Peroxidase isozyme polymorphism in Grape Cultivars infected by *Grapevine fan leaf virus* (GFLV) and *Tomato ring spot virus* (ToRSV).

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Abstract: Two different viruses were obtained from vines exhibiting typical symptoms of viral infection. One group of the collected samples was characterized with fan leaf shape, vein banding, double node and general malformation which are typical to *Grapevine fanleaf virus* (GFLV). Another group of samples showed stunting, short internodes, chlorotic mottling symptoms characteristic to infection by *Tomato ring spot virus* (ToRSV). The two virus isolates were identified as GFLV and ToRSV depending on symptoms and serological test (ELISA). ToRSV and GFLV were found to be widely spread in grapevine propagated material and are considered as economically important grapevine viruses in Egypt. Eight Grape cultivars were tested for their reactions to GFLVand ToRSV. All of these cultivars were found to be varied in their susceptible to the viruses and various symptoms were observed on the inoculated plants. Analysis of peroxidase (POD) isozymes of ToRSV and GFLV infected and healthy plants for eight grapevine cultivars showed increased peroxidase activity in ToRSV and GFLV diseased plants of cultivar Superior cultivar (five markers), followed by Flame seedless cultivar (four markers), then King Rupy (three unique markers), finally Black monukka (one isozyme marker). In the contrast, Thompson Seedless, Rich Baba, Matrouh Aswed and Beauty seedless cultivars were not found any POD-activity can be note. Increasing in peroxidase activity was induced resistance in grapevine for ToRSV and GFLV infection. Healthy or infected Superior and Matrouh Aswed achieved the best yield and its components as well as the best physical properties of bunch and improved the chemical characteristics of berries and ensured the best vegetative growth parameters in comparison with healthy or infected other cultivars, with caution that virus diseases can have a serious impact on vine health, yield and quality of the fruit.
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1. Introduction:

Grape is one of the most popular fruits all over the world and in Egypt. In Egypt, graps rank the second position in exportation after citrus. The total planted area of the vineyards in Egypt reached 167296 feddan with a production of 1370241 tons according to the latest statistics of Ministry of Agriculture (2009). The cultivars in Egypt cover approximately the whole season, these cultivars help in increasing exports to European, Arab and Asian countries.

Grape suffer from invasion by several graft transmissible diseases caused by viruses and virus like agents (Choueiri *et al*., 1996; El-Banna, 1998). *Grapevine fan leaf virus* (GFLV) *and Tomato ring spot virus* (ToRSV) causes an economically important disease in vineyards worldwide (Brown *et al.,* 1993 and Shaista *et al*., 2008). (GFLV) and (ToRSV) are members of the *Nepoviruses* . Based on their nematodes transmissibility (Brunt *et al*., 1996).The first report of GFLV in Egypt was by El-Kady *et al*(1991) . Although, ToRSV is the first time to study on grapevines under the Egyptian conditions in 2005 by Darwish.

The induction of the expression peroxidas isozyme associated with the infection of plant tissues has been reported for several species (Goodman *et al*.1986 and Rigden and Coutts 1988).Some of these changes resemble those occurring during natural ageing (Visedo *et al*.1990). Also the participation of physiological process in the plant defense against pathogens has been pointed out (Yang and Hoffman 1984). Involvement of protein components and peroxidase activity in plant diseases resistance has been documented in several plant patho-systems (Carvalho *et al*., 2006). Different kinds of proteins were found to play certain roles in the plant defense mechanism and the resistance to plant pathogens (Belkhadir *et al*., 2004). Peroxidase was recorded as one of the first enzymes responding and providing fast defense against plant pathogens. Infection with plant pathogens led to an induction in Peroxidase activity in plant tissues and a greater increase was recorded in resistant plants compared to the susceptible ones (Mydlarz & Harvell, 2006).

This research aimed to determine the presence of most wide spread viruses, external symptoms and

serodiagnosis in the growing season. Peroxidase activity and isozyme patterns were used to test the degree of susceptibility of eight grape cultivars to GFLV and ToRSV infection as well as the virus effect on grape characters. Such obtained results would be useful in breeding for resistance against such viruses in the future. Also, study the association of GFLV and ToRSV in the most common grapevine and the effect of GFLV in vine health, yield and quality of fruit of eight grape cultivars.

2. Material and Methods:2.1.Source of diseased materials sampling:

Samples from naturally infected grapevine (*Vitis vinifera* cv. Superior) leaves showing typical symptoms of GFLV (Severe deformation of young leaves and conspicuous vein-clearing on expanded leaves were observed on leaves of bud-grafted) and ToRSV (chlorotic spots and malformation) were sampled from vineyard located at 58 km of Cairo-Alexandria desert road during the growing season. The vines were 8 years old in a sandy loam soil, spaced at 2x3 meters apart. Irrigated by the drip irrigation system, cane pruned and trellised by Spanish parron shape system. Three replicates for each cultivar were taken each replicate consisted of nine vines and subjected to the some culture practices usually carried out for these cultivars to compare the healthy and infected vines with viruses. Each sample was treated separately in the horticulture subsequent experiments. Obtained random samples (total 512 samples from eight cultivars) including leaves of grapevines stored in refrigerator and used to extract and detect the viruses and varietal susceptibility in the natural infection.

2.2. Isolation and identification of the virus isolate:

GFLV and ToRSV-infected young leaves of grapevines were ground in a sterilized mortar in 2 ml of 0.05 M potassium phosphate buffer, pH 7.0, containing 1% (v/v) nicotine alkaloid (2 ml/g of tissue) and then rub-inoculated onto five seedlings of *C. quinoa* and *Chenopodium amaranticolor* plants at the first two leaves stage previously dusted with carborundum (600 mesh), Single local lesions (Kuhn, 1964) were used for biological purification of the viruses isolate from Grapevine (*Vitis vinifera* cv. Superior) which was used as propagative host plant and served as the source of virus infection for the subsequent experiments.

2.3.Serolgical reaction:

Leaves and leaf blades of tested cultivars were examined serologically using commercial Kits supplied by SANOFI (Sante Animale, Paris, France). Double –antibody sandwich ELISA (DAS- ELISA) for GFLV and ToRSV (Clark and Adams, 1977). Also, infected trees were checked for external symptoms for virus presence.

In the horticulture parameters studies work was tended to an inventory of the virus in the farm and found that there is the focus of GFLV and ToRSV with no significant differences between the two viruses in the severity of injury. However, GFLV was more prevalent in the farm. So the focus was in the results on the GFLV only which widespread in vineyards.

2.4.Graft transmission

Bark tissue from young shoots of the infected Grapevine (*Vitis vinifera* cv. Superior) tree was side grafted on potted Grapevine (*Vitis vinifera* cv. Superior) seedlings of free virus symptoms after testing by ELISA using GFLV and ToRSV antisera supplied by SANOFI (Sante Animale, Paris, France) for routine testing in Virus & Phytoplasma Res. Dept., In each trail, at least 10 seedling were used, inoculated rootstocks and scions were tied together with plastic strips. Three to four months after inoculation were checked for external symptoms and by DAS- ELISA test for virus presence. Infected seedling and healthy seedlings control were used in analysis of peroxidase (POD) isozymes.

2.5. Analysis of peroxidase:

2.5.1. Extraction of peroxidase (POD):

One g of young leaf Samples from eight grapevine cultivars (ToRSV and GFLV infected trees and the healthy control) were analyzed for POD-activity according to Anderson *et al*. (1995). Samples were homogenized in 0.01 M sodium phosphate buffer (pH 6.0) as (1:2 w/v). The extracts were centrifuged at 10,000 x g at 4ºC for 20 min and the supernatant served as the enzyme source.

2.5.2. Peroxidase (POD) isozymes electrophoresis:

Peroxidase isozymes were analyzed using the native polyacrylamide gel electrophoresis (native-PAGE) 10%, according to Vallejos (1983). The gels were run for 2 h at 10ºC and 30 mA in a vertical electrophoresis unit. POD-isozymes were detected by incubating the gels for 5-20 min in a reaction mixture containing 0.5 mM benzidiney drochloride and 10 mM H_2O_2 in 0.05 M acetate buffer, pH 4.9.

Peroxidase isozymes were designated by their migration position (mm of the origin line) on the gel.

2.5.3. Gel analysis

The gel analysis was applied by programme (UVI geltec version 12.4, 1999-2005, USA).

2.6. Parameters were measured to evaluate the tested varieties:

2.6.1. Yield and physical characteristics of bunches

Yield/vine (kg) was determined as number of bunches/vine X average bunch weight (g). Representative random samples of 6 bunches/vine were harvested at maturity. The following characteristics were determined: average bunch weight (g) and bunch width and length (cm).

2.6.2. Chemical characteristics of berries:

Berry total soluble solids in berry juice (T.S.S.) (%) by hand refractometer and total titratable acidity as tartaric acid (%) (A.O.A.C.1985). Hence TSS /acid ratio and total anthocyanin of the berry skin (mg/g fresh weight) according to Husia et al., (1965) were calculated.

2.6.3. Morphological and chemical characteristics of vegetative growth

At growth cessation, the following morphological and chemical determinations were carried out on 4 shoots / the considered vine:

1-Average shoots length (cm).

2- Number of leaves.

3- Average leaf area (cm2) of the apical 5th and 6th leaves using a planimeter.

2.6.4. Statistical analysis:

The complete randomized block design was adopted for the experiment the statistical analysis of the present data was carried out according to Snedecor and Cochran (1972). Were compared using the new L.S.D values at 5% level.

3. RESULTS AND DISCUSSION:

3.1. Isolation and Identification:

Virus isolates were obtained from naturally infected grapevine plants eight cultivars showing severe mosaic and malformed leaves (Fig.1 A2:A5 and B1:B5) were collected from vineyard located at 58 km of Cairo-Alexandria desert road during the growing season. The symptoms were very similar to those illustrated by Dias (1975) and Dias and Cation (1976). Subsequent work clearly proved that the viruses under study are GFLV and ToRSV. These results were based mainly on symptomatology and serology. Such obtained results would be useful in breeding for resistance against such viruses in the future.

3.1.1.Serological reaction:

Positive reaction obtained using specific antiserum against GFLV and ToRSV confirmed the identification of the viruses under study. Serological tests, such as ELISA provide rapid and convenient methods for the identification and estimation of plant viruses in leaves (Németh, 1986 and Esmenjaud *et al*., 1993). Viruses detection in eight cultivars Leaves and leaf blades of tested hosts were examined serologically.

Data in Table (1) illustrated that, from 512 samples examined by ELISA, 129 samples (25.2%) infected with GFLV while 51 samples (10%) infected with ToRSV, these results were in an agreement with (Shalaby *et al*,2007). Also, data in Table (1) revealed that Superior and Flame seedless cvs. were the lowest sensitivity to GFLV followed by Matrouh Aswd while King Ruby and Rich Baba were the highest sensitivity. The remained cultivars ranged among between them. On the other hand. Superior, Flame seedless and Matrouh Aswd cvs. were the lowest sensitivity to ToRSV followed by Beauty, King Ruby and Black Monukka that approached one another with the results in (Shalaby *et al*,2007).

3.2. Analysis of peroxidase

Peroxidase activity and isozyme patterns were investigated in eight grapevines cultivars inoculated with ToRSV and GFLV viruses which produced systemic symptoms. Peroxidase isozyme (POD) patterns displayed a total of 12 bands at different *Rf* values varying from 0.078 to 0.935, whereas 10 bands were polymorphic and the two other bands at *Rf* values (0.631 and 0.710) were found to be monomorphic among ToRSV and GFLV the infected plants of the eight grapevine cultivars compared with the healthy control as presented in **(Fig.2)**. The relative front (*Rf*) value of each band was calculated depending on this. It was concluded that, between healthy as well as ToRSV and GFLV infected plants there was significant difference in isozyme activity. In the case of, ToRSV-infected plants were found a clear extra three bands of Flame seedless cultivar at *Rf* value 0.078, 0.360 and 0.516. Also, ToRSV-diseased plants in Superior cultivar scored two isozyme markers with *Rf* 0.360 and 0.576. In addition, one unique marker induced in the ToRSV-diseased plants of Black-Monukka and King Rupy cultivars with *Rf* value (0.161) and (0.465), respectively and disappeared in the control.

On the other hand, GFLV-susceptible plants of superior cultivar scored three major bands with *Rf* 0.360, 0.465 and 0.576 which disappeared in the healthy control. Also, two unique markers were existed in GFLV-infected plants of king Rupy cultivar at *Rf* values 0.465 and 0.834. In addition to, the isozyme profile of POD revealed the disappearance of some bands in diseased plants which were present in their respective controls such as four bands revealed in the healthy plants of Thompson Seedless cultivar at *Rf* (0.834, 0.900, 0.915 and 0.935), three bands appeared in the healthy plants of Matrouh Aswed with *Rf* (0.360, 465 and 0.516) and Beauty seedless at *Rf* (0.078,

0.465 and 0.834) and one band was existed in the control plant of Black monukka, Flame seedless and superior, at *Rf* 0.360, 0.834 and 0.935 respectively. Moreover, it was not changed in isozyme activity of the healthy and ToRSV and GFLV infected plants of Rich-Baba cultivar (Fig.2) Consequently, The highest POD-activity was recorded in Superior cultivar (five markers), followed by Flame seedless cultivar (four markers), then King Rupy (three unique markers), finally Black monukka (one isozyme marker). In the contrast, Thompson Seedless, Rich Baba, Matrouh Aswed and Beauty seedless cultivars were not found any POD-activity can be note (Table 2). Increasing in peroxidase activity was accompanied by alteration in isozyme patterns and induced resistance in grapevine. These results were in an agreement with Nadlong and Sequeira (1980) suggested that the increased POD-activity following virus infection whereas up-regulated peroxidases might be responsible for growth reductions and malformations in virus-infected plants. Since enzymes control biochemical reactions, and their syntheses are under the control of specific gene, any change in the activity of an enzyme would reflect the pattern of gene expressions and corresponding metabolic events in the cell. Hence, enzymes can be used as tools to study the induced responses of plants showing disease symptoms at the biochemical level (Neog et al., 2004). In addition, phenol-oxidizing enzymes such as Peroxidase (POD) and polyphenoloxidase (PPO) are associated with many diseases (Pegg, 1985). POD participates in a variety of plant defense mechanisms (Mareschbacher et al., 1986) in which H_2O_2 is often supplied by an oxidative burst, a common event in defense responses (Dixon and Lamb, 1990). Also, Solymosy et al., (1967) who compared the changes in isozyme spectrum in various host virus combinations and indicated that the change was determined mainly by the host tissue and not by virus. Isozyme analysis is a powerful tool for estimating genetic variability identifying cultivars and germplasm accessions. The differences in the isozyme binding patterns are due to variation in the amino acid content of the molecule, which in turn is dependent on the sequence of nucleotides in DNA (Micales et al., 1986). Different bands obtained indicate different electrophoretic mobilities of the isozymes, which are coded by different alleles or separate genetic loci. Therefore, such studies are useful in identifying and characterizing resistance in *Vitis sp.* Caused by infection both of ToRSV and GFLV.

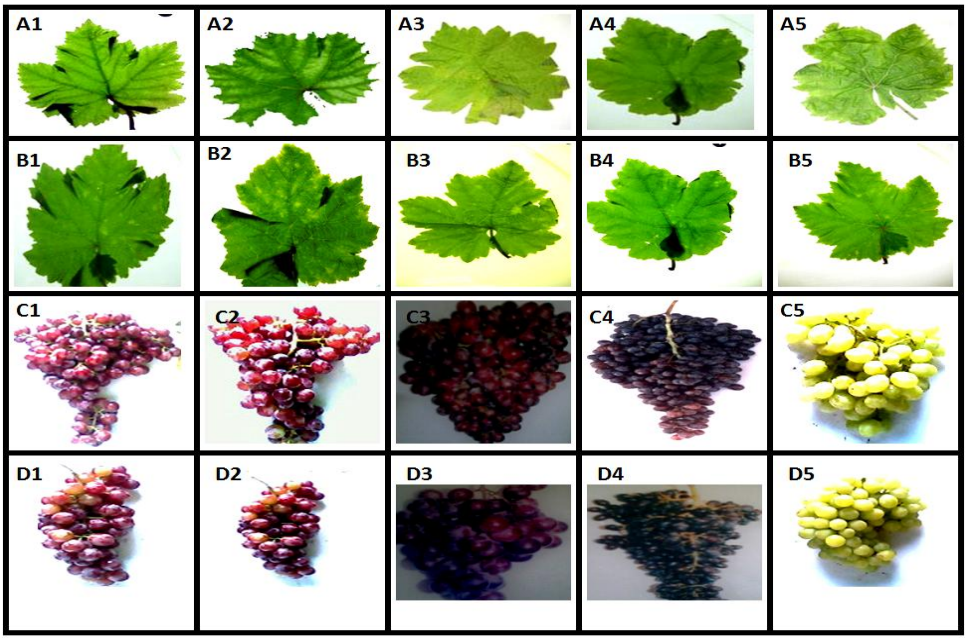


Fig (1): Symptoms on vine leaves A1:A5 in different cultivars susceptible of GFLV and from B1:B5 susceptible of ToRSV,(Leaves symptoms range from slight chlorosis , yellowing and feathering of leaf veinlets to mottled leaves with widened sinuses).C1:C5 healthy berries and from D1 : D5 GFLV infected berries (virus is responsible for uneven size and color of the berries on this vine).

Table 1. Incidence of *Grapevine fan leaf virus* (ToRSV) and *Tomato ringspot virus* (ToRSV) in highbush grape cultivars.

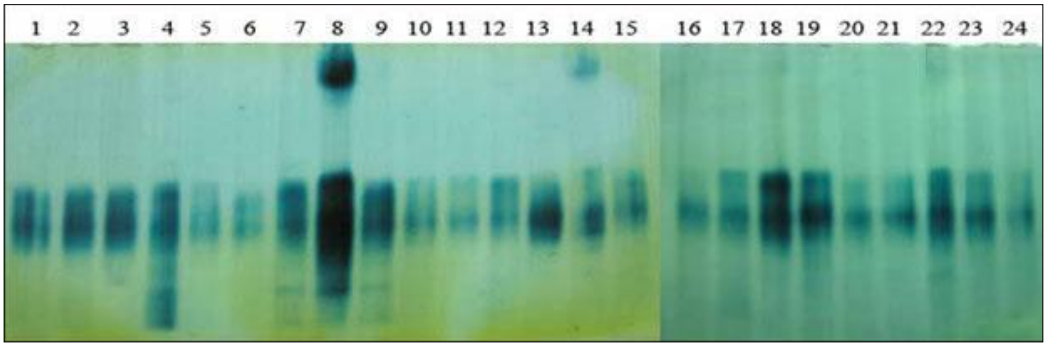
Grape cultivars	No. of bushes sampled	GFLV infection		ToRSV infection	
		No. of infection	%	No. of infection	%
Flame	90	10	11.1	4	4.4
Superior	55	6	10.9	3	5.5
Beauty	80	23	28.8	6	8.6
Thompson	60	14	23.3	10	16.7
B. Monukka	50	20	40	7	14
Matrouh Aswd	70	10	14.3	4	5.7
Rich Baba	42	18	42.9	11	26.2
king Ruby	65	28	43.1	6	9.2
Total samples	512	129	25.2 GFLV	51	10 ToRSV

Table 2: POD-isozyme marker of the eight Grapevine cultivars infected with ToRSV and GFLV

Band No.	***Rf***	**King Rupy**	**Thompson**	**Black Monukka**	**Rich Baba**	**Flame seedless**	**Superior**	**Matrouh Aswed**	**Be aut y**
Tomato ring spot virus									
1	**0.078**					+			
2	**0.161**			+					
3	**0.360**					+	+		
4	**0.465**	+							
5	**0.516**					+			
6	**0.576**						+		
Total = 6		**1**	**0**	**1**	**0**	**3**	**2**	**0**	**0**
Grapevine fan leaf virus									
1	**0.360**						+		
2	**0.465**	+					+		
3	**0.516**					+			
4	**0.576**						+		
5	**0.834**	+							
Total= 5		**2**	**0**	**0**	**0**	**1**	**3**	**0**	**0**
Total =11		**3**	**0**	**1**	**0**	**4**	**5**	**0**	**0**

***Table 3, 4, and 5 are at the end of the article following references.**

+ = Presence of band



Fig(2) : POD-isozyme polymorphism profile of eight grapevine cultivars infected with ToRSV and GFLV compared with the healthy control.
-1,4,7,10,13,16,19,22 the healthy plants of King Rupy, Thompson Seedless , Black Monukka, Rich-Baba, Flame seedless, Superior, Matrouh Aswed , Beauty seedless cultivars, respectively.
-2,5,8,11, 14,17,20,23 TRSV-susceptible plants of King Rupy, Thompson Seedless , Black Monukka, Rich-Baba, Flame seedless, Superior, Matrouh Aswed, Beauty seedless cultivars, respectively.
-3,6,9,12,15,18, 21, 24 GFLV-infected plants of King Rupy, Thompson Seedless, Black Monukka, Rich-Baba, Flame seedless, Superior, Matrouh Aswed , Beauty seedless cultivars, respectively.

3.3. Parameters were measured to evaluate the tested varieties:

3.3.1. Yield and physical characteristics of bunches

Data in Table (3) illustrated that yield and physical characteristics of bunches of eight grape cultivars i.e. Flame seedless, Superior, Beauty seedless, Thompson seedless, Black Monukka, Rich Baba, Matrouh Aswd, and King Ruby.

The values of number of bunches ranged from 17.33 to 23.67 and 19.00 to 25.67 in the two season respectively, King Ruby gave the greatest number of bunches, while Beauty seedless and Rich Baba gave the lowest one. Also, healthy vines gave number of bunches the highest higher than infected vines in all cultivars. The values of yield ranged between 6.98 to 12.15 and from 8.69 to 14.46 kg in the two seasons respectively.

The highest yield was obtained from Flame Seedless (12.15& 14.46 kg) and King Ruby (11.50 & 13.04 kg), followed in descending order by Thompson Seedless, Black Monukka, Superior, Matrouh Aswad, Rich Baba and Beauty Seedless grapevines.

The highest bunch weight (gm) was obtained by Flame Seedless (576.7 & 601.7 gm), while Beauty Seedless gave the lowest bunch weight (355.0 & 403.3 gm).

GFLV causes a reduce bunch weight especially in Beauty Seedless cv. In first season. The bunch weight decrease lead to a decrease also in yield / vine With respect to bunch dimensions, the effect of GFLV on bunch width and length was statistically significant in both seasons. Black Monukka gave the highest bunch length (30.17 & 32.50cm), while Beauty Seedless gave the lowest bunch length (18.42 & 19.83 cm) in the two seasons, respectively. The remaining cultivars gave values ranged between them.

Regarding to bunch width; Flame Seedless and Thompson Seedless gave the highest bunch width (20.50 & 22.40 cm) & (21.83 & 22.33 cm), respectively.

On the other hand, the lowest values were obtained by Beauty Seedless (18.33 & 18.83cm) and Rich Baba (13.00 & 16.50cm) in the two seasons.

Generally, the infected vines with GFLV gave least bunch weight, length and width so, yield in all cultivars in comparison with healthy vines.

These results were in an agreement with Credi and Babini (1997) who found that virus decrease yield by 14.2 to 72.9 %.

3.3.2. Chemical characteristics of berries:

Data in Table (4) show the percentages of total soluble solids, total titratable acidity of berry juice, as well as T.S.S/acid ratio. The values ranged from 16.83 to 20.33 and from 17.50 to 21.83 in the two seasons, respectively.

The greatest TSS values were obtained by Thompson Seedless cv. (20.33 & 21.83 %) and Flame Seedless cv. (19.00 & 20.83 %). On the other hand, the lowest values were obtained by Beauty Seedless (16.83&17.50%) at two seasons, respectively.

With respect to acidity, Flame Seedless resulted in the lowest percentage of acidity (0.29 & 0.35 %), while Superior (0.82 & 0.74 %)and Beauty seedless (0 .64 & 0.72 %) recorded the greatest Acidity % in the two seasons.

The effect of tested vines (healthy and infected) was insignificant in the first season only.

Regarding T.S.S/acid ratio, data revealed that Flame Seedless cv. Gave the highest TSS/acid ratio (65.23 & 59.47 %), followed by King Ruby cv. (62.17 & 67.36 %), while Beauty Seedless cv. Was gave the lowest TSS/acid ratio values (26.27 & 24.20 %) in the two seasons, respectively. The other cultivars gave values between them.

As regard to, Anthocyanin content of berry skin for Flame Seedless, Beauty Seedless, Black Monukka, Matrouh Aswad and King Ruby, the values ranged from 27.30 to 30.00 in the first season and from 28.92 to 33.67 mg .g f.w. in the second season .The greatest values of anthocyanin were obtained from Matrouh Aswad (30.00 & 33.67) . The lowest values were obtained from Flame Seedless in (27.50 &29.08 mg/100g f. w.) and beauty seedless (28.00 & 28.92 mg/g f.w.).

3.3.3. Vegetative growth:

Data in Table (5) indicated that No. of leaves per shoot, leaf area and shoot length. The values ranged from 20.50 to 29.5 & 22.83 to 32.17 for no. of leaves/per shoot, from 94.33 to 189.00 & 100.00 to 198.50 for leaf area (cm) and from 148.70 to 194.0 & 162.5 to 197.5 for shoot length (cm) in the two seasons, respectively.

Data show that the highest values of vegetative growth parameters responded positively to the healthy vines (free virus) as compared to infected vines was found to have the lowest ones of this respect in both seasons for eight cultivars under study.

Flame Seedless gave the highest shoot length (194.0 & 197.5cm) and leaf area 189.0 & 198.5 in the two seasons, respectively.

Regard to No. of leaves per shoot Flame Seedless and Superior gave the highest values of No. of leaves (29.5 & 31.33 cm), (29.33 & 31.33 cm), while Rich Baba gave the lowest No. of leaves (20.5 & 22.83) in the two seasons, respectively.

These results were on line with Credi and Babini (1997) who recorded that Virus causes growth losses of 21.2% and 23.1%.

4. Conclusion

In conclusion, Flame Seedless and Superior were highly cvs. resistance to GFLV and ToRSV, they achieved the best yield and its components as well as the best physical properties of bunches, improved the physical and chemical characteristics of berries and ensured the best vegetative growth parameters in comparison to other varieties specially Beauty Seedless and Rich Baba which gave the lowest values of these parameters.

The obtained results revealed that growth vigor (shoot length, leaf area and No. of leaves per shoot) were clearly affected by van leaf virus which reduce the shoot length by reducing the internodes length as a result of reducing leaf area due to injury of leaves affected by virus.

The positive effect of healthy vines on chemical characteristics of berries may be due to its increasing effect on photosynthesis process and promoter hormones such as cytokinin closely involved in cell division, proteins, carbohydrates and chlorophylls. While the effect of virus injury on infected vines might be due to the lower ability of injured leaves to do their photosynthesis process which affected directly on leaf pigments and affected bunches. Malakeberhan and Ferris (1989) and El –Nagdi *et al* (2009).

Generally, we can be overcome injury with virus diseases by expansion in the cultivation of resistant varieties and attention to balanced nutrition for the vines.

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Table (3): Effect of *Grapevine fan leaf virus* on yield and physical characteristics of bunches of eight grape cultivars

Variety		No. of bunches						Yield (kg / vine)					
		1st season 2010			2nd season 2011			1st season 2010			2nd season 2011		
		I	H	Av. (A)	I	H	Av. (A)	I	H	Av. (A)	I	H	Av. (A)
Flame Seed less		20.00	22.00	21.00	23.00	25.00	24.00	10.93	13.37	12.15	13.59	15.34	14.46
Superior		17.67	18.33	18.00	18.67	19.33	19.00	8.31	8.80	8.56	9.40	9.92	9.66
Beauty Seedless		19.67	19.67	19.67	20.33	22.67	21.50	6.22	7.74	6.98	7.78	9.60	8.69
Thompson Seedless		19.67	21.67	20.67	22.33	24.33	23.33	8.57	11.26	9.92	11.53	14.20	12.86
Black Monukka		18.33	19.67	19.00	20.00	22.67	21.33	8.36	10.42	9.39	10.33	13.85	12.09
Matrouh Aswd		17.33	19.33	18.33	21.33	22.33	21.83	6.94	8.57	7.76	9.69	10.89	10.29
Rich Baba		16.67	18.00	17.33	18.67	20.67	19.67	7.26	8.10	7.68	8.34	9.82	9.08
king Ruby		23.00	24.33	23.67	24.67	26.67	25.67	10.42	12.59	11.51	11.50	14.59	13.04
Av. (B)		19.04	20.38		21.13	22.96		8.38	10.11		10.27	12.27	
New L.S.D.	A Var.	0.9586			1.227			0.8964			1.012		
	B Treat	0.4793			0.6135			0.4482			0.5061		
	A*B	1.356			1.735			1.268			1.432		

*I = infected vines *H= Healthy vines

Table (3) *continued*: Effect of *Grapevine fan leaf virus* on yield and physical characteristics of bunches of eight grape cultivars

Variety		Bunch weight (gm)						Bunch length (cm)						Bunch Width (cm)					
		1st season 2010			2nd season 2011			1st season 2010			2nd season 2011			1st season 2010			2nd season 2011		
		I	H	M. (A)	I	H	M (A)	I	H	M (A)	I	H	M (A)	I	H	M (A)	I	H	M (A)
Flame Seed less		546.7	606.7	576.7	590.0	613.3	601.7	22.67	24.67	23.67	29.67	31.00	30.33	19.50	21.50	20.50	22.00	22.83	22.42
Superior		470.0	480.0	475.0	503.3	513.3	508.3	19.83	20.77	20.30	21.00	22.33	21.67	13.33	13.33	13.33	14.67	15.33	15.00
Beauty Seedless		316.7	393.3	355.0	383.3	423.3	403.3	18.00	18.83	18.42	19.33	20.33	19.83	18.00	18.67	18.33	18.33	19.33	18.83
Thompson Seedless		436.7	520.0	478.3	516.7	583.3	550.0	20.50	21.33	20.92	20.67	23.00	21.83	21.00	22.67	21.83	21.67	23.00	22.33
Black Monukka		456.7	530.0	493.3	516.7	610.0	563.3	29.67	30.67	30.17	32.67	32.33	32.50	15.00	16.33	15.67	15.67	16.67	16.17
Matrouh Aswd		400.0	443.3	421.7	453.3	486.7	470.0	20.67	23.00	21.83	24.67	26.33	25.50	14.67	16.00	15.33	16.67	17.67	17.17
Rich Baba		435.0	450.0	442.5	446.7	475.0	460.8	19.33	21.33	20.33	22.67	23.67	23.17	12.33	13.67	13.00	15.67	17.33	16.50
king Ruby		453.3	516.7	485.0	466.7	546.7	506.7	20.53	20.57	20.55	20.53	21.60	21.07	17.37	17.67	17.52	17.83	18.50	18.17
Av. (B)		439.4	492.5		484.6	531.5		21.4	22.65		23.9	25.08		16.4	17.48		17.81	18.83	
New L.S.D.	A Var.	29.18			31.44			1.192			1.966			0.8744			0.9837		
	B Treat	14.59			15.72			0.5960			0.9832			0.4372			0.4918		
	A*B	41.27			44.47			1.686			2.781			1.237			1.391		

*I = infected vines *H= Healthy vines

Table (4): Effect of *Grapevine fan leaf virus* on chemical characteristics of berries of eight grape cultivars

Variety		T.S.S. (%)						Acidity (%)					
		1st season 2010			2nd season 2011			1st season 2010			2nd season 2011		
		I	H	Av. (A)	I	H	Av. (A)	I	H	Av. (A)	I	H	Av. (A)
Flame		18.33	19.67	19.00	20.17	21.50	20.83	0.30	0.28	0.292	0.38	0.33	0.353
Superior		16.50	17.17	16.83	18.33	19.00	18.67	0.84	0.82	0.828	0.76	0.73	0.747
Beauty		16.33	17.33	16.83	17.33	17.67	17.50	0.67	0.62	0.645	0.72	0.73	0.725
Thompson		19.67	21.00	20.33	21.33	22.33	21.83	0.63	0.61	0.617	0.65	0.59	0.617
B. Monukka		17.83	19.33	18.58	19.67	21.67	20.67	0.34	0.40	0.373	0.41	0.42	0.415
Matrouh Aswd		17.50	17.83	17.67	17.67	18.00	17.83	0.57	0.54	0.557	0.48	0.40	0.438
Rich Baba		17.67	18.00	17.83	18.33	19.33	18.83	0.51	0.44	0.475	0.43	0.40	0.415
king Ruby		18.67	19.33	19.00	19.83	21.50	20.67	0.29	0.33	0.307	0.32	0.29	0.308
Av. (B)		17.81	18.71		19.08	20.13		0.519	0.504		0.518	0.486	
New L.S.D.	A Var.	0.6845			0.7550			0.03729			0.04085		
	B Treat.	0.3422			0.3775			N.S.			0.02042		
	A*B	0.9680			1.068			0.05273			0.05776		

*I = infected vines *H= Healthy vine

Table (4) *continued*: Effect of *Grapevine fan leaf virus* on chemical characteristics of berries of eight grape cultivars

Variety		T.S.S / Acid ratio						Anthocyanin (mg/100g F.W)					
		1st season 2010			2nd season 2011			1st season 2010			2nd season 2011		
		I	H	Av. (A)	I	H	Av. (A)	I	H	Av. (A)	I	H	Av. (A)
Flame		61.14	69.42	65.28	53.10	65.84	59.47	26.60	28.00	27.30	28.50	29.67	29.08
Superior		19.68	21.04	20.36	24.03	26.06	25.04						
Beauty		24.38	28.16	26.27	24.17	24.24	24.20	27.33	28.67	28.00	28.17	29.67	28.92
Thompson		31.38	34.61	32.99	33.12	38.08	35.60						
B. Monukka		52.49	47.95	50.22	48.01	51.59	49.80	27.67	29.33	28.50	31.33	34.00	32.67
Matrouh Aswd		30.74	32.84	31.79	37.09	45.08	41.08	28.67	31.33	30.00	32.67	34.67	33.67
Rich Baba		34.44	41.23	37.84	42.95	47.93	45.44						
king Ruby		65.16	59.19	62.17	61.35	73.37	67.36	28.83	30.00	29.42	33.83	32.67	33.25
Av. (B)		39.93	41.8		40.48	46.52		27.82	29.47		30.9	32.13	
New L.S.D.	A Var.	2.756			2.266			1.179			1.686		
	B Treat.	1.378			1.133			0.7454			1.066		
	A*B	3.898			3.204			1.667			2.384		

*I = infected vines *H= Healthy vine

Table (5): Effect of *Grapevine fan leaf virus* on leaf parameters of eight grape cultivars

Variety		No of leaves per shoot						Leaf area (cm)^2					
		1st season 2010			2nd season 2011			1st season 2010			2nd season 2011		
		I	H	Av. (A)	I	H	Av. (A)	I	H	Av. (A)	I	H	Av. (A)
Flame		27.00	32.00	29.50	28.33	34.33	31.33	187.3	190.7	189.00	193.3	203.7	198.50
Superior		28.67	30.00	29.33	30.67	32.00	31.33	159.3	160.7	160.00	164.3	168.0	166.17
Beauty		21.33	25.33	23.33	26.33	28.00	27.17	91.0	97.7	94.33	96.7	103.3	100.00
Thompson		26.33	30.00	28.17	28.67	32.00	30.33	157.7	161.3	159.50	167.3	175.3	171.33
B. Monukka		24.67	26.00	25.33	27.33	31.33	29.33	160.7	163.3	162.00	162.7	169.7	166.17
Matrouh Aswd		24.67	26.33	25.50	27.67	31.67	29.67	154.7	159.3	157.00	161.3	163.3	162.33
Rich Baba		19.67	21.33	20.50	21.67	24.00	22.83	132.0	142.0	137.00	160.3	171.7	166.00
king Ruby		24.67	28.67	26.67	30.67	33.67	32.17	108.3	116.7	112.50	118.0	121.7	119.83
Av. (B)		24.63	27.46		27.67	30.88		143.9	149		153	159.6	
New L.S.D.	A Var.	1.445			1.449			4.338			5.785		
	B Treat.	0.7223			0.7247			2.169			2.892		
	A*B	2.043			2.050			6.135			8.181		

*I = infected vines *H= Healthy vine

Table (5) *continued*: Effect of *Grapevine fan leaf virus* on leaf parameters of eight grape cultivars

Variety		Shoot length (cm)					
		1st season 2010			2nd season 2011		
		I	H	Av. (A)	I	H	Av. (A)
Flame		190.33	197.67	194.0	190.00	205.00	197.5
Superior		178.67	180.67	179.7	180.67	183.00	181.8
Beauty		143.33	154.00	148.7	160.00	165.00	162.5
Thompson		175.33	184.67	180.0	186.00	190.33	188.2
B. Monukka		178.00	182.67	180.3	187.33	188.67	188.0
Matrouh Aswd		172.00	174.00	173.0	179.67	183.67	181.7
Rich Baba		134.33	142.67	138.5	140.67	153.33	147.0
king Ruby		178.33	183.00	180.7	164.67	171.00	167.8
Av. (B)		168.8	174.9		173.6	180	
New L.S.D.	A Var.	4.269			3.447		
	B Treat.	2.135			1.723		
	A*B	6.038			4.875		

*I = infected vines *H= Healthy vine