

## Influence of WSMV Infection on Biochemical Changes in Two Bread Wheat Cultivars and in Their F<sub>2</sub> Populations

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### ABSTRACT

*Wheat Streak Mosaic Virus* (WSMV) causes extensive economic damage to wheat (*Triticum aestivum* L.) in many regions of the world. The present study was conducted to find out if the variations in biochemical changes in reaction to WSMV infection in F<sub>2</sub> generation of either potentially resistant (Adl-Cross) or susceptible (Marvdasht) cultivars are genetically inherited. A factorial experiment was employed with two factors of: genotypes (Adl-Cross, Marvdasht, resistant F<sub>2s</sub> and susceptible F<sub>2s</sub>), and inoculation (either infected or non infected) at Shiraz University, Iran during 2007-2009. Leaves of seedlings were harvested at different time intervals for total protein, total phenolic compounds and peroxidase activity analysis. Results indicated that virus infection caused stress in all genotypes. Total protein reduction in the inoculated resistant Adl-Cross and in its F<sub>2s</sub> was not significant whereas it was significant in the inoculated susceptible Marvdasht and its susceptible F<sub>2s</sub>. Viral infection reduced peroxidase activity in the susceptible Marvdasht cultivar and in its susceptible F<sub>2s</sub> whereas in Adl-Cross and in its resistant F<sub>2s</sub> the activity was increased. It is speculated that peroxidase enzyme may affect synthesis of compounds effective in resistance to *wheat streak mosaic virus*. The trend in the increase in phenolic compounds indicated that their formation and accumulation is faster in the resistant genotypes as compared with the susceptible ones. It appears that the extent of total protein, total phenolic compounds as well as peroxidase activity changes in response to WSMV are inherited by the next generations and these biochemical changes in a genotype could be adopted as selective factors in the preliminary experimental stages of selection for tolerance to the virus.

**Keywords:** Peroxidase activity, Phenolic compounds, Total protein, WSMV.

### INTRODUCTION

*Wheat streak mosaic virus* (WSMV) is a serious pathogen of wheat in the United States, Canada, Iran and in some other wheat-producing countries. Losses due to WSMV are usually sporadic. The loss averaged 1% of the produce in Kansas during 1988-1998, equivalent to over 120 million US dollars lost in production (Appel *et al.*, 1999). During epidemic infection periods, a similar loss could happen in a single cropping season. Extensive research has been conducted to find resistant

genotypes in many countries including in Iran. Adl-Cross was introduced as a potentially resistant genotype during an extensive screening program in Iran (Yassaie *et al.*, 2002). Hassani and Assad (2004) evaluated the F<sub>2</sub> population from a cross between Adl-Cross and Marvdasht and concluded that resistance in Adl-Cross was apparently controlled by one dominant gene.

Resistance to phytopathogenic microorganisms may include changes in total protein synthesis (Roby *et al.*, 1985), activation or synthesis of defense peptides and proteins (Castro and Fontes 2005), the

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fast production of reactive to oxygen species (ROS) (De Gara *et al.*, 2003) as well as synthesis of phenolics (Matern and Kneusal 1988). Phenols are found in plants in the form of glycosides, which act as a mobilized defense system and can be translocated by plants and enzymatically converted to defensive substances at the site of attack (Kovalvi and Nassuth, 1995). Polyphenol oxidase and peroxidase, the enzymes involved in the oxidation of phenols to more toxic quinines, have been reported to be increased in infected plants (Yamamoto *et al.*, 1978). Peroxidases are associated with the active defence reactions in higher plants in response to foreign organisms. They are involved in the oxidation of phenolic compounds in cell walls, polymerization of lignin and suberin, and in several other oxidation processes (Fossdal *et al.*, 2001). Hosseini Nezhad *et al.* (2008) demonstrated that the amount of total protein in Adl-Cross was higher as compared with Marvdasht in both inoculated and non inoculated conditions. However, phenolic compounds were higher in Adl-Cross, as compared with Marvdasht in all conditions. Zinati (2009) reported that temperatures above 32°C decreased total protein in all genotypes with mosaic symptoms appearing in Adl-Cross. However, high temperature decreased phenolic compounds and peroxidase's activity in all genotypes. The objectives of this study were: (i) to investigate the relationships between development of systemic WSM symptoms and biochemical changes in Adl-Cross, Marvdasht and in their F<sub>2</sub> generations, (ii) to see the consistency of biochemical changes in F<sub>2</sub> generation.

## MATERIALS AND METHODS

Seeds of Adl-Cross and of Marvdasht were obtained from Agricultural Research Center, Zarghan, Iran. F<sub>1</sub> and F<sub>2</sub> were produced in Plant Virology Research Center, Shiraz University during 2007-2009. The WSMV isolate employed in the experiment

was the same as that used by Yassaie *et al.* (2002) in their screening experiments.

A factorial experiment was employed with two factors of: (1) genotypes (Adl-Cross, Marvdasht, resistant F<sub>2s</sub> as well as susceptible F<sub>2s</sub>); (2) inoculation (infected and non infected conditions) in a completely randomized design of three replications. The sample populations were planted in 3:1:1 soil mix consisting of field soil, sand and manure in 50 cm diameter pots. Plants were grown in growth chamber at 25/20°C day/night. Seedlings in each genotype were divided into two groups; seedlings in one group were inoculated with WSMV ten days past planting, while seedlings in the other group served as control. Leaves of each seedling were harvested on five different times, e.g., one hour, 1 day, 2 days, 4 days and 8 days after inoculation. Sample leaves were harvested, weighed and stored in -70°C for later protein assessment, free phenolic extraction and peroxidase analysis. WSMV damage (leaf chlorosis) was assessed using the damage ratings of 0 to 7 (Masumi *et al.*, 1999). The rating scale was based on symptoms observed on lower leaves and systemic spread of the virus to upper leaves in 9 to 10 weeks after inoculation. For symptomatology, the lower four to five leaves were rated on a scale of 0 to 7. Seedlings showing chlorotic spots (0-2); those with no streaking and leaf rolling were considered as tolerant while the ones suffering from leaf streaking and rolled leaves (3-7) were considered as susceptible.

Leaves were used to prepare tissue extracts. The leaf tissue (0.5 g fresh weights) was homogenized in 1 ml of 50 mM Tris-HCl, pH 8.0 at 25°C, 10 mM MgCl<sub>2</sub>, 2.5 mM dithioerythritol and 10% glycerol (v/v). The homogenates were clarified through centrifugation at 1,000g for 20 minutes. The resultant extracts were used for measuring protein content and for peroxidase activity. The procedure of Bradford (1976) was employed to determine total soluble protein. Peroxidase activity was assessment by the method described by Poll *et al.* (1994). Enzyme extracts (50 µl) were mixed with 5

ml extraction buffer (100 mM  $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$  pH 7), containing 20 mM Guaiacol and 10 mM  $\text{H}_2\text{O}_2$ . Peroxidase activities were determined spectrophotometrically at 436 nm. Free phenolics were extracted according to Campell and Ellis (1992). Half a gram (0.5 g) samples were extracted in 2 volumes of 50% methanol for 1.5 hours at 80°C. The extract was centrifuged for 5 minutes at 3,000g and the supernatants used for an evaluation of the phenolic content. Phenolic content was assessed by the method of SeEVERS and DALY (1970). Half of milliliter (0.5 ml) volumes of extract were diluted to 8 ml with water, mixed with 0.4 ml of Folin reagent along with 1.8 ml of  $\text{Na}_2\text{CO}_3$ . The absorbance of samples was measured at 725 nm at room temperature and after 1 hour past. Phenolic contents were determined using a standard curve prepared through use of caffeic acid.

SAS general linear model (SAS Institute, Carry, NC, USA, Version 6.06) was employed for a completely randomized design. Differences among genotypes and mean total protein, phenolic compounds and peroxidase activity changes were analyzed using Duncan's multiple ranges.

## RESULTS

Typical streak mosaic symptoms developed on inoculated plants in approximately 2 weeks after inoculation. The range and mean WSMV damage rating of the tolerant and susceptible genotypes and as well,  $F_2$  populations are shown in Table 1. Adl-Cross showed the least mean of damage rating. Izadi (2008) noted that this level of damage did not reduce yield as compared with yield obtained from non inoculated plots. However, yield got reduced in

susceptible Shiraz and Azadi-Cross genotypes by 35.4 and 38.8 percent respectively when the genotypes were inoculated with WSMV.

Total protein, total phenolic compounds and peroxidase activity changes in Adl-Cross, Marvdasht, and  $F_2$  populations are shown in Table 2. Total protein in tolerant genotypes (Adl-Cross and resistant  $F_{2s}$ ) was higher as compared with susceptible genotypes (Marvdasht and susceptible  $F_{2s}$ ) in either inoculated or non inoculated conditions. Hosseini Nezhad *et al.* (2008) also reported similar results. Infection decreased total protein in all populations, but significant protein reduction was only recorded in Marvdasht and susceptible  $F_{2s}$ . Zinati (2009) showed that total protein reduction in inoculated Adl-Cross was not significant at 25°C but temperatures above 32°C resulted in both processes of breaking resistance and decrease in total protein. However, total protein reduction in infected Marvdasht was significant at 25°C and of temperatures above 32°C (Table 2). The loss of leaf soluble proteins in viral infected leaves had been shown to be due in part to either degenerated chloroplasts, or protein synthetic inhibition (Bertamini *et al.*, 2005). Castro and Fontes (2005) demonstrated that quick defense responses include the synthesis of defense peptides and proteins with antimicrobial properties. The main groups of antimicrobial peptides found in plants are thionins, defensins and lipid transfer proteins. They constitute the interesting candidates to engineer disease resistance in plants.

A comparison among means of phenolic compounds indicated that their accumulation in inoculated tolerant Adl-Cross and resistant  $F_{2s}$  is more than in uninoculated

**Table 1.** Range and mean of WSMV damage rating in Adl-Cross, Marvdasht and their  $F_2$  populations.

Parents and progeny	Range and mean of WSMV damage rating	Observed		Expected ratio	$\chi^2$	P
		Resistant	Susceptible			
Adl-Cross	0-2 (0.8)	38	0			
Marvdasht	3-7 (3.8)	0	45			
$F_2$	0-7 (1.85)	82	31	3:1	0.35	0.58

**Table 2.** Influence of WSMV infection on total protein content, total phenolic content and peroxidase activity in Adl-Cross and Marvdasht and in their F<sub>2</sub> populations.

	Adl-Cross		Marvdasht			F <sub>2</sub>	
	non-infected	Infected	non-infected	Infected	non-infected	Infected	
						Resistant F <sub>2s</sub>	Susceptible F <sub>2s</sub>
Total soluble protein (mg protein/g fresh weight)							
1 hour	4.3 Ac	4.2 Ac	3.1 Aa	2.93 Ca	3.61 Ab	3.29 Aa	3.43 Aab
1 days	4.5 Ac	4.36 ABc	3.32 Ab	2.6 Ba	4.2 Bc	3.9 Bbc	3.2 Aab
2 days	5.1 Bb	4.61 Bb	3.94 Bb	2.4 Ba	4.3 Bb	4.1 BCb	2.6 Ba
4 days	5.41 Cc	5.3 Cc	4.1 Bbc	2.36 Aa	4.6 Cc	4.37 Cc	2.5 Bab
8 days	5.8 Db	5.71 Cb	4.4 Cb	2.15 Aa	5.6 Cb	5.45 Db	2.2 Ca
Phenolic content (µg phenolics/g fresh weight)							
1 hour	2289 Ad	2292 Ad	2210 Aa	2225 Aab	2265 Acd	2288 Ad	2242 Bbc
1 days	2310 Bd	2315 Bd	2221 Aa	2240 Bab	2279 Ac	2311 Bd	2257 Cbc
2 days	2318 Bb	2348 Cc	2257 Ba	2263 Da	2291 Bb	2329 Bbc	2269 Da
4 days	2343 Cbc	2385 Dc	2278 Ca	2251 Ca	2328 Cb	2371 Cc	2239 Ba
8 days	2358 Cc	2398 Dc	2291 Db	2236 Ba	2338 Cbc	2392 Dc	2227 Aa
Peroxidase activity (units/g fresh weight)							
1 hour	282 Ab	283 Ab	280 Ab	279 Cb	272 Aa	272 Aa	269 Ca
1 days	285 Bbc	289 Bc	283 Ab	276 Cb	274 Aab	277 Ab	264 BCa
2 days	283 Ad	292 Bd	281 Acd	267 Bab	280 Bbc	287 Bd	260 Ba
4 days	285 Bb	296 Cc	285 Bb	263 Ba	281 Bb	289 Bbc	253 Ba
8 days	286 Bb	295 Cb	288 Cb	254 Aa	289 Cb	301Cb	248 Aa

Means within each column (row) followed by same capital (small) letters are not significantly different (DMRT,  $\alpha=0.05$ ).

plants (Table 2). Infected leaves of Adl-Cross and of resistant F<sub>2s</sub> apparently produced phenolic compounds from 1 hour to 8 days after inoculation. The highest induction was recorded at the eighth days after inoculation. When Marvdasht and susceptible F<sub>2s</sub> were infected by WSMV, a significant increase in phenolic compounds was observed in leaves but there was a lag phase between 2 days and 4 days after inoculation with phenolic compounds declining thereafter. Hosseini Nezhad *et al.* (2008) reported that synthesis of phenolic compounds was higher in Adl-Cross as compared with that in Marvdasht in all conditions. Zinati (2009) emphasized on these results and also demonstrated that temperature above 32°C decreased phenolic compounds in Adl-Cross. Apparently, the formation and accumulation of phenolic

compounds were higher in Adl-Cross and resistant F<sub>2s</sub>. Kofalvi and Nassuth (1995) also reported that WSMV influenced phenylpropanoid metabolism and the accumulation of phenolics as well as lignin in wheat. These results may indicate that plants respond to infection by synthesis of phenolic compounds, to prevent proliferation and spreading of WSMV. Rapid synthesis of antibiotic phenols and their polymerization in the cell wall is considered as part of an active defense response (Nicholson and Hammerschmidt 1992). These results emphasize the role of phenolic compounds in preventing viral movement and spread in Adl-Cross and resistant F<sub>2s</sub>.

Peroxidase activity increased in Adl-Cross and in the resistant F<sub>2s</sub>, 3.14 and 4.15 percent respectively (Table 2). However, Marvdasht

and susceptible F<sub>2s</sub> showed a reduction of the activity by 11.8 and 14.1 percent respectively. Hosseini Nezhad *et al.* (2008) did not observe any regular trend of formation and accumulation of peroxidase activity in Adl-Cross and Marvdasht in any condition. However, Zinati (2009) found a significant increase in peroxidase activity in inoculated Adl-Cross as compared with uninoculated control at 25°C. Increased peroxidase activity has been observed in a number of resistance involved interactions involving plant-pathogenic fungal and bacterial interaction (Reimers *et al.*, 1992; Young *et al.*, 1995). Peroxidase is important in defense mechanism against pathogens, through its role in the oxidation of phenolic compounds to quinones, causing increasing antimicrobial activity. It is believed that peroxidase may be directly involved in stopping pathogen development (Melo *et al.*, 2006; Shimizu *et al.*, 2006); accelerating the cellular death of cells close to the infection site, preventing the advance of infection and/or by generating a toxic environment which will inhibit the growth of the pathogen inside the cells (Bi and Felton 1995). Peroxidase enzyme probably affects synthesis of compounds effective in conferring resistance. Therefore, reduction of enzyme activity may reduce these compounds as well as quinone production. Quinones are more poisonous to pathogens than phenolic compounds, Also, reduction in enzyme activity increases reactive oxygen species (ROS) and this leads to oxidative damage (Malolepsza and Rozalaska 2005; De Gara *et al.*, 2003).

It may be concluded that the variation of biochemical changes in response to WSMV infection in F<sub>2</sub> generation of potentially resistant and susceptible cultivars is genetically inherited. In addition, the level of total protein, total phenolic compounds and peroxidase activity could be adopted as selective criteria in preliminary stages of selection for tolerance to wheat streak mosaic virus.

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تأثیر آلودگی WSMV بر روی تغییرات بیوشیمیایی دو رقم گندم نان و نسل F<sub>2</sub> آن‌ها

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## چکیده

ویروس موزائیک رگه‌ای گندم باعث خسارت اقتصادی شدید محصول مناطق تولید گندم (*Triticum aestivum* L.) در جهان می‌شود. به منظور مطالعه وراثت تغییرات عوامل بیوشیمیایی در واکنش به آلودگی ناشی از ویروس موزائیک رگه‌ای گندم (WSMV) در نسل F<sub>2</sub> دو رقم گندم مقاوم (کراس عدل) و حساس (مرودشت)، آزمایشی انجام شد. آزمایش فاکتوریل با دو فاکتور، شامل: ژنوتیپ (کراس عدل، مرودشت، F<sub>2</sub>های مقاوم و F<sub>2</sub>های حساس) و مایه‌زنی (مایه‌زنی با WSMV و بدون مایه‌زنی) در دانشگاه شیراز بین سال‌های ۱۳۸۸-۱۳۸۶ انجام گرفت. برگ گیاهچه‌ها در زمان‌های مختلف برای بررسی تغییرات میزان پروتئین، فعالیت آنزیم پراکسیداز و میزان مواد فنلی برداشت شدند. نتایج نشان دادند که آلودگی حاصل از WSMV باعث ایجاد تغییرات بیوشیمیایی در تمام ژنوتیپ‌ها می‌شود. کاهش میزان پروتئین کل در رقم کراس عدل و F<sub>2</sub>های مقاوم مایه‌زنی شده معنی‌دار نبود، اما در رقم مرودشت و F<sub>2</sub>های حساس مایه‌زنی شده باعث کاهش پروتئین شد. در رقم کراس عدل و F<sub>2</sub>های مقاوم مایه‌زنی شده فعالیت آنزیم پراکسیداز افزایش یافت ولی در رقم مرودشت و F<sub>2</sub>های حساس مایه‌زنی شده، فعالیت آنزیم پراکسیداز کاهش یافت. آنزیم پراکسیداز ممکن است در تولید ترکیبات موثر در مقاومت به ویروس موزائیک رگه‌ای گندم نقش داشته باشد. روند افزایش مواد فنلی در ژنوتیپ‌های مقاوم و حساس در شرایط مایه‌زنی نشان داد که بطور کلی در ژنوتیپ‌های مقاوم ساخته شدن و میزان تجمع مواد فنلی بیشتر از ژنوتیپ‌های حساس است. به نظر می‌رسد تغییرات میزان پروتئین و فعالیت آنزیم پراکسیداز و فنل کل در پاسخ به WSMV، به نسل‌های بعد، به ارث می‌رسد و میزان این ترکیبات در یک ژنوتیپ می‌تواند به عنوان معیار انتخاب در مراحل اولیه گزینش برای مقاومت به ویروس در نظر گرفته شود.