

Influence of WSMV Infection on Biochemical Changes in Two Bread Wheat Cultivars and in Their F₂ Populations

S. R. Sahhafi¹, M. T. Assad^{1*}, M. Masumi², H. Razi¹, and A. Alemzadeh¹

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 F₂ 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_{2s} and susceptible F_{2s}), 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_{2s} was not significant whereas it was significant in the inoculated susceptible Marvdasht and its susceptible F_{2s}. Viral infection reduced peroxidase activity in the susceptible Marvdasht cultivar and in its susceptible F_{2s} whereas in Adl-Cross and in its resistant F_{2s} 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 F₂ 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

¹ Department of Agronomy, College of Agriculture, Shiraz University, Shiraz, Islamic Republic of Iran.

* Corresponding author; e-mail: assad@shirazu.ac.ir

² Plant Virology Research Center, College of Agriculture, Shiraz University, Shiraz, Islamic Republic of Iran.



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 F₂ generations, (ii) to see the consistency of biochemical changes in F₂ generation.

MATERIALS AND METHODS

Seeds of Adl-Cross and of Marvdasht were obtained from Agricultural Research Center, Zarghan, Iran. F₁ and F₂ 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_{2s} as well as susceptible F_{2s}); (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 MgCl₂, 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 H_2O_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 Na_2CO_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 | χ^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 F₂ populations.

| | Adl-Cross | | Marvdasht | | F ₂ | | |
|---|--------------|----------|--------------|----------|----------------|------------------------------|--------------------------------|
| | non-infected | Infected | non-infected | Infected | non-infected | Infected | |
| | | | | | | Resistant F _{2s} | Susceptible F _{2s} |
| 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_{2s} 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_{2s} 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_{2s}. 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_{2s}.

Peroxidase activity increased in Adl-Cross and in the resistant F_{2s}, 3.14 and 4.15 percent respectively (Table 2). However, Marvdasht

and susceptible F_{2s} 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 F₂ 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.

REFERENCES

1. Appel, J. A., Bowden, R. L. and Eversmeyer, M. G. 1999. Preliminary 1999 Kansas Wheat Disease Loss Estimates. *Extension Report*, Kansas State University, Manhattan, Kansas.
2. Bertamini, M., Malossini, U., Muthuchelian, K. and Nedunchezian, N. 2005. Physiological Response of Field Grown Grapevine (*Vitis vinifera* L. cv. Marzemino) to Grapevine Leaf roll-Associated Virus (GLRaV-1). *Phytopathologia Mediterranea*, **44**: 256-265.
3. Bi, J. L. and Felton, G. W. 1995. Foliar Oxidative Stress and Insect Herbivory: Primary Compounds, Secondary Metabolites and Reactive Oxygen Species as Components of Induced Resistance. *J. Chem. Ecol.*, **21**: 1511-1530.
4. Bradford, M. M. 1976. A Rapid and Sensitive Method for the Quantification of Microgram Quantities of Protein Utilizing the Principles of Protein-dye Binding. *Analytical Biochem.*, **72**: 248-254.
5. Campbell, M. M. and Ellis, B. E. 1992. Fungal Elicitor-mediated Responses in Pine Cell Cultures. *Planta*, **186**: 409-417.
6. Castro, M. S. and Fontes, W. 2005. Plant Defense and Antimicrobial Peptides. *Protein Peptide Letters*, **12**: 11-16.
7. De Gara, L., de Pinto M. C. and Tommasi, F. 2003. The Antioxidant Systems *via-á-via* Reactive Species during Plant-pathogen Interaction. *Plant Physiol. Biochem.*, **41**: 863-870.
8. Fossdal, C. C., Sharma, P. and Lonneborg, A. 2001. Isolation of the First Putative Plant Peroxidase cDNA from a Conifer and the Local and Systemic Accumulation of Related Proteins upon Pathogen Infection. *Plant Mol. Biol.*, **47**: 423-435.
9. Hassani, F. and Assad, M. T. 2004. Inheritance and Allelism of Wheat Streak Mosaic Virus Resistance in Two Iranian Wheat Lines. *Euphytica*, **140**: 213-216.
10. Hosseini Nezhad, S. M., Assad, M. T. and Masumi, M. 2008. Evaluation of Some Biochemical Factors in Resistance to Wheat Streak Mosaic Virus. *Iran Agr. Res.*, **31**: 39-54.
11. Izadi, M. 2008. Genotype Environment Interaction in Reaction of Bread Wheat to



- Wheat Streak Mosaic Virus. M.Sc. Thesis. Shiraz University, Shiraz, Iran.
12. Kovalvi, S. A. and Nassuth, A. 1995. Influence of *Wheat Streak Mosaic Virus* infection on phenylpropanoid metabolism and the accumulation of phenolics and Lignin in Wheat. *Physiol. and Mol. Plant Pathol.*, **47**: 365-377.
 13. Melo, G. A., Shimizu, M. M. and Mazzafera, P. 2006. Polyphenoloxidase Activity in Coffee Leaves and Its Role in Resistance against the Coffee Leaf Miner and Coffee Leaf Rust. *Phytochem.*, **67**: 277-285.
 14. Masumi, M., Kamran, R., Shiravani, A. and Izadpanah, K. 1999. Reaction of Wheat Genotypes to Wheat Streak Mosaic Virus in Iran. *Iranian J. Plant Pathol.*, **35**: 9-18.
 15. Matern, U. and Kneusal, R. E. 1988. Phenolic Compounds in Plant Disease Resistance. *Phytopath.*, **16**: 153-170.
 16. Malolepsza, U. and Rozalaska, S. 2005. Nitric Oxide and Hydrogen Peroxide in Tomato Resistance: Nitric Oxide Modulates Hydrogen Peroxide Level in O. hydroxyethylrutin-induced Resistance to *Botrytis cinerea* in Tomato. *Plant Physiol. Biochem.*, **43**: 623-635.
 17. Nicholson, R. L. and Hammerschmidt, R. 1992. Phenolic Compound and Their Role in Disease Resistance. *Ann. Rev. Phytopath.*, **30**: 369-389.
 18. Polle, A., Otter, T. and Seifert, F. 1994. Apoplastic Peroxidases and Lignification in Needle of *Picea abies*. *Plant Physiol.*, **106**: 53-60.
 19. Reimers, P. J., Gue, A. and Leach, J. E. 1992. Increased Activity of a Cationic Peroxidase Associated with an Incompatible Interaction between *Xanthomonas oryzae* pv. *oryzae* and Rice (*Oryza sativa*). *Plant Physiol.*, **99**: 1044-1050.
 20. Roby, D., Toppan, A. and Esquerré-Tugayé, M. T. 1985. Cell Surface in Plant-microorganism Interactions: V. Elicitors of Fungal and of Plant Origin Trigger the Synthesis of Ethylene and of Cell Wall Hydroxyproline-rich Glycoproteins in Plants. *Plant Physiol.*, **77**: 700-704.
 21. Seevers, P. M. and Daly, J. M. 1970. Studies on Wheat Stem Rust Resistance Controlled at the *Sr6* Locus: The Role of Phenolic Compounds. *Phytopath.*, **60**: 1322-1328.
 22. Shimizu, N., Hosogi, N., Hyon, G. S., Jiang, S., Inoue, K. and Park, P. 2006. Reactive Oxygen Species (ROS) Generation and ROS Induced Lipid Peroxidation Are Associated with Plasma Membrane Modifications in Host Cells in Response to AK-toxin 1 from *Alternaria alternate* Japanese Pear Pathotype. *J. General Plant Pathol.*, **72**: 6-15.
 23. Yamamoto, H., Hokin, H. and Tani, T. 1978. Peroxidase and Polyphenol Oxidase in Relation to the Crown Rust Resistance of Oat Leaves. *Phytopath.*, **91**: 193-202.
 24. Yassaie, M., Masumi, M., Amin, H. and Izadpanah, K. 2002. Evaluation of *Wheat Streak Mosaic Virus* Response in Commercial and Native Wheat Ancestors. In: *Proceedings of the 15th Iranian Congress of Plant Pathology*, 7-11 September University of Razi, Kermanshah, Iran. PP.32
 25. Young, S. A., Guo, A., Guikema, J. A., White, F. F. and Leach, J. E. 1995. Rice Cationic Peroxidase Accumulates in Xylem Vessels during Incompatible Interactions with *Xanthomonas oryzae* pv. *oryzae*. *Plant Physiol.*, **107**: 1333-1341.
 26. Zinati, Z. 2009. The Effect of Temperature on Biochemical Changes in Inoculation Period in Resistant and Susceptible Bread Wheat Genotypes to *Wheat Streak Mosaic Virus*. M.Sc. Thesis. Shiraz University, Shiraz, Iran.

تأثیر آلودگی WSMV بر روی تغییرات بیوشیمیایی دو رقم گندم نان و نسل F₂ آن‌ها

س. ر. صحافی، م. ت. آساد، م. معصومی، ه. راضی و ع. عالم زاده

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 F₂ دو رقم گندم مقاوم (کراس عدل) و حساس (مرودشت)، آزمایشی انجام شد. آزمایش فاکتوریل با دو فاکتور، شامل: ژنوتیپ (کراس عدل، مرودشت، F₂های مقاوم و F₂های 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 F₂های مقاوم مایه‌زنی شده معنی‌دار نبود، اما در رقم مرودشت و F₂های حساس مایه‌زنی شده باعث کاهش پروتئین شد. در رقم کراس عدل و F₂های مقاوم مایه‌زنی شده فعالیت آنزیم پراکسیداز افزایش یافت ولی در رقم مرودشت و F₂های 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.