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EFFECT OF HOT CLIMATE ON CITRUS TRISTEZA VIRUS DAMAGES IN JAHROM REGION (IRAN)

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ABSTRACT

Citrus are strategically and economically important crop in Iran and around the world. Tristeza is considered among the most important viral diseases of citrus, producing severe economic losses worldwide. Thermal therapy is a management method to viral diseases of plants. Jahrom has the hot dry climate. In order to assess the effect of the environment heat on Citrus Tristeza Virus (CTV) in the field conditions, 20 CTV free plants and 20 CTV infected plants were selected from each variety (Mexican lime, Washington-navel orange and Kinnow tangerine varieties). Different characteristics of healthy and diseased trees were compared. In all varieties, the results showed that virus infection reduces the fruit yield. In most cases, however, characteristics of the Mexican lime fruits were not significantly changed but important characteristics such as sugar contents, contents of the vitamin C in fruit juice, juice pH, peel/flesh ratio and juice percentage (purity) showed significant differences between healthy and infected trees of orange and Kinnow tangerine. In general, Citrus Tristeza Virus makes damages in Plants that ripening of their fruits comes with cooler temperature of autumn. But in Mexican lime that is an early fruit, tristeza damage is inconsiderable.

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KEY WORDS

Citrus Tristeza Virus, Effect of Virus on physiology, Hot Temperature

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INTRODUCTION

Citrus are considered as the strategic products in the global agricultural industry. They are used in more than one hundred industries. Because of its destructive effects on citrus industries in several countries, CTV is economically regarded as the most important virus of citrus [1]. To date, the disease has destroyed millions of citrus trees grafted onto the sour orange rootstocks in Argentina, Brazil, Peru, America, Spain and Venezuela. In Florida, CTV was reported first in 1950s but the severe strain of the virus speeded in the 1980s and made heavy damages [2].

Under field conditions, in general, CTV symptoms exhibit a complex range based on the virus isolates, citrus varieties, infection time and environmental circumstances [3-5]. CTV leads in three types of economically important symptoms including quick decline in trees grafted on sour orange rootstock, stem pitting on scion - regardless of the rootstock- and seedling yellows causing mass losses in nurseries and greenhouse conditions [6]. Citrus Tristeza Virus (CTV) belongs to the genus Closterovirus (family: Closteroviridae). CTV has a flexuous filamentous particle with the size of 2000 × 10^{-11} nm. Two capsid proteins (P25 & P27) cover 97% and 3% of the viral particle, respectively [1, 7, 8]. Each particle contains a ss(+) RNA that is about 20 kb [4, 9-11] and 6.3-6.9 × 10^6 in molecular weight [12].

In Iran, CTV was first reported from unshu tangerine seedlings imported from Japan in Mazandaran province, in 1978 [13]. CTV distribution in southern Iran was identified in 1998. Infected regions included Khafr, Kazeroun, Fasaroud, Jannat shahr, Dalaki, Rood fariab, Khiz and Jam [14]. In 2006, the first evaluation on distribution and genetic diversity of CTV isolates was performed in Kerman province, Iran [15]. During the same year, three monoclonal antiserums (3CA5, 3DF1 & MCA13) and three polyclonal antiserums (Bioreda, Agritest &Shiraz)

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were used in two indirect ELISA methods (I-ELISA) and (I-TPIA) to identify the CTV infection in all Iranian southern provinces (Fars, Kohgeluyeh-Boyer Ahmad, Hormozgan, Sisstan & Baluchestan and Kerman) [16].

The optimum temperature for viral infection and replication is 15-25 °C. Examinations showed that under thermal treatment, infected seedlings had less viral particles compared to the control samples [17]. In addition, some strains don't generate symptoms like seedling yellows in response to the thermal conditions. Further investigations suggested that the heat effect on the virus depends on the heat tolerance of the host plant [18].

Thermal therapy is a control method to viral diseases. Heat reduces the concentration of viruses within the plants. In the spring and summer, temperatures are very high in Jahrom and the maximum temperatures of above 40 ° C have been recorded for most of its summer days. Despite the high investments on CTV research efforts in Fars province, damages of the virus have not been surveyed in Jahrom to date. Given the importance of citrus in Jahrom, the global importance of CTV and the controlling role of the heat on the plant viruses, it is necessary to conduct research efforts in the field of CTV induced damages in different citrus varieties in Jahrom. Information on the damage extents and their economically assessing can be helpful in future research policies. If the disease's damages are not economically significant, there will be no need to perform costly researches.

MATERIALS AND METHODS

In order to evaluate and select the healthy and CTV infected trees in Jahrom, randomly leaf sampling was conducted from several Mexican lime, kinnow tangerine and Washington-navel orange yards in autumn and winter 2013 and spring 2014. The samples were placed in plastic bags. After recording the characteristics, samples were transported to the laboratory in cooling chambers.

ELISATest

According to the manufacturer's instructions, ELISA test (DAS-ELISA) [19] was performed using the virus specific antiserum (Bioreba) to identify the infected plants. Then, optical density was measured at 405 nm by ELISA reader (Mikura). Positive sample was defined as having two fold OD_{405} value of the negative control sample.

RNAExtraction

Total RNA was extracted using the RNA extraction kit (RNase plant mini kit) according to the manufacturer's instructions. Extracted RNA was stored at -20°C for further uses.

cDNASynthesis

To synthesize the cDNA with the final volume of 20 µl, 9 µl of total RNA was heated with 1 µl of Oligo(dt) at 65 °C for 5 minutes. The mix was immediately transferred to ice and was added by other reaction compounds including 2 µl of 10X buffer, 2 µl of dNTPs and 1 µl (200U) of Reverse transcriptase enzyme (Sinagen). The reaction mix underwent 25 ° C for 10 minutes, 42 °C for 60 min and 70 °C for 10 min. Obtained cDNA was stored at -20 °C for future uses.

Polymerase chain reaction (PCR)

PCR was performed in a total volume of 25 µl. Reaction compounds included 2.5 µl of 10X buffer, 0.75 µl of each primer (CP1 & CP2), 0.75 µl of $Mgcl_2$, 0.5 µl of mixed dNTPs, 1.25 units of Taq DNA polymerase enzyme and 4 µl of cDNA. Polymerase chain reaction consisted of a cycle at 94 °C for 3 min, 35 cycles at 94 °C for 1 min, 56 °C 1 min, 72 °C 1 min and 72 °C for 10 minutes in the last stage. The PCR amplified fragments were separated on 1.2% agarose gel and stained in 0.5µg/ml with ethidium bromide and analyzed using BIO imaging system.The primers used in this research were CP1: 5´- ATGGACGACGAAACAAAGAA-3´ and CP2: 5´- TCAACGTGTGTTGAATTTC-3´.

Sugar contents

Fruit juice sugar contents was measured by volumetric method of Lane-Eynon.

Vitamin C contents

After juicing the fruits, 2 ml of 1% starch and 20 ml of distilled water were added to 10 ml of filtered juice. The mix was titrated using Lugol to create the olive green color. Finally, the vitamin C contents in 10 ml of fruit juice were calculated based on the following formula: Used Lugols solution × 0.88.

Titratable acid (TA)

5 ml of the filtered fruit juice was added by 2-5 drops of phenolphthalein. The mix was titrated using 3.0 normal sodium hydroxide to create the light red color. Finally, citric acid contents in 100 ml of fruit juice were calculated based on the following formula:

mg of acid in 100 ml fruit juice= ml of NaOH × normality of NaOH × Equivalent Weight of dominant acid×100/Sample Weight × 1000

Fruit Juice pH

After filtering the fruit juices, their pH values were read by using of a pH meter.

Fruit firmness

Fruit firmness was measured by a handy penetrometer (Mc Cormic FT-327) with the 11 mm tip. The firmness value was expressed in kilograms.

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Tree yield

Trees products were completely harvested and weighted.

Peel thickness

The fruits were longitudinally peeled off and the thicknesses values of their various parts were measured by a caliper. Mean values of peel thicknesses were obtained.

Juice density

A glass hydrometer was used to measure the juice density values.

Peel/ flesh ratio

Fruits were peeled, the flesh and peel of the fruit were weighted by a digital scale (0.01 gr) and the ratio was calculated.

Percentage of Fruit juice

Percentage of fruit juice was measured by this formula: weight of fruit juice/weight of fruit ×100.

Total Soluble Solids Content (TSS) (Brix)

TSS content of fruit juice was measured by handy refractometer. The measured values were expressed in percentage.

Data analysis

The results were analyzed in a randomized complete block design and the means were compared using the LSD test.

RESULTS

Selecting the healthy and infected trees

After collecting the samples, ELISA and PCR tests were performed. From Mexican lime, Washington-navel orange and kinnow tangerine varieties, 20 CTV free trees and 20 infected ones were selected for further studies.

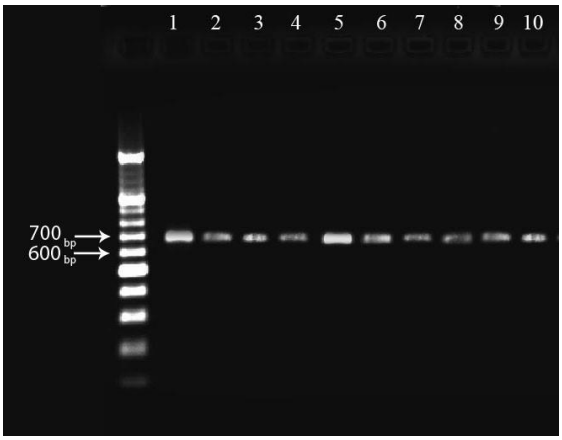

Fig: 1. Amplified coat protein genome of Citrus Tristeza Virus isolates from some infected trees

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Measuring the various factors

Results of data analysis of Citrus Tristeza Virus free and CTV infected trees

Table: 1. Data analysis of Washington navel orange

		Mean Squars (MS)										
Sources of variations	**DF**	**Fruit sugar**	**Vitamin C**	**TA**	**pH**	**Fruit firmness**	**Yield**	**Peel thickness**	**Juice Density**	**Peel/Flesh**	**% Fruit juice**	**TSS**
Variety	1	1.892**	883.6**	0.00625**	0.930**	0.225**	5040.0**	0.016^{ns}	0.178**	0.0176**	469.2**	2.116**
Error	38	0.020	3.8	0.00025	0.011	0.008	37.9	0.011	0.003	0.0002	2.8	0.013
%Coefficient Variance	%(CV)	2.0	3.0	1.4	2.2	3.0	3.7	2.3	3.8	2.7	2.1	1.2

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** and ns = significant and Non-significant at 1%

Table: 2. Comparison of measurements data of healthy and tristeza infected Washington navel orange

Factors / Plant	%Fruit sugar	Vitamin C (mg)	TA (mg)	pH	Fruit firmness (kg/cm^2)	Yield (kg)	Peel thickness (mm)	Juice Density (g/cm^3)	Peel/Flesh	% Fruit juice	%TSS
CTV free	7.44a	69.8a	1.148b	4.90a	3.00b	178.3a	4.46a	1.424a	0.438b	83.8a	9.58a
CTV infected	7.00b	60.4b	1.173a	4.60b	3.14a	155.8b	4.42a	1.290b	0.480a	76.9b	9.12b
LSD %5	0.092	1.248	0.010	0.066	0.059	3.942	0.066	0.033	0.008	1.077	0.074

*- In each column numbers with the same alphabetic are non-significant at %5 of LSD test.

Table: 3. Data analysis of Mexican lime

		Mean Squars (MS)										
Sources of variations	DF	Fruit sugar	Vitamin C	TA	pH	Fruit firmness	Yield	Peel thickness	Juice Density	Peel/Flesh	% Fruit juice	TSS
Variety	1	0.056ns	2.50ns	0.00072ns	0.081**	0.001ns	950.6**	0.036ns	0.064**	0.0048**	8.10*	1.056**
Error	38	0.015	2.47	0.00284	0.006	0.009	15.7	0.009	0.007	0.0001	1.74	0.023
%Coefficient Variance %(CV)		2.6	2.9	3.7	3.6	6.2	2.8	5.3	7.3	3.3	1.7	2.2

** = significant at 1%; *= significant at 5%; ns = Non-significant at 5%

Table: 4. Comparison of measurements data of healthy and tristeza infected Mexican lime

Factors / Plant	%Fruit sugar	Vitamin C (mg)	TA (mg)	pH	Fruit firmness (kg/cm^2)	Yield (kg)	Peel thickness (mm)	Juice Density (g/cm^3)	Peel/Flesh	% Fruit juice	%TSS
CTV free	4.79a	54.6a	1.435a	2.20a	1.51a	145.6a	1.78a	1.210a	0.348b	79.2a	7.07a
CTV infected	4.72a	54.0a	1.426a	2.12b	1.52a	135.8b	1.84a	1.130b	0.370a	78.2b	6.74b
LSD %5	0.079	1.006	0.034	0.050	0.060	2.536	0.061	0.055	0.008	0.846	0.097

*- In each column numbers with the same alphabetic are non-significant at %5 of LSD test.

Table: 5. Data analysis of kinnow tangerine

		Mean Squars (MS)										
Sources of variations	DF	Fruit sugar	Vitamin C	TA	pH	Fruit firmness	Yield	Peel thickness	Juice Density	Peel/Flesh	% Fruit juice	TSS
Variety	1	33.581**	354.02**	0.243**	0.552**	0.272**	2325.6**	1.849**	0.225**	0.0292**	180.6**	5.625**
Error	38	0.123	4.18	0.003	0.011	0.006	25.0	0.077	0.001	0.0005	1.8	0.063
%Coefficient Variance %(CV)		3.7	6.5	4.8	2.3	3.6	4.4	8.9	3.5	6.3	1.6	2.2

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** = significant at 1%

Table: 6. Comparison of measurements data of healthy and tristeza infected kinnow tangerine

Factors / Plant	%Fruit sugar	Vitamin C (mg)	TA (mg)	pH	Fruit firmness (kg/cm^2)	Yield (kg)	Peel thickness (mm)	Juice Density (g/cm^3)	Peel/Flesh	% Fruit juice	%TSS
CTV free	10.31a	34.3a	1.116b	4.56a	2.14b	121.4a	2.90b	1.292a	0.320b	85.8a	12.06a
CTV infected	8.48b	28.4b	1.272a	4.32b	2.30a	106.1b	3.33a	1.145b	0.374a	81.6b	11.32b
LSD %5	0.224	1.308	0.036	0.066	0.052	3.198	0.178	0.027	0.014	0.865	0.161

*- In each column numbers with the same alphabetic are non-significant at %5 of LSD test.

Tables1, 3 and 5 show data analysis and tables 2, 4 and 6 represent the comparative results of various mean data from Washington navel oranges, Mexican limes and Kinnow tangerines, respectively. According to the results, contents of sugar and vitamin C were significantly higher in fruit juices from healthy trees of Washington navel oranges and Kinnow tangerines than the CTV infected trees. The mentioned parameters showed no significant differences between healthy and infected Mexican limes. Compared to the healthy trees, total acidity (TA) values were significantly lower in CTV infected fruits of Washington navel orange and Kinnow tangerine trees. However, TA values didn't show a statistically significant difference between healthy and infected Mexican limes. The pH values were significantly higher in fruit juices from healthy trees of Washington navel orange, Kinnow tangerine and Mexican lime than the CTV infected trees. The fruit firmness values of the healthy orange and tangerine trees were significantly lower than the CTV infected trees, while no statistically significant differences were observed between the healthy and CTV infected lime trees. In all species, total yields of the healthy trees were significantly higher than the infected ones. Peel thickness values of the healthy trees were higher than the infected ones but the difference was only significant in Kinnow tangerines. In all species, the juice density and TSS values of the healthy trees were significantly higher than the infected ones. In all species, the peel/flesh ratios of the healthy trees were significantly lower than the CTV infected trees. Moreover, CTV infected trees had significantly lower juice percentages than the healthy trees.

DISCUSSION

Based on the research, leaves of the virus-infected plants had higher respiration rates than the healthy plants. This led in carbon partial loss and less starch production. However, older infected leaves didn't produce more carbon dioxide values [20].According to the tables 2, 4 & 6, sugar contents in fruit juices from healthy trees of Washington navel orange and Kinnow tangerine were significantly higher than the CTV infected trees but healthy and infected Mexican lime trees showed no significant differences in sugar contents. Reduced sugar contents can be the result of higher respiration rates of infected plants. On the other hand, Whitehead examined the impacts of the leaf curl virus on the harvested potato tubers. Based on this study, respiration rates of the infected tubers were much higher than the healthy ones at the beginning of the harvest stage. Respiratory rates gradually decreased below the values of the healthy tubers [20]. Resulted from the higher respiration rates of CTV-infected fruits, sugar content reduction consisted to Whitehead's study. Sugar contents were not statistically significant different between healthy and infected lime fruits. According to unpublished data from Jahrom weather station, the mean maximum temperatures in the months of 2014 were as follows respectively: 14.1, 17.4, 22.8, 27, 33.3, 39.5, 41.4, 41, 37.8, 33.9, 24.7 and 20.7 ˚C. In Jahrom, lime fruits are harvested in September while oranges and Kinnow tangerines are picked after December. Therefore, growth and ripening seasons of lime fruits occur in the hot weather of June, July and August. Intense heat reduces the virus concentrations and thus, the viral impacts on the plant. This makes no statistically significant differences in sugar contents of the healthy and infected fruits. On the contrary, orange and tangerine fruits grow and ripen in cool autumn temperatures, increasing the virus concentration within the plants. Therefore, the viral impacts lead in a significant difference between fruit sugar contents of the healthy and infected trees.

Many researchers have evaluated total carbohydrate contents in various cases. A study suggested that abnormally high contents of carbohydrates accumulate in leaves of the mulberry trees which are infected by Mulberry dwarf.

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Investigation on several potato varieties showed higher total amount of carbohydrates in leaves of the infected plants than the healthy ones and their lower contents in tubers. In addition, carbohydrate contents in leaves of the infected spinach plants were higher than the healthy plants. In a research, it is proved that dark mosaic areas have higher sugar than the healthy tissues. Another study showed that leaves of the infected spinach plants contain 25-250% higher sugar contents than the healthy tissues. The contents of sucrose and other disaccharides have also been investigated in several studies. Mulberry leaves infected by leaf curl virus have exhibited higher sucrose contents than the healthy plants. In another experiment, sugar contents of infected grape leaves were significantly lower than the healthy plants. No significant differences were found in sugar contents between sugarcane plants infected by stripe virus and the healthy ones. In several studies, the contents of starch in infected leaves were higher than the healthy ones. Various reasons have been addressed for higher carbohydrates in leaves of the infected plants compared to the healthy plants. One of the reasons is reduced movement of carbohydrates within the plant, resulted from the phloem necrosis or changed permeability of the protoplasts. In such conditions, carbohydrates are not able to leave the leaf. Based on the studies, the carbohydrate accumulation takes place before the visible necrosis of the vessels [20]. The results are consistent with our findings. In the present study, sugar contents were higher in healthy fruits as well as the density and TSS values of fruit juices. Therefore, CTV also has led in lower contents of carbohydrates and sugar in infected fruits as the probable result of the blocked transfer from the leaves to fruits. Reduced respiration may be considered as another reason. An experiment was performed to examine the photosynthesis levels in healthy grape plants and those infected by Fan Leaf and Rupestris stem pitting viruses. The results showed that photosynthetic activity is reduced in infected plants [21]. The effects of CTV on photosynthetic activity and antioxidant metabolism of the sensitive lime plants were studied too. In this experiment, two severe (T318) and mild (T385) strains were used. CTV infection reduced the photosynthetic capacity [22]. These studies confirmed our results.

In contrast to the lime trees, vitamin C contents and total acidity of fruit juices and texture firmness were significantly different between healthy and infected trees of Kinnow tangerine and Washington navel orange. As explained above, ripening and picking the lime fruits occur in warm months with the mean maximum temperatures around 40 ˚C but it happens during cooler months for oranges and Kino tangerines. Thus, no differences were observed between healthy and infected lime trees. Given the permanent presence of the virus and its increased concentrations in cooler periods within the plants, characteristics such as total yield, fruit peel thickness, peel/flesh ratio and juice percentage are affected regardless of ripening in hot months. These results are consistent with Bassanezi et al. (2007) results [23]

CONCLUSION

Citrus Tristeza virus (CTV) is considered as a destructive viruses of citrus around the world. Since the reductive effects of high temperatures on the virus concentrations within the plants, summer maximum temperatures over 40 °C reduce the viral concentrations. Given the summer temperatures in Jahrom, early fruit trees are less physiologically affected by virus regardless of their virus resistances. However, CTV affects the quality and quantity of the fruits, leading in economic damages. Accordingly, it is necessary to control the disease in Jahrom city as a hot region.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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None declared.

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