ویروس‌های ایجادکننده پیچیدگی بوته چغندرقند در ایران: تنوع و پراکنش در گیاهان و مناطق جغرافیایی*

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چکیده

ویروس پیچیدگی شدید بوته چغندرقند (Beet severe curly top virus, BSCTV)، که اخیراً به عنوان سویه شدید ویروس پیچیدگی بوته چغندرقند معرفی شده است و ویروس ایرانی پیچیدگی بوته چغندرقند (Beet curly top Iran virus, BCTIV) دو عامل اصلی ایجادکننده بیماری پیچیدگی بوته در چغندرقند و چندین میزبان دولپه‌ای دیگر در ایران محسوب می‌شوند. تنوع ژنتیکی و پراکنش این ویروس‌ها در ارتباط با میزبان، منطقه جغرافیایی و سن گیاه میزبان اصلی (چغندرقند) مورد بررسی قرار گرفت. بدین منظور واکنش زنجیره‌ای پلیمراز (PCR) با استفاده از آغازگرهای اختصاصی برای تکثیر بخشی از ژنوم BSCTV و BCTIV در آموده‌های دی‌ان‌ای استخراج شده از ۷۳٤ نمونه گیاهی جمع‌آوری شده از میزبان‌های مختلف در پنج استان ایران انجام گرفت. قطعات تکثیر یافته به اندازه حدود ۵۰۰ و ۷۰۰ جفت‌باز به ترتیب از ۱۸ و ۱۰ گیاه آلوده به BSCTV و BCTIV تعیین توالی شده و واکاوی فیلوژنتیکی در مورد این قطعات صورت گرفت. اثر منطقه جغرافیایی بر تنوع ژنتیکی تنها در مورد جدایه‌های BCTIV مشاهده شد. نوع گیاه میزبان با تنوع ژنتیکی هیچ کدام از دو ویروس ارتباطی نشان نداد. در این مطالعه بیشترین میزان وقوع هر دو ویروس در استان فارس حادث شد. تجزیه واریانس ناپارامتری داده‌های حاصل از ۷۳٤ نمونه نشان داد که شیوع هر ویروس در مزارع مختلف متفاوت است و به نوع گیاه میزبان، تاریخ کشت و منطقه جغرافیایی بستگی دارد. در حالیکه شیوع BCTIV در چغندر قند بیشتر از BSCTV بود، BSCTV از شیوع بیشتری در سایر میزبان‌ها نظیر گوجه‌فرنگی، فلفل و لوبیا برخوردار بود.

کلیدواژه: تاریخ کشت، تنوع ژنتیکی، منطقه جغرافیایی، میزبان، ویروس پیچیدگی شدید بوته چغندرقند، ویروس ایرانی پیچیدگی بوته چغندرقند

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Beet curly top viruses in Iran: Diversity and incidence in plants and geographical regions*

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Abstract

Beet severe curly top virus (BSCTV, recently named BCTV-Svr) and Beet curly top Iran virus (BCTIV) are the causal agents of curly top disease in sugar beet and several other dicotyledonous plants in Iran. In this investigation, genetic diversity and prevalence of curly top viruses in relation to host, geographical distribution and age of the main host plant (sugar beet) were studied. Polymerase chain reaction using specific primer pairs to amplify a part of the genome of BSCTV and BCTIV was applied to 734 plant samples collected from various hosts in five provinces in Iran. DNA fragments approximately 500 and 700 bp in size were amplified from 18 BSCTV and 10 BCTIV infected plants, respectively. Amplified fragments were subjected to sequencing and phylogenetic analysis. Effect of geographical location on genetic diversity of viruses was observed only for BCTIV isolates. Type of host plant had no correlation with genetic diversity of either BSCTV or BCTIV. The highest incidence of both viruses occurred in Fars province. Nonparametric analysis of PCR data showed that the prevalence of each virus varied in different fields and depended on the host, date of cultivation and geographical region. While the incidence of BCTIV in sugar beet plants was higher than BSCTV, BSCTV was more frequent in other plants such as tomato, pepper and bean.

Keywords: Beet curly top Iran virus, Beet severe curly top virus, date of cultivation, host, genetic diversity

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Introduction

Curly top is a serious disease of sugar beet (*Beta vulgaris*) in semiarid parts of the world including the Middle East and countries bordering the Mediterranean Sea (Briddon *et al.* 1998, Chen & Gilbertson 2009). This disease has been considered economically important in Iran since its first report in 1967 (Gibson 1967). The symptoms of curly top in sugar beet include stunting, leaf curling, vein swelling (enation), stiffness of leaves and hyperplasia and necrosis of the phloem (Bennett 1971). Other susceptible crops include common bean (*Phaseolus vulgaris*), pepper (*Capsicum* spp.), spinach (*Spinacia oleracea*), and tomato (*Solanum lycopersicum*) (Bennett 1971).

Beet curly top is caused by several viruses belonging to *Curtovirus* and *Becurtovirus* genera (family: *Geminiviridae*) (Adams *et al.* 2013). The viruses are transmitted by leafhoppers (*Circulifer* spp.) and can infect dicotyledonous hosts from 44 families and at least 300 species (Bennett 1971). Each virus contains a circular single-stranded DNA of approximately 3 kb in size that is encapsulated in a twinned icosahedral capsid. Curtoviruses and becurtoviruses, however, differ considerably in the number of open reading frames of their genome, genome organization and nucleotide sequences (Brown *et al.* 2012, Heydarnejad *et al.* 2013, Soleimani *et al.* 2013).

Molecular studies revealed that three distinct curtovirus species are associated with beet curly top disease in the western United States: *Beet curly top virus* (BCTV, the type species previously designated Cal/Logan strain), *Beet mild curly top virus* (BMCTV, previously designated Worland strain of BCTV), and *Beet severe curly top virus* (BSCTV, previously known as CFH strain of BCTV and including an Iranian isolate) (Stenger & Ostrow 1994, Stenger & McMahon 1997, Strausbaugh *et al.* 2008). Later four new species including *Horseradish curly top virus* (HrCTV, Klute *et al.* 1996), *Spinach curly top virus* (SpCTV) (Baliji *et al.* 2004), *Pepper curly top virus* (PepCTV, Lam *et al.* 2009) and *Beet curly top Iran virus* (BCTIV, Heydarnejad *et al.* 2007) were added to the list of *Curtovirus* species (Brown *et al.* 2012). In more recent ICTV classification proposal (Adams *et al.* 2013) BMCTV, BSCTV, PepCTV and SpCTV are considered as strains of BCTV. Iranian isolate of BSCTV was assigned as BCTV-C strain and more recently as severe strain of BCTV (BCTV-Svr [IR-SVR-86]). Likewise, BCTIV with *Spinach curly top Arizona virus* (SCTAV) are reassigned to the genus *Becurtovirus* (Adams *et al.* 2013, Varsani *et al.* 2014a and b, http://talk.ictvonline.org/files/ictv_official_taxonomy_updates_since_the_8th_report/m/plant-official/4454.aspx).

BCTIV has been reported only from Iran (Bolok-Yazdi *et al.* 2008). In spite of having unusual gene content on complementary-sense strand of its genome and low genome full-length sequence identity relative to curtoviruses, BCTIV is similar to members of the genus *Curtovirus* in biological characteristics such as wide host range (Heydarnejad *et al.* 2007) and transmission by leafhoppers (Soleimani *et al.* 2013,Taheri *et al*, 2012). Although BCTIV is reported to have higher incidence in sugar beet (Heydarnejad *et al.* 2007), there is no solid information on the effect of geographical location and host species on the prevalence of this virus and curtoviruses. The objective of the present study was to address these points and to obtain information on the possible genetic diversity of the two curly top viruses in Iran.

Methods and Materials

Collection of samples

To determine the prevalence of BSCTV and BCTIV, a total of 734 samples of plants were collected in fields of four provinces including Khorasan-Razavi (Sabzevar, Neyshabour, Chenaran and Jovein), Fars (Marvdasht, Eghlid, and Nourabad), Bushehr (Borazjan and Ahram) and Kohguiluyeh-Boyerahmad (Yasouj) in 2011 and 2012. To collect samples from sugar beet and tomato fields, the entire diagonal of each field was walked and hypothetical plots of 10-meter length with 20-30 meter intervals were used for sampling and recording the number of healthy and curly top infected plants based on symptom appearance. Leaf samples of all plants within each plot were collected and individually subjected to polymerase chain reaction (PCR) analysis.

To study the genetic diversity of these viruses, plant samples were randomly collected from the fields of four provinces listed above plus the fields

Table 1. Details of specific primers used in this study (Ebadzad Sahrai 2008).

Primer	Size (nt)	[a]Nucleotide position	Sequence from 5′ to 3′	Expected fragment size(bp)
BSCTV-IR358[V]	26	358-383	GTGGATCAATTTCCAGACAATTATC	496
BSCTV-IR853[C]	26	828-853	CCCCATAAGACCCATATCAAACTTC	
BCTIV 474[V]	21	474-494	TACAAGAAGTATGGCGGTTC	682
BCTIV1155[C]	22	1134-1155	AAGAATAGCATTCTCCTTCAC	

[a]Nucleotide position of Iranian isolate of Beet severe curly top virus (BSCTV-IR) and Beet curly top Iran virus (BCTIV) from the GenBank database (accession numbers X97203.1 and JQ707939, respectively).
[C]Complementary-sense strand primer
[V]Virion-sense strand primer

of Kermanshah province (Kermanshah, Mahidasht, Bistoon and Eslamabad regions) during 2011-2012. Symptomatic crop samples and symptomless weeds were collected. Sample were taken from sugar beet, tomato, pepper, bean, turnip, radish, eggplant, *Physalis alkekengi*, *Amaranthus retroflexus*, *Petunia* sp., *Solanum nigrum*, *Convolvolus arvensis*, *Chenopodium album* and *Sisymbrium* sp.

DNA extraction and polymerase chain reaction

Total DNA of collected samples was extracted from plant tissues as described previously (Behjatnia *et al.* 1996). Viral DNA was subjected to PCR using specific primer pairs BSCTV-IR358[V]/BSCTV-IR853[C] and BCTIV474[V]/BCTIV1155[C] (Table 1) to amplify a part of the genome of each virus. PCR reactions were carried out in 25 µl reaction mixtures each containing 10-15 ng DNA template, 0.4 mM oligonucleotide primers, 0.2 mM dNTPs, 1.5 mM $MgCl_2$ and 1.5 Units of *Taq* DNA polymerase (Cinagen, Iran) in the reaction buffer provided by the same source. The mixture was denatured for 3 min at 95 °C and subjected to 30 cycles of PCR using a program of 30 sec at 94 °C, 45 sec at 49 °C for BCTIV and 54 °C for BSCTV, and 1 min at 72 °C. The final cycle was followed by a 10 min incubation at 72 °C. The reaction mixture was then loaded directly onto a 1% agarose gel, stained with ethidium bromide and visualized by UV light.

Analysis of nucleotide sequences

Amplicons produced from 28 isolates (from fields of Khorasan-Razavi, Fars, Kermanshah, Boushehr and Kohguiluyeh-Boyerahmad provinces) were purified by a PCR purification kit (Qiagen, Germany) and sent to Bioneer (South Korea) or TechDragon (Hong Kong) for sequencing. DNA sequences were compared to the sequences of known accessions of each curtovirus and Becurtovirus species available in GenBank to confirm species identity using BLAST program. In addition, DNA sequences were aligned and compared with other isolates of *Curtovirus* and *Becurtovirus* genera by Vector NTI Explorer advanced 11.0 and ClustalX version 1.83. Phylogenetic trees were plotted using neighbor joining method by Mega5 program. Confidence intervals in tree topologies were estimated by bootstrap analysis with 1000 replicates. Only nodes with bootstrap values over 50% were considered significant. Characteristics of curtoviruses, becurtoviruses and other geminiviruses used in sequence analysis in this study are shown in Table 2.

Frequency Analysis

Infection of each collected plant with BSCTV and BCTIV was analysed by PCR using specific primers listed in Table 1. To verify if there are any statistical associations in viral infection incidence between types of viruses, geographical region and their interaction, Friedman two-way nonparametric ANOVA was used (Conover 1999). Mean of frequency of occurrence of BSCTV and BCTIV in sugar beet fields was compared between viruses, regions and their interaction effects using SAS software (SAS Institute 1996). Incidence rate of these viruses in sugar beet and tomato fields was also determined.

Table 2. Characteristics of geminiviruses used in the phylogenetic analyses of curly top viruses.

Genus	Virus [isolate name*]	Host plant	Location	Accession number
Becurtovirus	BCTIV [BCTIV-K]	*Beta vulgaris*	Kerman province, Iran	**NC010417**
	BCTIV [IR:Yaz:B35 K:Sug:06]	*Beta vulgaris*	Yazd province, Iran	**JQ707951**
	BCTIV [IR:Shi:B18 K:Sug:06]	*Beta vulgaris*	Shiraz, Fars province, Iran	**JQ707939**
	BCTIV [IR:Kav:B22 K:Sug:08]	*Beta vulgaris*	Kavar, Fars province, Iran	**JQ707941**
	BCTIV [BCTIV-Kh]	*Beta vulgaris*	Khorasan province, Iran	**EU263012**
	BCTIV [BCTIV-Kj]	*Beta vulgaris*	Karaj, Alborz province, Iran	**EU263013**
	SCTAV [09-10 spinach]	*Spinacia oleracea*	Arizona, USA	**NC015051**
	BCTIV [IR: Ney: sug:01]	***Beta vulgaris***	**Neyshabour, Khorasan-Razavi province,Iran**	**KM402780**
	BCTIV [IR: Che: sug:01]	***Beta vulgaris***	**Chenaran, Khorasan-Razavi province, Iran**	**KM402781**
	BCTIV [IR: Jov: sug:01]	***Beta vulgaris***	**Jovein, Khorasan-Razavi province, Iran**	**KM402782**
	BCTIV [IR: Sab: sug:01]	***Beta vulgaris***	**Sabzevar, Khorasan-Razavi province, Iran**	**KM402783**
	BCTIV [IR: Bis: sug:01]	***Beta vulgaris***	**Bisotun, Kermanshah province, Iran**	**KM402784**
	BCTIV [IR: Esl: sol:01]	***Solanum nigrum***	**Eslamabad-e Gharb, Kermanshah province, Iran**	**KM402785**
	BCTIV [IR: Mar: pep:01]	***Capsicum annuum***	**Marvdasht, Fars province, Iran**	**KM402786**
	BCTIV [IR: Mar: phy:02]	***Physalis alkekengi***	**Marvdasht, Fars province, Iran**	**KM402787**
	BCTIV [IR: Mar: tom:03]	***Solanum lycopersicum***	**Marvdasht, Fars province, Iran**	**KM402788**
	BCTIV [IR: Mar: ama:04]	***Amaranthus retroflexus***	**Marvdasht, Fars province, Iran**	**KM402789**
	BCTIV [IR: Mar: sug:05]	***Beta vulgaris***	**Marvdasht, Fars province, Iran**	**KM402790**
	BCTIV [IR: Mar: sol:06]	***Solanum nigrum***	**Marvdasht, Fars province, Iran**	**KM402791**
	BCTIV [IR: Mar: con:07]	***Convolvulus arvensis***	**Marvdasht, Fars province, Iran**	**KM402792**
	BCTIV [IR: Yas: sug:01]	***Beta vulgaris***	**Yasouj, Kohguiluye-Boyerahmad province, Iran**	**KM402793**
	BCTIV [IR: Yas: sug:01]	***Beta vulgaris***	**Yasouj, Kohguiluye-Boyerahmad province, Iran**	**KM402794**
	BCTIV [IR: Yas: sug:02]	***Beta vulgaris***	**Yasouj, Kohguiluye-Boyerahmad province, Iran**	**KM402795**
	BCTIV [IR: Yas: sug:03]	***Beta vulgaris***	**Yasouj, Kohguiluye-Boyerahmad province, Iran**	**KM402796**
	BCTIV [IR: Yas: sug:04]	***Beta vulgaris***	**Yasouj, Kohguiluye-Boyerahmad province, Iran**	**KM402797**
Curtovirus	BMCTV [USA , Worland]	*Beta vulgaris*	Worland, USA	**NC004753**
	BCTV [California, Logan]	*Beta vulgaris*	California, USA	**NC001412**
	BSCTV [Iran:1986]	*Beta vulgaris*	Shiraz, Fars province, Iran	**X97203**
	BSCTV [US:Cfh]	*Beta vulgaris*	USA	**U02311**
	HrCTV [horseradish]	*Raphanus sativus*	USA	**NC002543**
	SPsCTV [09-10-8]	*Spinacia oleracea*	Arizona, USA	**NC014631**
	BSCTV [IR: Bis: sug:02]	***Beta vulgaris***	**Bisotun, Kermanshah province, Iran**	**KM402770**
	BSCTV [IR: Che: sug:02]	***Beta vulgaris***	**Chenaran, Khorasan-Razavi province, Iran**	**KM402771**
	BSCTV [IR: Jov: sug:02]	***Beta vulgaris***	**Jovein, Khorasan-Razavi province, Iran**	**KM402772**
	BSCTV [IR: Mah: sug:01]	***Beta vulgaris***	**Mahidasht, Kermanshah province, Iran**	**KM402773**
	BSCTV [IR: Sab: sug:02]	***Beta vulgaris***	**Sabzevar, Khorasan-Razavi province, Iran**	**KM402774**
	BSCTV [IR: Esl: che:02]	***Chenopodium album***	**Eslamabad-e Gharb, Kermanshah province, Iran**	**KM402775**
	BSCTV [IR: Ker: phy:01]	***Physalis alkekengi***	**Iran ,Kermanshah**	**KM402776**
	BSCTV [IR: Mah: phy:02]	***Physalis alkekengi***	**Mahidasht, Kermanshah province, Iran**	**KM402777**
	BSCTV [IR: Bou: tom:01]	***Solanum lycopersicum***	**Boushehr province, Iran**	**KM402778**
	BSCTV[IR: Bou: che:02]	***Chenopodium album***	**Boushehr province, Iran**	**KM402779**
Turncurtovirus	TCTV	*Brassica napus*	Homayejan, Fars province, Iran	**JQ742019**

*Isolates shown in bold characters are from the present study

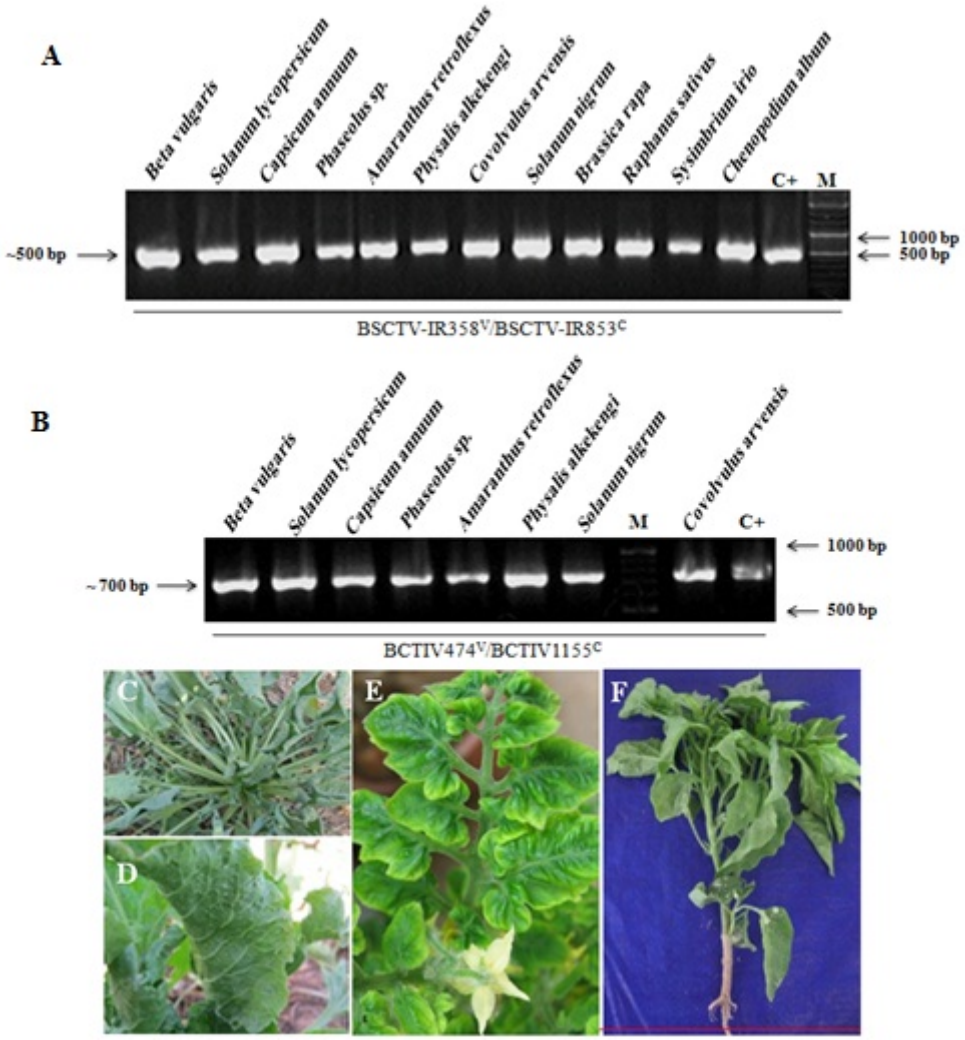

Fig 1. A and B Electrophoresis pattern of PCR products of BSCTV (panel A) and BCTIV (panel B) amplified from total DNA extracts of naturally infected plants as outlined at the top of each lane using specific primer pairs outlined at the bottom of each panel. C^+, positive control (cloned DNA of BSCTV, Ebadzad Sahrai, 2008 and cloned DNA of BCTIV, Soleimani *et al.* 2012). M= Marker (DNA ladder Mix, Fermentas, Lithuania). C. Stunting and leaf curling symptoms in an infected sugar beet plant. D. Vein swelling on the lower leaf surface of sugar beet. E. Leaf curling, yellowing of leaf margins and severe malformation of the leaves in tomato. F. Plant stunting, bunchy top growth and mild discoloration of the leaves in pepper.

Results

Detection of curly top viruses

Incidence of curly top viruses in collected samples from five provinces was investigated by PCR using specific primer pairs (Table 1). DNA fragments of the expected size, approximately 500 bp for BSCTV (Fig 1A) and 700 bp for BCTIV (Fig 1B) were amplified from most symptomatic crop plants and some weeds. Mixed infection with BSCTV and BCTIV was detected in single samples of sugar beet, tomato, pepper, bean, *C. arvensis*, *P. alkekengi* and *A. retroflexus*. Other examined hosts including *C. album*, *S. nigrum*, *Sysmberium* sp., Radish and Eggplant were infected only by one of the viruses. Naturally infected sugar beet plants collected from different regions showed typical curly top disease symptoms (Fig 1C and D). Symptoms in tomatoes consisted of leaf curling, yellowing of leaf margins, thickening and severe malformation of leaves (Fig 1E), while infected peppers showed stunting, bunchy top growth and mild discoloration of the leaves (Fig 1F). Infected turnip and eggplant showed mild yellowing while in infected beans, in

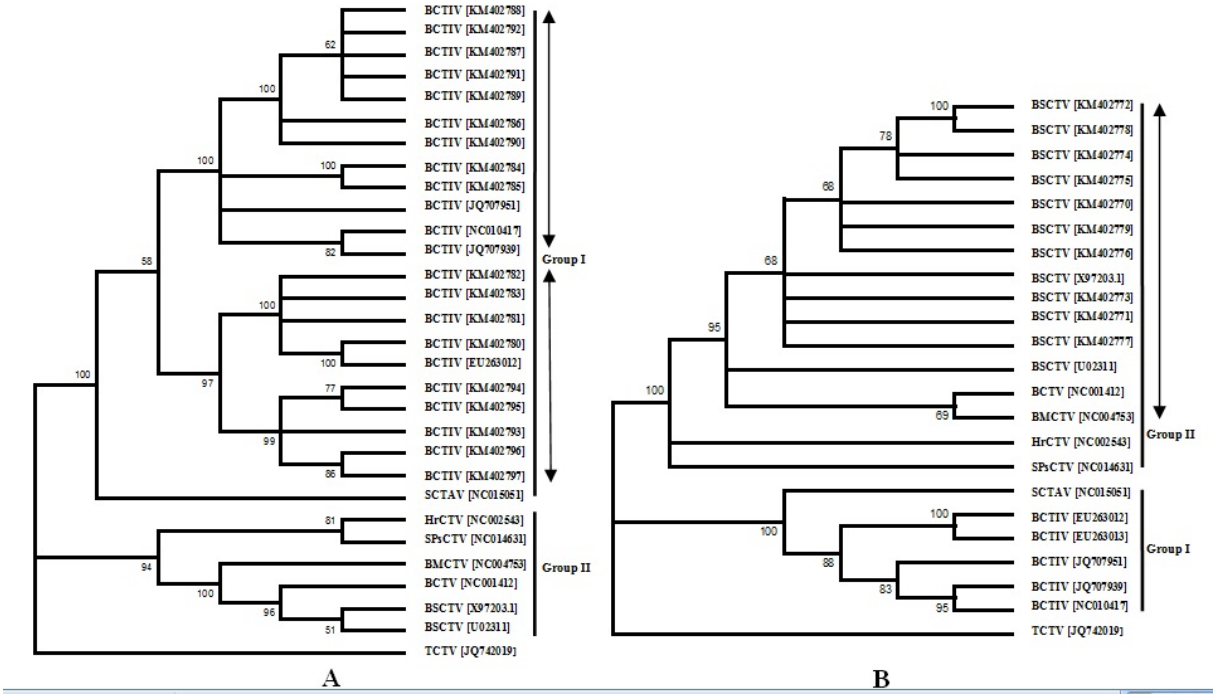

Fig 2. Phylogenetic relationships of 18 samples of Beet curly top Iran virus (BCTIV) based on CP gene (682 bp, nt 474 to nt 1155 encompassing V1 ORF) (dendrogram A) and 10 sampels of Beet severe curly top virus (BSCTV, 496 bp, nt 358 to nt 853 encompassing the V2 ORF and 290 bp of 5′ end region of V1 ORF) (dendrogram B) with the same region of genome of selected geminiviruses using neighbor joining method. Subgroups indicated by double arrows. See Table 2 for isolates abbreviation and the accession number of the sequences. Only nodes with bootstrap values over 50% were considered significant.

addition to yellowing, leaf rolling was also observed.

Genetic diversity among isolates of BSCTV and BCTIV

Nucleotide sequence of partial genome of 10 samples of BSCTV (496 bp, nt 358 to nt 853 encompassing the V2 ORF and 290 bp of 5′ end region of V1 ORF) were aligned with the nt sequence of the same region of the genome of an already described Iranian isolate of BSCTV (BSCTV-IR[CFH(beta)]), a BSCTV isolate reported from USA and various curly top virus species available in the GenBank (Table 2). Based on the analysis 86.1% to 99.7% sequence identities were observed among BSCTV isolates. The latter shared 71.0–95.7% nt homology with other curtoviruses, i.e., BCTV, BMCTV, HrCTV and Spinach severe curly top virus (SpsCTV). Comparison of nt sequence of the CP region (682 bp, nt 474 to nt 1155 encompassing V1 ORF) of 18 BCTIV samples with the nt sequence of the same region of other curly top virus species available in the GenBank (Table 2) revealed 86.3 to 100 % nt sequence homology among BCTIV isolates.

A dendrogram (Fig 2A) obtained by phylogenetic analysis of the nt sequence of the 682 bp fragment of BCTIV isolates with the same region of genome of various curly top virus species available in the GenBank (Table 2) using ClustalX and Mega5 programs revealed two main groups and one separated line of viruses causing curly top diseases in dicotyledons plants worldwide (Fig 2A). The first main group (Fig 2A, Group I) encompasses BCTIV isolates and SCTAV. BCTIV isolates are divided into two subgroups; the first subgroup is represented by BCTIV isolates from Fars and Kermanshah provinces, characterized in this study, were placed together with BCTIV isolates from Yazd, Kerman and Shiraz (strain A) deposited already into GenBank. Another subgroup is represented by BCTIV isolates of Khorasan-Razavi and Kohguiluyeh-Boyerahmad provinces. BCTIV isolates of each province in both subgroups clustered together, regardless of the host species. The second main group (Group II) in this dendrogram is represented by curtoviruses including BCTV, BMCTV, HrCTV, SpsCTV and BSCTV isolates. TCTV is placed in a separate line

Table 3. Incidence rates of BSCTV and BCTIV in various hosts and regions of Iran during 2011-2012.

Province	Region	Host	Symptoms	Total no. of samples	Incidence rate (%)	
					BCTIV	BSCTV
Khorasan-Razavi	Chenaran	sugar beet	Typical curly top symptoms	61	21.0	37.8
	Jovein	sugar beet		361	28.6	27.2
		tomato	Thickening and yellowing of leaves	20	47.0	51.9
	Neyshabur	sugar beet	Typical curly top symptoms	28	47.2	0.0
	Sabzevar	sugar beet		7	57.0	28.5
					40.2*	**29.1**
Fars	Eghlid	sugar beet	Typical curly top symptoms	46	4.9	17.0
		tomato	Thickening and yellowing of leaves	6	50.0	0.0
	Nourabad	tomato		16	0.0	31.2
	Marvdasht	tomato		13	38.4	84.6
		sugar beet	Typical curly top symptoms	63	79.2	43.5
		pepper	Stunting, yellowing and ring spot symptoms	18	55.5	88.8
					38.0	**44.2**
Kohguiluyeh-Boyerahmad	Yasouj	pepper	Yellowing of leaves, Leaf roll	11	9.0	72.7
		bean		7	70.0	57.1
					39.5	**64.9**
Bushehr	Ahram	*Chenopodium* sp.	Leaf curling	23	0.0	8.7
		tomato	Thickening and yellowing of the leaf	18	0.0	0.0
	Borazjan	tomato		36	0.0	7.1
					0.0	**5.2**

*Numbers in bold are total infection rate of each province

distinct from the two main groups.

Another dendrogram (Fig 2B) obtained by phylogenetic analysis of the nt sequence of the 496 bp fragment of BSCTV isolates and the same region of the genome of various curly top virus species available in the GenBank (Table 2). Again, it showed two main groups, represented by *Curtovirus* and *Becurtovirus* members, and one separated line, represented by TCTV. SCTAV grouped again with BCTIV isolates (Group I) even though it was distinct by forming a separate line in the group. In the *Curtovirus* group (Group II), BCTV, BMCTV and BSCTV isolates clustered together in a subgroup while HrCTV and SpsCTV formed two separated lines. This clustering of curtoviruses is consistent with the recent ICTV classification proposal for the genus *Curtovirus*. Based on this proposal, BMCTV, BSCTV, PepCTV and SpCTV are removed from *Curtovirus* as species and all their isolates are considered the strains of BCTV (Adams *et al.* 2013). This proposal seems to be also valid with the output obtained by phylogenetic analysis of the nt sequence of a small fragment of the genome (either 496 bp or 682 bp fragments) in this study.

Prevalence of BSCTV and BCTIV in different regions of Iran

Different plants from four regions in Iran were screened for the presence of BSCTV and BCTIV. Use of PCR with specific primer pairs for each virus (Table 1) showed that both viruses contribute to curly top syndrome in various regions of the country. Of 734 suspected samples collected in the 2011–2012 growing seasons, 226 and 215 samples were infected with BSCTV and BCTIV, respectively. The incidence rates of BSCTV and BCTIV in different hosts and different areas are shown in Table 3 and Fig 3.

Based on these data, the highest rate of BSCTV (88.8%) and BCTIV (79.2 %) infectivity was observed in pepper and sugar beet fields in Marvdasht region (Fars province), respectively (Table 3, Fig 3). Regardless of host species, the highest total infection of host plants by BSCTV

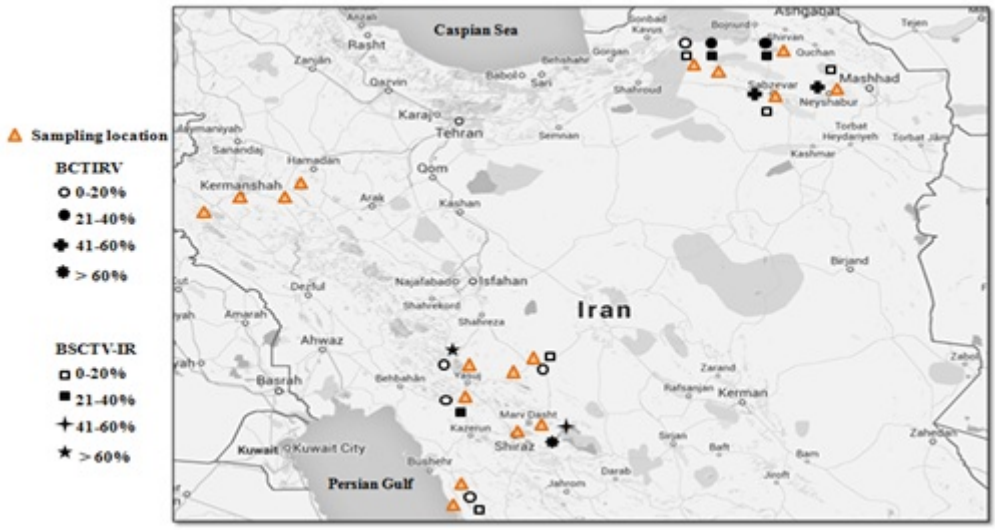

Fig 3. A map showing locations of sampling and infection rate of BSCTV and BCTIV in surveyed provinces of Iran.

Table 4. Incidence rates of BSCTV and BCTIV in various hosts.

Plant / Virus	Sugar beet (%)	Tomato (%)	Pepper (%)	Weeds (%)
BSCTV	26.2	29.3	80	53.8
BCTIV	36.6	22.9	32.2	34.2

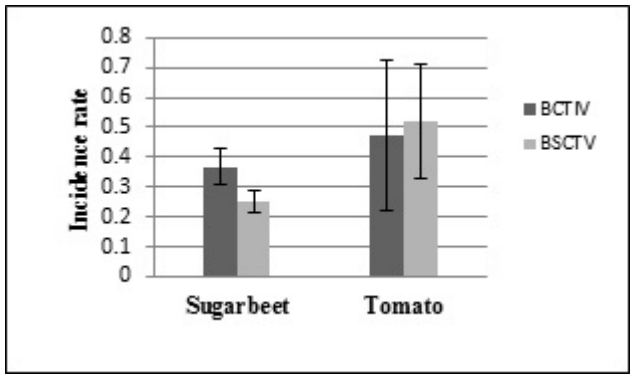

Fig 4. Incidence rates of BSCTV and BCTIV in sugar beet and tomato.

(64.9%) and BCTIV (40.2%) occurred in Kohguiluye-Boyerahmad and Khorasan-Razavi provinces, respectively (Fig 3).

Frequency of BSCTV and BCTIV infection in different hosts

The highest incidence rate of BCTIV and BSCTV was found in sugar beet and pepper, respectively (Table 4). The incidence of both viruses on tomato plants was not significantly different while BCTIV infection was higher than BSCTV in sugar beet plants (Fig 4). *Physalis alkekengi* and *Solanum nigrum* with the infection rate of 87% (8 infected plants from 12 tested plants) and 66% (13 infected plants from 15 tested plants) were the most significant weed hosts of BCTIV and BSCTV, respectively.

Incidence rate of BSCTV and BCTIV in different regions

Virus incidence rate was assessed by Friedman two-way nonparametric ANOVA in the fields of four regions; Neyshabour, Chenaran, Bahrabad and Motahari (the latter two regions are from Jovein) in Khorasan-Razavi and two regions; Marvdasht and Eghlid in Fars province. The results of this analysis are shown in Fig 5. In Neyshabour, all three fields investigated were infected with BSCTV (p value = 0.001) and no BCTIV infection was detected. The incidence of BCTIV was higher than BSCTV in Bahrabad region (p value = 0.031). In this region, sugar beet was planted in late June and the sampling date was four months after planting date. Conversely, incidence of BSCTV was higher in Motahari region (p value = 0.0094).

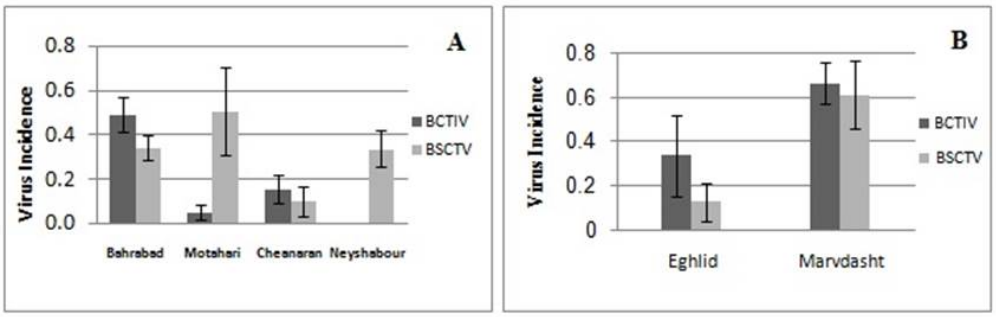

Fig 5. Total present detection of BSCTV and BCTIV in fields of Khorasan-Razavi (A) and Fars (B) assessed by Friedman two-way nonparametric ANOVA method.

Planting date in this region was late March and sampling date was seven months after planting date (Fig 5A). No significant difference between BSCTV and BCTIV incidence rate was observed in Chenaran region (p value = 0.51) (Fig 5A) and in Marvdasht (p value = 0.063) and Eghlid (p value = 0.083) regions (Fig 5B).

Discussion

Although members of *Curtovirus*, *Becurtovirus* and *Turncurtovirus* genera are similar in biological properties including symptomatology, natural and experimental host ranges, pathogenicity, vector specificity and cell and tissue tropism, they are highly divergent in genome sequence and, in some cases, have unique genome architectures (Briddon *et al.* 2010, Chen *et al.* 2010, Soleimani *et al.* 2013). Phylogenetic analyses in the present study indicated that geographical separation and host diversity do not affect genetic diversity of BSCTV isolates (Fig 2). BSCTV isolates characterised in this study share 86.1% to 99.7% sequence identity and along with a BSCTV isolate previously reported from Iran formed a subgroup, closer to BCTV and BMCTV isolates reported form USA than to other two virus species, i.e., HrCTV and SPsCTV, supporting the theory of continuum of variants (Strausbaugh *et al.* 2008). BSCTV has already been reported to infect different crops and weeds in different regions in Fars province (Ebadzad Sahraei 2008, Ghodoum Parizipour 2011). In addition to Fars province, this study revealed the distribution of BSCTV in some regions of Khorasane-Razavi, Bushehr and Kohguiluye-Boyerahmad provinces. Sequencing of complete genome of BSCTV isolates from different regions in Iran will clear genetic diversity of this virus.

In the present study, BCTIV was isolated from sugar beet, tomato, pepper and bean fields in Fars, Khorasan-Razavi and Kohguiluye-boyerahmad. Previous reports (Bolok Yazdi *et al.* 2008, Heydarnejad *et al.* 2013, Gharouni Kardani *et al.* 2013) have also indicated the incidence of BCTIV in Fars, Khorasan-Razavi, Northern Khorasan, East Azerbaijan, West Azerbaijan, Kerman, Yazd and Alborz provinces.

Phylogenetic analyses of the complete genome sequences of nine isolates of BCTIV isolated from Khorasan-Razavi, Northern Khorasan, East Azerbaijan, West Azerbaijan and Fars provinces revealed 11 % diversity amongst BCTIV isolates (Gharouni Kardani *et al.* 2013). BCTIV samples used in this study showed 86.3 to 100 % nt sequence homology in coat protein region with the other curly top virus species available in the GenBank (Table 2). Phylogenetic tree of BCTIV isolates showed separation of the Fars isolate from others. Khorasan-Razavi, Kermanshah and Kohguiluyeh-Boyerahmad isolates were also separately placed in their own subgroup (Fig 2), pointing out geographical divergence of BCTIV isolates. This would indicate either a longer evolutionary history of BCTIV or its more rapid evolutionary rate.

Both BSCTV and BCTIV were found in sugar beet and other crop and weed hosts in surveyed locations in Iran. Presence of both viruses in the same plant indicates absence of cross protection. The incidence rate of the viruses, however, differed from region to region and from host to host. In Khorasan-Razavi province in a 4 months old sugar beet field in Bahrabad region, BCTIV incidence was higher than BSCTV incidence, while in 7 months old-field in Motahari region BSCTV was identified more frequently than BCTIV. It seems that the frequency of each virus

varies in different fields and depends on the host, date of cultivation and geographical region. Chen *et al.* (2010) indicated that presence of inoculum sources (e.g., weeds or crop plants) and geographical locations with higher proportion of curly top virus–positive reservoir hosts influenced the frequency of curly top viruses, while Strausbaugh *et al.* (2008) suggested that new species and vector population are involved in curly top disease severity.

It was observed that BCTIV was more frequent in sugar beet plants while in the other plants including tomato, pepper and weeds BSCTV was detected more frequently than BCTIV. However, the impact of each virus on the curly top disease of dicotyledonous plants needs to be investigated further.

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