2016). Moreover, virus accumulation in resistant genotypes that were not significantly different from RC was assessed *via* semi-quantitative Polymerase Chain Reaction (semi-qPCR).

Semi-Quantitative PCR

In order to quantify the virus accumulation in the tested genotypes that were in the same group with the resistant control, DNA was extracted from leaf samples at the end of the 6^{th} and 10^{th} weeks following agroinoculation (Doyle, 1987) and then semi-qPCR was performed. Primers and programs for semi-qPCR are shown in Tables 1 and 2. PCR products obtained were also analyzed by electrophoresis on a 1% agarose gel in 1X TBE buffer (90 mMTris-borate, 2 mM EDTA). Furthermore, DNA molecular weight markers (GeneRuler^{TM} 1 kb DNA ladder, Fermentas, Lithuania) were used to determine the approximate size of amplified fragments.

Experimental Design and Statistical Analysis

A completely randomized design, with at least 20 replicates per line, was used under greenhouse conditions and then plant agroinoculation was carried out with the infectious clone of each virus as described by Montazeri *et al*. (2016). Viruses used in this study were BCTV-Svr, GenBank accession no. X97203 (Briddon *et al.*, 1998) provided by Dr. S. A. A. Behjatnia (Shiraz University, Shiraz, Iran), and BCTIV-[IR:Neg:B33P:-Sug:08], GenBank accession no. JQ707949 (Heydarnejad *et al.*, 2013) provided by Dr. J. Heydarnejad from Shahid Bahonar University of Kerman, Kerman, Iran. The experimental material consisted of 17 genotypes (including 14 selected resistant genotypes and two RC and one SC lines) were planted on 6 May 2016 in randomized complete block design with 6 replications in 7 m long rows 50 cm apart. Disease symptoms were recorded two times, 15 and 19 weeks after planting.

The data was analyzed *via* SAS (version 9.1) (SAS Institute 2002) using the General Linear Model Procedure (Proc GLM). The

Table 1. Oligonucleotide primers used to measure virus accumulation in resistant lines.

Virus and primer names	Forward primer 5′-3′	Reverse primer 5′-3′	Predicted product size (Base pairs)	Reference
BCTV-Svr-IR V1V/V1C	*5′-AGAAAATATACAAGAAATC-3′*	*5′-TTAATAAAAATA ACATCTAC-3′*	750 bp	(Ebadzad *et al.*, 2008)
BCTIV-1559-F/FL-R	*5′-CAC TCATACAAG GTATCCAG TCCA-3′*	*5′-ACG GAG CTC TCCAAA CAGTATT GGC-3′*	792 bp	(Heydarnejad, un published) [a]
DNA18S1/S2	*5′-AACGGCTACCACATCCAAG-3′*	*5′-TCATTACTCCGATCCCG AA -3′*	500 bp	(Faria *et al.*, 2006)

[a] Shahid Bahonar University of Kerman, Kerman, Iran; $P > F$ was the probability associated with the F value. LSD = Fisher's protected least significant difference value with α=0.05.

[DOR: 20.1001.1.16807073.2021.23.2.12.7]

Table 2. Semi-quantitative PCR programs for *Beet Curly Top Virus* (BCTV-Svr) and *Beet Curly Top Iran Virus* (BCTIV).[a]

PCR cycle	BCTV-Svr		BCTIV		
	Temperature (°C)	Time	Temperature (°C)	Time	Cycle
First denaturing	94	5 min	94	3 min	1
Denaturing	94	1 min	94	1 min	18
Annealing	48	40 s	59	1 min	18
Extension	72	1 min	72	1.5 min	18
Final extension	72	10 min	72	10 min	1

[a] $P > F$ was the probability associated with the F value. LSD = Fisher's protected least significant difference value with α=0.05.

Least Significant Difference (LSD) test at 5% probability level was also employed for comparison of means.

RESULTS

In all experiments, curly top symptoms were observed in susceptible line (191) 1-4 weeks after inoculation of plants with either of the two viruses. The resistance reaction in the genotypes differed not only in terms of symptom appearance but also in terms of severity. The symptoms of BCTIV and BCTV-Svr in the tested genotypes were the same as described for infectious clones (Ebadzad Sahraei *et al.*, 2008; Heydarnejad *et al.*, 2013). It was found that some genotypes showed mild symptoms and some others, despite inoculation, showed no symptoms at all.

Reaction to BCTV-Svr in Greenhouse Experiments

Results showed that the time of first symptom appearance (latent period) varied from 7 to 20 days, depending on the genotypes (Table 3). The studied genotypes had different responses to BCTV-Svr inoculation and the DSI varied from 2.72 to 8.78 four weeks after inoculation (Table 3). The highest DSI was recorded in OT NB5, 16402 × CL510, S1-91043, and S1-91035 genotypes, whereas lowest DSI was observed in S1 91019 and 13668× (16042× CL511) genotypes. Disease severity index recorded within four weeks varied significantly in different genotypes ($P \leq$ 5). The comparison of genotypes also revealed that genotypes S1 91023, S1 91029, 13668× (16042× CL511), S1 91019, 16402-66, SC (607× 474-78-40-90), S1 91025, 13668× (16042× CL634), S1 91028, and S1 91022 grouped in the same cluster with the resistant control. These genotypes had the lowest percentage of infected plants, the highest latent period, the highest recovery, and the lowest DSI (Table 3), such that genotypes S1 91019, S1 91023, and 13668× (16042 × CL511) had 27, 30, and 30% infected plants, respectively. In semi-qPCR, the resistant genotypes showed the lowest accumulation of viruses (Figure 1). Since accumulation of virus in the resistant genotypes was at low levels, incubation period could be longer and the rate of recovery could be faster (Salehi *et al.*, 2006). The genotypes that had the same statistical group with the resistant line were inoculated once, and genotypes reaction did not change in comparison with the first experiment, except S1 91025. In the first experiment, line S1 91025, which showed resistance to the virus (Table 4), fell in the same group with the susceptible line and its latent period decreased from 18 days (first experiment) to 8 days.

Reaction to BCTIV in Greenhouse Experiments

The latent period in the studied genotypes after inoculation with BCTIV varied from 14 to 29 days (Table 5). The reaction of genotypes to the virus was assessed six weeks after inoculation to BCTIV (Table 5). In this respect, data analysis demonstrated

[DOR: 20.1001.1.16807073.2021.23.2.12.7]

Table 3. Curly top disease severity index after inoculation and latent period in 38 sugar beet genotypes inoculated with BCTIV infectious clone under greenhouse. [a]

Accession no	DSI weeks		Latent period	Accession no	DSI weeks		Latent period
	4	8			4	8	
16396-66	5.58	4.36	10	S1 91025	3.88	2.5	18
16398-66	5.81	4.23	8	S1 91026	5.90	4.3	15
16402-66	3.91	2.1	15	S1 91027	6.4	5.13	9
O.T NB5	8.78	7.7	7	S1 91028	3.93	2.13	17
16402 × CL634	8.44	7.23	7	S1 91029	3.21	1.34	20
16402 × CL510	8.66	7.6	7	S1-91032	7.00	5.25	10
16402 × CL511	7.73	6.56	8	S1-91033	7.00	5.3	8
13637 × (16042 × CL634)	7.80	6.75	10	S1-91034	7.58	6.8	7
13637 × (16042 × CL510)	5.94	4.27	7	S1-91035	8.46	7.5	7
13637 × (16042 × CL511)	6.90	5.24	9	S1-91036	5.71	3.72	13
13668 × (16042×CL634)	4.33	2.9	14	S1-91040	7.50	6.5	14
13668 × (16042×CL510)	7.21	5.71	8	S1-91041	7.60	5.96	12
13668 × (16042×CL511)	2.84	1.2	18	S1-91042	8.44	6.75	10
S1 91019	2.72	1.5	20	S1-91043	8.50	6.96	9
S1 91020	6.11	4.85	10	SC (607 × 436-100-42-90)	8.08	7	8
S1 91021	5.62	4.12	10	SC (607 × 41-25-19-90)	6.07	4.5	9
S1 91022	4.63	2.8	15	SC (607 × 474-78-40-90)	3.62	1.7	15
S1 91023	3.00	1.25	17	F – 20718	3.75	2.5	15
S1 91024	6.11	4.11	14	191	6.81	5.8	8
$P > F$, LSD ($P < 0.05$) = 1.47							

[a] $P > F$ was the probability associated with the F value. LSD = Fisher's protected least significant difference value with α=0.05.

Table 4. Curly top disease severity index at the end of 4 week after inoculation with BCTV-Svr infectious clone under greenhouse condition.

Accession number	DSI in the fourth week	Statistical group*
S1 91019	2.66	B
13668× (16042× CL511)	2.56	B
S1 91023	1.71	B
S1 91029	2.29	B
SC (607× 474-78-40-90)	3.25	B
F-20718	3.20	B
16402-66	2.58	B
S1 91028	3.13	B
13668× (16042× CL634)	3.23	B
S1 91022	3.35	B
S1 91025	5.83	A
191	6.25	A

* Means followed by the same letter did not differ based on Fisher's protected least significant difference (LSD) value with α = 0.05.

[DOR: 20.1001.1.16807073.2021.23.2.12.7]

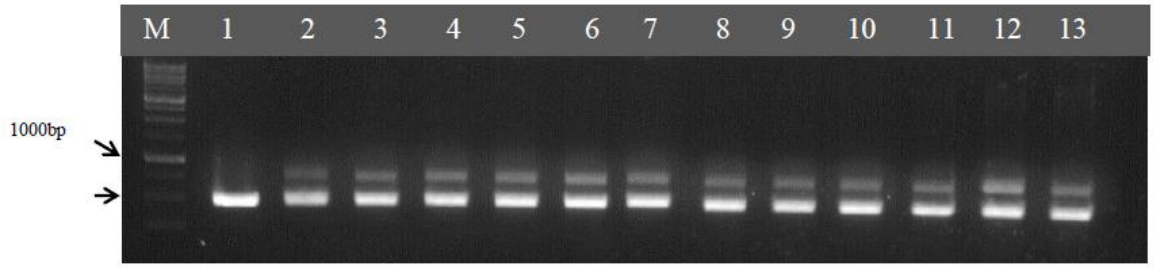

Figure 1. Semi q-PCR for quantitation of BCTV-Svr in genotypes resistant to BCTV-Svr. Specific 750 bp for BCTV-Svr and 500 bp for *18S rDNA* fragments in the sugar beet lines. Electrophoresis was carried out on a 1 % agarose gel. M: Size marker (GeneRuler^{TM} 1 kb DNA ladder, Fermentas); Lane 1: Non-inoculated sugar beet, Lane 2: RC (F–20718), Lane 12: SC (191), Lanes 3-11 and 13: (Tested genotypes)= 13668* (16042* CL511), S1 91019, 16402-66, SC (607* 474-78-40-90), S1 91025, S1 91029, S1 91028, 13668* (16042* CL634), S1 91023 and S1 91022, respectively.

Table 5. Curly top disease severity index after inoculation and latent period in 38 sugar beet genotypes inoculated with BCTIV infectious clone under greenhouse. [a]

Accession no	DSI weeks		Latent period	Accession no	DSI weeks		Latent period
	6	12			6	12	
16396-66	3.86	3.72	17	S1 91025	4.33	2.45	17
16398-66	4.05	1.56	20	S1 91026	4.27	3.79	17
16402-66	5.75	2.90	17	S1 91027	5.23	4.90	15
O.T NB5	5.7	1.43	15	S1 91028	2.77	2.46	22
16402× CL634	5.61	1.37	16	S1 91029	2.16	2.50	21
16402× CL510	5	1.08	18	S1-91032	4.2	3.85	17
16402× CL511	3.37	3.95	17	S1-91033	3.9	3.90	18
13637× (16042× CL634)	3.87	1.27	17	S1-91034	4.2	3.56	17
13637× (16042× CL510)	5.86	1.05	15	S1-91035	4	3.74	18
13637 × (16042 × CL511)	6.09	1.09	14	S1-91036	4.2	3.91	18
13668× (16042× CL634)	5.69	4.50	17	S1-91040	5.2	3.60	16
13668 × (16042× CL510)	3.14	2.00	20	S1-91041	4.2	3.75	14
13668× (16042× CL511)	2.6	2.10	23	S1-91042	2.5	2.91	22
S1 91019	2.79	1.80	22	S1-91043	5.12	4.65	14
S1 91020	5.86	5.50	14	SC (607× 436-100-42-90)	6.21	5.68	14
S1 91021	5.12	4.50	15	SC (607× 41-25-19-90)	5.05	5.00	16
S1 91022	4.5	4.17	17	SC (607× 474-78-40-90)	2.6	4.00	21
S1 91023	2.41	4.11	21	F – 20718	2.9	2.00	29
S1 91024	4.9	4.05	19	191	6.23	4.96	15
$P > F$, LSD ($P < 0.05$) = 1.04							

[a] $P > F$ was the probability associated with the F value. LSD = Fisher's protected least significant difference value with α=0.05.

[DOR: 20.1001.1.16807073.2021.23.2.12.7]

that the DSI was significantly different among the genotypes (P≤ 0.05). Moreover, the genotypes 13637× (16042× CL511), S1 91020, and SC (607× 436-100-42-90) had the highest, while the genotypes S1-91042, S1 91029, and S1 91023 had the lowest DSI. Severe stunting was also observed in the genotypes 13637× (16042× CL510) and 13637× (16042× CL511). On comparing means, it was also revealed that genotypes S1 91019, SC (607× 474-78-40-90), S1 91029, S1-91042, 13668× (16042× CL511), S1 91023, 16398-66, 13668× (16042× CL510), and S1 91028 did not differ significantly from the resistant control. This statistical group had the lowest DSI as well as the highest incubation period among the assessed genotypes (Table 5). Moreover, these resistant genotypes had different accumulations of viruses when compared with the susceptible control (Figure 2). Despite being in the same statistical groups with the resistant control and having the lowest DSI, accumulation of viruses in genotypes 16398-66 and 13668× (16042× CL510) was higher than the resistant control. These genotypes were identified as tolerance genotypes, the term 'tolerance was used for the genotypes in which resistance to symptom formation or yield loss, rather than to virus multiplication (Fraser, 1990). To evaluate the resistant genotypes, the experiment was repeated. Although in some genotypes the DSI increased, they had the same statistical group with resistant control (Table 6).

Field Experiment

To find genotype(s) resistant to both viruses, based on greenhouse studies, 14 sugar beet genotypes were selected to test

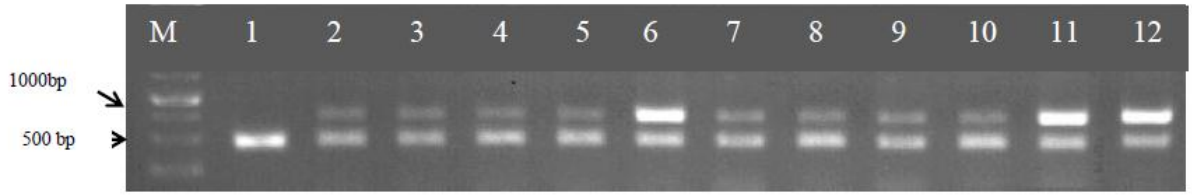

Figure 2. Semi q-PCR for quantitation of BCTIV in genotypes resistant to BCTIV, specific 790 bp for BCTIV and 500 bp for *18S rDNA* fragment in the sugar beet lines, electrophoresis on a 1% agarose gel and staining by ethidium bromide. M: Size marker (GeneRuler^{TM} 1 kb DNA ladder, Fermentas); Lane 1: Non-inoculated sugar beet, Lane 2: RC (F–20718), Lane 12: SC (191), Lanes 3–11: (Tested genotypes)=13668* (16042* CL511), S1 91019, S1 91028, 13668* (16042*CL510), S1 91029, S1-91041, S1 91023, SC (607* 474-78-40-90) and 16398-66.

Table 6. Curly top disease severity index at the end of 6 weeks after inoculation with BCTIV infectious clone under greenhouse condition.

Accession number	DSI in the sixth week	Statistical group*
S1 91029	2.30	C
S1 91042	2.64	CB
13668× (16042× CL511)	2.70	CB
SC (607 × 474-78-40-90)	2.70	CB
S1 91028	2.80	CB
S1 91019	2.87	CB
F – 20718	2.96	CB
13668× (16042× CL510)	3.20	CB
16396-66	3.29	CB
S1 91023	3.42	B
191	6.41	A

* Means followed by the same letter did not differ based on Fisher's protected least significant difference (LSD) value with α = 0.05.

[DOR: 20.1001.1.16807073.2021.23.2.12.7]

against the two viruses under field condition. The genotypes (Table 7), namely, 13668× (16042× CL511), S1 91019, S1 91023, S1 91028, S1 91029 and SC (607× 474-78-40-90), which were resistant to both viruses, along with 13668× (16042× CL634), S191022, 16402-66, and S1 91025 resistant to BCTV-Svr, as well as 16398-66, [13668× (16042× CL510] and S1-91042 genotypes, which were resistant to BCTIV, were evaluated. The results showed significant differences among genotypes. Genotypes S1 91019, S1 91022, S1 91023, S1 91028, S1 91029 and S1-91042 belong to the same group as the resistant control. Among these, the genotypes S1 91022 and S1-91042 were resistant to BCTV-Svr and BCTIV, respectively, under greenhouse condition; however, in field experiment, these two along with four other genotypes (S1 91019, S1 91023, S1 91028, S1 91029) showed resistance reaction to both viruses (Table 7). Several genotypes, for example, 13668× (16042× CL511), SC (607× 474-78-40-90), S1 91025, 13668× (16042× CL634), 13668× (16042× CL510), and 16398-66, which had shown different reactions (resistance or susceptibility) to the two viruses in greenhouse assay, demonstrated similar reactions in the field experiment and all of them were susceptible.

DISCUSSION

Since BCTV-Svr and BCTIV are the main causal agents of CTD in Iran (Bolok Yazdi *et al*., 2008; Briddon *et al*., 1998; Varsani *et al*., 2014a; Varsani *et al*., 2014b), and the most promising approach of their management is the use of resistant genotypes. Therefore, this research was carried out to find sugar beet genotypes resistant to both CTD causal agents. In view of this, the reaction of 38 sugar beet genotypes to CTD was evaluated and, finally, 10 and 7 genotypes resistant to, respectively, BCTV-Svr and BCTIV under greenhouse condition and six genotypes resistant to mixed infections under field conditions were identified.

The reactions of different genotypes varied

Table 7. Curly top disease severity index, number of infected genotypes and statistical group of sugar beet genotypes evaluated under field condition. [a]

Accession number	No plant	No. infected plant	DSI	Statistical group
F-20718 (RC)	321	26	1.21	F
S1-91019	318	44	1.23	F
S1-91022	258	34	1.27	F
S1-91023	267	51	1.30	EF
S1 91029	265	40	1.33	DEF
S1-91042	266	42	1.35	DEF
S1 91028	284	40	1.36	DEF
F-20364 (RC)	334	68	1.44	DEF
S1-91039	127	33	1.52	DE
13668× (16042× CL511)	279	61	1.55	D
S.C (607× 474-78-40-90)	304	92	1.94	C
S1 91025	279	66	1.97	CB
13668× (16042× CL634)	288	98	2.03	CB
16398-66	269	81	2.10	CB
13668× (16042× CL510)	289	87	2.18	B
16402-66	296	108	2.44	A
SBSI-2 (SC)	304	129	2.55	A
P > *F*, LSD (*P* < 0.05) = 0.2412				

[a] *P* > *F* was the probability associated with the F value. LSD = Fisher's protected least significant difference value with α=0.05.

[DOR: 20.1001.1.16807073.2021.23.2.12.7]

significantly to viruses. Even in a single same accession, different resistance reactions to BCTV-Svr and BCTIV were observed. For example, 16398-66 genotype was susceptible to BCTV-Svr but resistant to BCTIV. Such reaction to these viruses (Montazeri *et al.*, 2016) and to BCTV-CFH (BCTV-C) that are different from BCTV-Logan (BCTV-H) have also been reported by Stenger *et al.* (1990). A similar reaction to RNA viruses was reported, where two pepper (*Capsicum chinense*) lines (CNPH275 and PI159236) were immune against one specific isolate (TSWV-BSB) but were susceptible to another isolate (TSWV-SP) (Boiteux *et al.*, 1993). Since the host plant and environmental conditions were the same in this study, it was concluded that the different reactions of the host plants would be related to the genomes of the viruses.

The symptoms reported for both infectious clones of BCTV-Svr and BCTIV (Fatahi *et al.*, 2012; Heydarnejad *et al.*, 2013) were observed in the susceptible line and other genotypes, confirming that infection assays of the genotypes has been correctly fulfilled. The genotypes also showed similar symptoms to both viruses such as stunted and distorted plant growth, leaf curling, and vein swelling as reported earlier by Jahanbin *et al.* (2015) and Soleimani *et al.*(2013). These results showed that BCTIV induced milder symptoms than BCTV-Svr. Also, latent period was different between the two viruses: it was 14-29 day post-inoculation (dpi) for BCTIV, in different genotypes, while it was 7-20 dpi for BCTV-Svr (Jahanbin *et al.*, 2015; Montazeri *et al.*, 2016). Several factors such as host, virus and environment could influence the symptoms severity and latent period. On the plant side, species, variety, and age of the host at the inoculation time, and on the virus side, species and strain of the virus; suppression of virus-induced host defenses such as RNA silencing and the method and site of inoculation have an effect on the incubation period (Favara *et al.*, 2019). In this study, the same genotypes were inoculated with both viruses and kept at the same condition, thus the difference in latent period could be referred to viruses.

Genotypes 13668× (16042× CL511), SC (607× 474-78-40-90), S1 91025, 13668× (16042× CL634), 13668× (16042× CL510), and 16398-66 were resistant under greenhouse condition but their reaction under field condition were susceptibility. It has been reported that sugar beet cultivars infected with both BCTV-Svr and BCTIV show severe symptoms (Fatahi *et al*., 2012; Majidi *et al*., 2015), and viruses accumulation increase (Majidi *et al*., 2017). Moreover, the mixed infection of viruses in resistant genotypes could change their reaction or the resistance of genotype could be compromised (Astaraki *et al*., 2020).

It should be noted that mixed infections of both viruses might occur naturally. The identified genotypes, namely, S1 91019, S1 91022, S1 91023, S1 91028, S1 91029, and S1-91041 resistant to both viruses under natural field conditions may further be evaluated for their agronomic traits and can be exploited in virus resistant breeding program of sugar beet.

ACKNOWLEDGEMENTS

This research was supported by Tarbiat Modares University.

REFERENCES

1. Astaraki, S., Safaie, N. and Shams-Bakhsh, M. 2020. Reaction of Sugar Beet, Pepper and Bean Plants to Co-Infection with *Cucumber Mosaic Virus* and *Beet Curly Top Viruses*. *Iran. J. Plant Pathol.*, **56(3)**: 1-16.
2. Bennett, C. W. 1971. Curly Top Disease of Sugarbeet and Other Plants. *Am. Phytopathol. Soc.*, St. Paul, **7:** 68-81.
3. Boiteux, L., Nagata, T., Dutra, W. and Fonseca, M. 1993. Sources of Resistance to *Tomato Spotted Wilt Virus (*TSWV) in Cultivated and Wild Species of Capsicum. *Euphytica*, **67(1-2)**: 89-94.

[DOR: 20.1001.1.16807073.2021.23.2.12.7]

4. Bolok Yazdi, H. R., Heydarnejad, J. and Massumi, H. 2008. Genome Characterization and Genetic Diversity of *Beet Curly Top Iran Virus*: A *Geminivirus* with a Novel Nonanucleotide. *Virus Genes*, **36(3)**: 539-545.
5. Briddon, R. W., Stenger, D. C., Bedford, I. D., Stanley, J., Izadpanah, K. and Markham, P. G. 1998. Comparison of a *Beet Curly Top Virus* Isolate Originating from the Old World with Those from the New World. *Eur. J. Plant Pathol.*, **104(1)**: 77-84.
6. Chakraborty, S. 2005. Potential Impact of Climate Change on Plant-Pathogen Interactions. Australas. *Plant Pathol.*, **34(4)**: 443-448.
7. Doyle, J. J. 1987. A Rapid DNA Isolation Procedure for Small Quantities of Fresh Leaf Tissue. *Phytochem. Bull.*, **19**: 11-15.
8. Duffus, J.E. and Skoyen, I. 1977. Relationship of Age of Plants and Resistance to a Severe Isolate of the *Beet Curly Top Virus*. *Phytopathology*, **67(2)**: 151-154.
9. Ebadzad, S. G., Bahjatina, S. A. A. and Izadpanah, K. 2008. Infectivity of the Cloned Genome of Iranian Isolate of *Beet Severe Curly Top Virus* in Experimental Hosts. *Iran. J. Plant Pathol.*, **44**: 176-183.
10. Faria, J. C., Albino, M. M., Dias, B. B., Cançado, L. J., da Cunha, N. B., Silva, L. D. M., Vianna, G. R. and Aragao, F. 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(5):** 565-571.
11. Fatahi, Z., Behjatnia, S. A. A., Afsharifar, A., Hamzehzarghani, H. and Izadpanah, K. 2012. Screening of Sugar Beet Cultivars for Resistance to Iranian Isolate of *Beet Severe Curly Top Virus* Using an Infectious Clone of the Virus. *Iran. J. Plant Pathol.*, **48(1)**: 111-121.
12. Favara, G. M., Bampi, D., Molina, J. P. E. and Rezende, J. A. M. 2019. Kinetics of Systemic Invasion and Latent and Incubation Periods of *Tomato Severe Rugose Virus* and *Tomato Chlorosis Virus* in Single and Co-Infections in Tomato Plants. *Phytopathology*, **109(3)**: 480-487.
13. Fraser, R. 1990. The genetics of Resistance to Plant Viruses. *Annu. Rev. Phytopathol.*, **28(1)**: 179-200.
14. Gibson, K. E. 1967. Possible Incidence of Curly Top in Iran: A New Record. *Plant Dis. Rep.*, **51**: 976-977.
15. Heydarnejad, J., Keyvani, N., Razavinejad, S., Massumi, H. and Varsani, A. 2013. Fulfilling Koch's Postulates for *Beet Curly Top Iran Virus* and Proposal for Consideration of New Genus in the Family Geminiviridae. *Arch. Virol.*, **158(2)**: 435-443.
16. Jahanbin, D., Izadpanah, K. and Behjatnia, S. A. A. 2015. Comparison of Natural and Experimental Host Range of *Beet Severe Curly Top, Beet Curly Top Iran* and *Tomato Yellow Leaf Curl Viruses*. *Iranian J. Plant Pathol.*, **51(4)**: 505-521.
17. Kamali, M., Heydarnejad, J., Massumi, H., Kvarnheden, A., Kraberger, S. and Varsani, A. 2016. Molecular Diversity of *Turncurtoviruses* in Iran. *Arch. Virol.*, **161(3)**: 551-561.
18. Majidi, A., Behjatnia, S. A. A. and Hamzehzarghani, H. 2015. Screening of Sugar Beet Cultivars Resistant to Beet Curly Top Iran Virus. Proceedings of the First International and 9th National Biotechnology Congress of Islamic Republic of Iran, Shahid Beheshti University, 24-26 May, Tehran, Iran.
19. Majidi, A., Hamzehzarghani, H., Izadpanah, K. and Behjatnia, S. A. A. 2017. Interaction between *Beet Curly Top Iran Virus* and the Severe Isolate of *Beet Curly Top Virus* in Three Selective Sugar Beet Cultivars. *J. Plant Pathol.*, **99**: 381-389.
20. 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(1)**: 31-40.
21. Panella, L., 2005. Objectives of Sugar Beet Breeding-Resistance to the *Curly Top Virus. Genetics Breed.*, **1**: 74-76.
22. Panella, L. and Strausbaugh, C. A. 2011. Beet Curly Top Resistance of USDA-ARS National Plant Germplasm System Plant Introductions, 2010. *Plant Dis. Manage. Rep.*, **5:** FC066.
23. Salehi, M., Nejat, N., Masumi, M., Niroomand, M. and Izadpanah, K. 2006. Greenhouse Evaluation of Sugar Beet Germplasm for Curly TopResistance. *Iran. J. Plant Pathol.*, **42(1)**: 55-70.

[DOR: 20.1001.1.16807073.2021.23.2.12.7]

24. Soleimani, R., Matic, S., Taheri, H., Behjatnia, S. A. A., Vecchiati, M., Izadpanah, K. and Accotto, G. P. 2013. The Unconventional *Geminivirus Beet Curly Top Iran Virus*: Satisfying Koch's Postulates and Determining Vector and Host Range. *Ann. Appl. Biol.*, **162(2)**: 174-181.
25. Stenger, D. C., Carbonaro, D. and Duffus, J. E. 1990. Genomic Characterization of Phenotypic Variants of *Beet Curly Top Virus. J. Gen. Virol.*, **71(10)**: 2211-2215.
26. Stern, N. and Stern, N. H. 2007. *The Economics of Climate Change: The Stern Review*. Cambridge University Press, PP. 1-2.
27. Strausbaugh, C. A. and Fenwick, A. L. 2018. Beet Curly Top Resistance in USDA-ARS Ft. Collins Germplasm, 2017. *Plant Dis. Manage. Rep.*, **12**: 84-85.
28. Sutic, D. D., Ford, R. E. and Tosic, M. T. 1999. *Handbook of Plant Virus Diseases.* CRC Press, PP 277-279.
29. Taheri, H., Izadpanah, K. and Behjatnia, S. A. A. 2012. *Circulifer haematoceps*, the Vector of the *Beet Curly Top Iran Virus*. *Iran. J. Plant Pathol.*, **48**(1): 141-145.
30. Varsani, A., Martin, D.P., Navas-Castillo, J., Moriones, E., Hernández-Zepeda, C., Idris, A., Murilo Zerbini, F. and Brown, J. K. 2014a. Revisiting the Classification of *Curtoviruses* Based on Genome-Wide Pairwise Identity. *Arch. Virol.*, **159(7)**: 1873-1882.
31. Varsani, A., Navas-Castillo, J., Moriones, E., Hernández-Zepeda, C., Idris, A., Brown, J.K., Murilo Zerbini, F., Martin, D. P., 2014b. Establishment of Three New Genera in the Family Geminiviridae: *Becurtovirus*, *Eragrovirus* and *Turncurtovirus*. *Arch. Virol.*, **159**: 2193-2203.
32. Varsani, A., Roumagnac, P., Fuchs, M., Navas-Castillo, J., Moriones, E., Idris, A., Briddon, R.W., Rivera-Bustamante, R., Murilo Zerbini, F. and Martin, D. P. 2017. *Capulavirus* and *Grablovirus*: Two New Genera in the Family Geminiviridae. *Arch. Virol.*, **162(6)**: 1819-1831.
33. Wintermantel, W. M. and Kaffka, S. R. 2006. Sugar Beet Performance with Curly Top Is Related to Virus Accumulation and Age at Infection. *Plant Dis.*, **90(5)**: 657-662.

شناسایی ژنوتیپ های چغندرقند (*Beta vulgaris* L.) مقاوم علیه بیماری پیچیدگی بوته

م. سعادتی، آ. رجبی، و م. شمس بخش

چکیده

جدایه‌ی شدید ویروس پیچیدگی بوته (*Beet curly top virus* (BCTV-Svr)) و ویروس ایرانی پیچیدگی بوته چغندرقند (*Beet curly top Iran virus* (BCTIV)) از مهمترین عوامل بیماری پیچیدگی بوته چغندرقند در ایران محسوب می شوند و آلودگی مخلوط BCTV-Svr و BCTIV در طبیعت رخ می‌دهد. از آنجایی که استفاده از رقم های مقاوم پایدارترین ابزار برای مدیریت بیماری می‌باشد، هدف پژوهش حاضر شناسایی ژنوتیپ های مقاوم به هر دو عامل بیماری بود. برای این منظور واکنش ۳۸ ژنوتیپ چغندرقند نسبت به هر دو ویروس به صورت جداگانه تحت شرایط گلخانه با استفاده از همسانه عفونت زای ویروس‌ها ارزیابی شد. برای انتخاب ژنوتیپ مقاوم دوره نهفتگی، بهبودی و شاخص شدت بیماری بررسی شد. در شرایط گلخانه به ترتیب ده و هفت ژنوتیپ

[DOR: 20.1001.1.16807073.2021.23.2.12.7]

مقاوم به BCTV-Svr و BCTIV برای ارزیابی‌های بعدی انتخاب شد. آزمایش‌های گلخانه‌ای به منظور ارزیابی ژنوتیپ‌های مقاوم تکرار شدند. در آزمایش مزرعه با آلودگی ویروسی طبیعی، ژنوتیپ-های مقاوم مورد ارزیابی قرار گرفتند و در نتیجه شش ژنوتیپ (S1 91019, S1 91022, S1 91023, S1 91028, S1 91029, and S1-91041) مقاوم به هر دو ویروس شناسایی شدند که می تواند در برنامه های اصلاحی بعدی مورد استفاده قرار گیرد.

[DOR: 20.1001.1.16807073.2021.23.2.12.7]