Determination of the effects of *Apple stem grooving virus* on some commercial apple cultivars

Birişik, N.[1], Baloğlu, S.[2]
[1] Plant Protection Research Institute. Adana, Turkey, nevzatbir@yahoo.com
[2] Plant Protection Department, University of Cukurova, Adana, Turkey. Email: saba@cu.edu.tr

Abstract

Studies to determine the effects of *Apple stem grooving Capillovirus* (ASGV) on external and physical characteristic of some commercial apple (*Malus dometsica* Borkh.) cultivars were carried out in the Adana Plant Protection Research Institute's screen house facility in Turkey during 2006-2008. The selected cultivars for this aim were 'Jersey Mac', 'Fuji', 'Golden Delicious', 'Summer Red', 'Granny Smith', 'Vista Bella', 'Galaxy Gala' and 'Starking'. The selection of the cultivars was based on their common use by growers in the country. All cultivars were grafted on M9 rootstock and potted in the screen house. Turkish io-50 ASGV isolate, which had been obtained from previous works from an 'Anna' apple tree, was used for inoculation by chip budding, and the success of inoculation was confirmed by DAS-ELISA. The trial was evaluated two years after inoculation, based on six external and two physical parameters of the inoculated trees. The results demonstrated that ASGV has no statistically important effects on tree length, number of the branches, average and total length of the branches, and leaf dry matter. However, ASGV decreased the trunk diameter by about 18%, and the woody dry matter in a statistically significant rate, whereas the angle of the branches from the trunk increased on average about 41% by ASGV infection. The cultivars reacted differently to virus inoculation and stem grooving symptoms were observed on some tested cultivars.

Keywords: ASGV, apple, cultivar, effectivness.

Introduction

Pome fruits are grown in nearly all agricultural areas of Turkey. Among the pome fruits, apple is the most important in the country with a yearly production of about 2.7 million tonnes. Several virus diseases of fruits have been reported on apple in different regions and on different cultivars. *Apple stem grooving* (ASGV) is known as one of the widest distributed latent pome fruit virus in all apple growing areas (Khan and Dijikstra, 2006). This virus has been reported in Turkey and is considered to exist in all the main apple production areas of Turkey, due to the use of infected rootstocks (Birisik et al, 2008*)*. ASGV is a member of *Capillovirus* genus from the *Flexiviridae* family (Anonymous, 2005). It has a ssRNA genome of 6496 nucleotides, with particles of 600-700 nm in length (Hirata et al, 2003). Research has demonstrated the economical importance of virus diseases even when they are mostly latent on commercial apple varieties with ASGV infection resulting in 23.4 % less growth and 13.7 % reduction in trunk diameter (Maxim et al, 2004). Virus diseases can be a considerable problem in intensive production systems and with correlating high temperature and fast grooving of sapling.

Materials and methods

Inoculum source: For the inoculation of the tested varieties, an ASGV isolate (io-50) from an infected 'Anna' apple in Adana region was used. This isolate has been detected and reported as Turkish ASGV isolate from previous research (Birişik et. al 2008).

Cultivar selection: The selection of cultivars from early, fall and winter type apples was made on the basis of their frequency in Turkey. 'Jersey Mac', 'Vista Bella' and 'Summer Red' are considered summer type apples, 'Galaxy Gala', 'Fuji' as fall harvest type apples and 'Granny Smith', 'Starking' and 'Golden' as winter type varieties.

Inoculation: Virus tested saplings were obtained from the Eğirdir Horticultural Research Institute (Isparta, Turkey) and inoculated by chip budding with ASGV according to Boscia et al., (1999) in June 2006. For each variety four inoculated and two healthy plants were used in the experiment.

Confirmation: DAS-ELISA tests were performed for the detection of ASGV one year after inoculation in order to understand the status of ASGV presence in inoculated trees (Clark and Adams, 1977). Before the final evaluation of the experiment, RT-PCR was used as a confirmatory test. For the RT-PCR assay total RNA was extracted from healthy and infected tissues using the Promega SV total RNA kit as described by suppler with slight modifications. The primers used in RT-PCR assays are the same as those reported by James, (1999) and Kummert et, al., (1998).

Evaluation of the experiment: The data were collected from the experiment after two vegetative years by means of measuring listed parameters for all plants. The counting of plant dry weight was done according to Walsh and Beaton (1973). All obtained data were processed in the SPSS statistical program.

Morphological characters:	**Physical characters:**
1. Length of trees.	1. Dry weight of the leaf tissues
2. Number of branches	2. Dry weight of the woody tissues
3. Average branch length	
4. Total branch length	
5. Trunk diameter	
6. Average branch angle	

Results

ASGV inoculation was made in May 2006, and plants were controlled for infection by ELISA and RT-PCR. In the latter, two different DNA bands (574 and 499 bp) were obtained , as expected size for the primers pairs used(Fig. 1). Data obtained after two years is presented in table 1. Results showed that; statistically ASGV has an effect on trunk diameter, branch angel and dry weight of woody tissues. Other characteristics of the inoculated plant were not different in comparison with healthy control plants. The selected apple cultivars did not react differently to ASGV inoculation based on their harvesting time.

The average trunk diameter of infected plants was calculated as 14.86 cm while control plants were 17.95 cm (Fig. 2). However the average branch angle of the inoculated trees was 66.5º, compared to the negative controls of 47.0º (Fig. 3). Moreover the dry weight of the woody tissue was 5.88 gr. for ASGV infected trees, and 5,99 gr. for control plants (Table 1). Obtained data were processed in the SPSS statistics program and are shown in Fig. 4. During the evaluation of the experiment stem grooving symptoms, which are characteristic for ASGV, have been observed on 'Starking' trees (Fig.6).

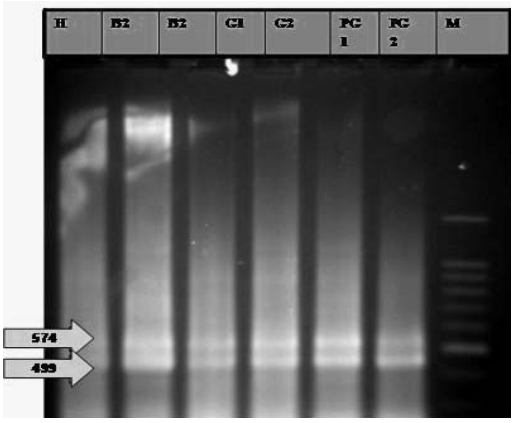

Fig. 1 RT-PCR results; ASGV-2/U 499 bp. (James, 1999) ASGV-4F/574 bp. (Kummert et. al1998)

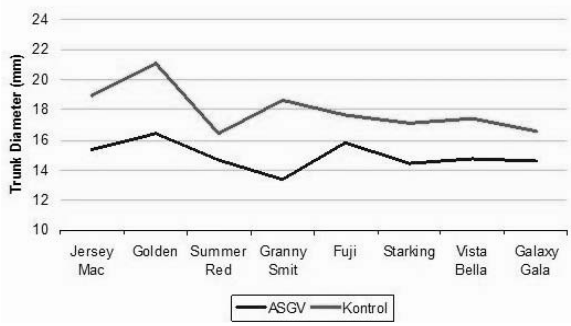

Fig. 2 Comparison of ASGV infected trees trunk diameter with control.

Tab. 1 Average of measured parameters for ASGV infected and control plants in the experiment.

	Characters															
Para-meters	**Jersey Mac**		**Golden**		**Summer Red**		**Granny Smith**		**Fuji**		**Starking**		**Vista Bella**		**Galaxy Gala**	
	ASGV	**Cont.**	**ASGV**	**Cont.**	**ASGV**	**Cont.**	**ASGV**	**Cont.**	**ASGV**	**Cont.**	**ASGV**	**Cont.**	**ASGV**	**Cont.**	**ASGV**	**Cont.**
Tree length (cm)	152	140	181	188	172	193	191	159	159	161	119	119	160	180	175	169
Number of branches	12,0	10,5	15,2	13,5	9,2	15,5	9,7	12,5	15,0	16,0	19,0	11,5	12,0	9,0	17,2	15,5
Total length of the branches (cm)	274	245	678	593	408	316	380	359	503	589	118	103	301	394	482	495
A. Length of the branches (cm)	24,2	23,8	45,5	44,6	46,5	20,5	41,8	16,9	33,7	36,8	6,3	9,0	25,2	44,3	28,2	32,2
Trunk diameter	15,3	18,9	16,3	21,0	14,6	16,4	13,3	18,6	15,8	17,6	14,4	17,1	14,7	17,4	14,5	16,6
A. angle of the branches	55,5	44,0	64,5	47,0	71,2	48,5	61,0	45,5	60,7	45,5	50,0	47,0	66,2	49,5	63,0	49,0
Dry weight of leaf tissue (gr)	4,15	4,01	4,03	4,13	3,85	3,78	4,27	4,44	4,39	4,37	4,36	4,57	4,14	4,41	4,20	4,29
Dry weight of woody tissue	5,81	5,94	5,81	5,93	5,87	5,99	5,81	5,94	5,91	5,98	6,05	6,16	5,97	6,09	5,84	5,95

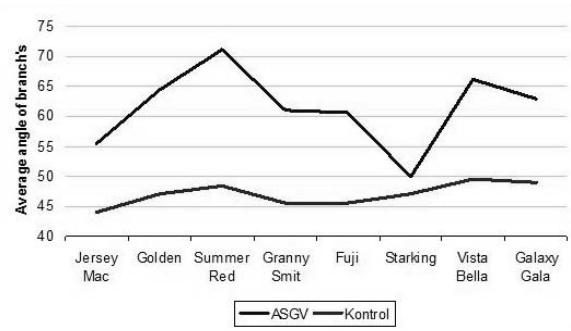

Fig. 3 Comparison of branches angle of ASGV infected trees with control.

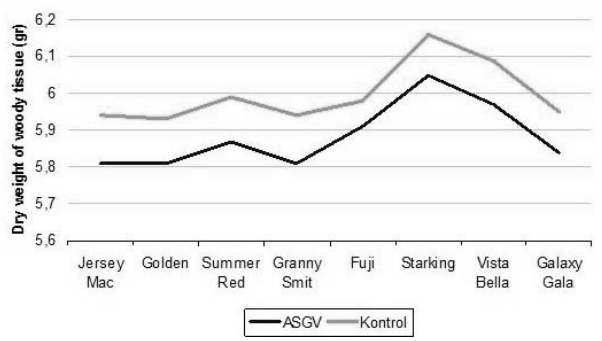

Fig. 4 Comparison of dry weight of ASGV infected woody tissues with control.

Fig. 5 ASGV infected Summer Red trees (right) have larger branch angle than healthycontrol (left).

Fig. 6 Stem grooving symptom observed on Starking.

Discussion

This study demonstrated that ASGV infection decreases the trunk diameter by about 18%, the dry weight of woody tissues by 2%, and increases the average branch angle to around 41%. Trunk diameter is an indicator for plant health: if the trunk growth is less vigorous compared to non-inoculated plants, then ASGV has a serious effect on tree size, a situation that will result in serious yield loss. Among the tested varieties 'Granny Smith' (39,8%) and 'Golden' (28,8%) were the most effected varieties in decreasing size of trunk diameter. These varieties are still popular in Turkey. The results of this study showed that ASGV infection has serious affect on plant dry weight. This fact could be very important in high density apple production systems using dwarfed trees because in these systems fruited branches are not as thick as classical trees. In case of ASGV infection branches could break at high yield. For woody dry weight, there were no big differences between the varieties. 'Granny Smith' and 'Jersey Mac' were most affected by 2.1% on average. Moreover ASGV infection increased the angle of the branches of all varieties but mostly 'Summer Red', ''Vista Bella' and 'Golden' with 46.8%, 37.2% and 34.6% on average, respectively (fig.5). This reaction might be due to shortage of dry material in woody tissues. As a result, trees could not be strong enough to carry long branches. ASGV infection induced stem grooving symptoms on 'Starking' which is not known as susceptible varieties to ASGV infection.

This result indicates that ASGV can induce some typical symptoms on many apple varieties in the appropriate growing conditions.

Acknowledgements

Thanks to The Scientific and Technological Research Council of Turkey (TUBITAK) for funding this research activity via scientific research project. No: 1001-16O303.

Literature

Anonymous; 2005. Catalog of virus taxonomy and nomenclature approved by the international Committee on Taxonomy of Viruses. http://www.ncbi.nlm.nih.gov/ICTVdb/ICTVdB/index.htm. 13.04.2009.

Birisik, N.; Myrta, A.; Hassan, M.; and Baloglu, S.;2008. A Preliminary Account on Apple Viruses in Mediterranean Region of Turkey. XX International Symposium on Virus and Virus-Like Diseases of Temperate Fruit Crops - Fruit Tree Diseases. Acta Horticulturae, **781**: 125-130.

Boscia D.; A, M, D'Onghia.; Di Terlizzi, B.; Fagioli, F.; and Osler, R.; 1999. Accertamenti fitosanitari sul materiale di propagazione. Atti del Convegno Nazionale su Certificazione delle Produzioni Vivaistiche. ds. V. Savino, P. La Notte, M. Saponari, L. Cavone, A. Bazzoni. 99-53. Bari, Italy.

Clark, M.F; and Adams, A.N.; 1977. Characteristics of the microplate method of enzyme linked immunosorbent assay for the detection of plant viruses. J. Gen.Virol. **34**: 475-483.

James, D.; 1999. A simple and reliable protocol fot the detection of *apple stem grooving virus* by RT-PCR and in a multiplex PCR assay. J. Virol. Methods, 83:1-9.

Hirata, H.; Lu, X.; Yamaji, Y.; Kagiwada, S.; Ugaki, M; and Namba, S.; 2003. A single silent substitution in the genome of *Apple stem grooving virus* causes symptom attenuation. J. Gen. Virol. **84,** 2579-2583.

Khan, J.A; and Dıjkstra, J.; 2006. Handbook of Plant Virology. Food Products Press. New York, London, Oxford 452p.

Kummert, J.; Morinho, V.L.A; Rufflard, G.; Colinet, D.; Lepoivre, P.; 1998. Sensitive detection of *apple stem grooving* and *apple stem pitting* viruses from infected apple trees by RT-PCR. Acta Horticulture, **472** (1):97-104.

Maxim, A.; Zagrai, L.; Zagrai, I.; Isac, M.; 2004. Studies on the Influence of Apple Stem Grooving Virus on Tree Growth of Various Apple Cultivars in the Nursery. XIX International Symposium on Virus and Virus-like Diseases of Temperate Fruit Crops - Fruit Tree Diseases. Acta Horticulture, **657**: 41-44.

Walsh, L.M.; and Beaton, J.D.; 1973. Soil Testing and Plant Analysis. Soil Sci. Soc. Of Am. Inc. Medison. Wisconsin. 512.