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

S. R. Sahhafi, M. T. Assad∗, M. Masumi, H. Razi, and A. Alemzadeh

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 F2s, and susceptible F2s), 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 resistant F2s was not significant whereas it was significant in the inoculated susceptible Marvdasht and its susceptible F2s. Viral infection reduced peroxidase activity in the susceptible Marvdasht cultivar and in its susceptible F2s whereas in Adl-Cross and in its resistant F2s, 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

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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 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 F2s as well as susceptible F2s); (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 assessed by the method described by Poll et al. (1994). Enzyme extracts (50 µl) were mixed with 5
ml extraction buffer (100 mM KH$_2$PO$_4$/K$_2$HPO$_4$ pH 7), containing 20 mM Guaiacol and 10 mM H$_2$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 Na$_2$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$_2$s) was higher as compared with susceptible genotypes (Marvdasht and susceptible F$_2$s) 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$_2$s.

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$_2$s is more than in uninoculated

<table>
<thead>
<tr>
<th>Parents and progeny of WSMV damage rating</th>
<th>Range and mean of WSMV damage rating</th>
<th>Observed</th>
<th>Expected ratio</th>
<th>$\chi^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adl-Cross</td>
<td>0-2 (0.8)</td>
<td>38</td>
<td>Resistant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marvdasht</td>
<td>3-7 (3.8)</td>
<td>0</td>
<td>Susceptible</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F$_2$</td>
<td>0-7 (1.85)</td>
<td>82</td>
<td>3:1</td>
<td>0.35</td>
<td>0.58</td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th></th>
<th>Adl-Cross</th>
<th>Marvdasht</th>
<th>F2</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>non-infected</td>
<td>Infected</td>
<td>non-infected</td>
</tr>
<tr>
<td>Total soluble protein (mg protein/g fresh weight)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 hour</td>
<td>4.3 Ac</td>
<td>4.2 Aa</td>
<td>3.1 Aa</td>
</tr>
<tr>
<td>1 days</td>
<td>4.5 Ac</td>
<td>4.36 AaBc</td>
<td>3.32 Ab</td>
</tr>
<tr>
<td>2 days</td>
<td>5.1 Bb</td>
<td>4.61 Bb</td>
<td>3.94 Bb</td>
</tr>
<tr>
<td>4 days</td>
<td>5.41 Cc</td>
<td>5.3 Cc</td>
<td>4.1 Bbc</td>
</tr>
<tr>
<td>8 days</td>
<td>5.8 Db</td>
<td>5.71 Cc</td>
<td>4.4Cb</td>
</tr>
</tbody>
</table>

Phenolic content (µg phenolics/g fresh weight)

|            |  |  |  |  |  |  |  |  |
| 1 hour    | 2289 Ad     | 2292 Ad    | 2210 Aa    | 2225 AaBb | 2265 Acd   | 2288 Ad   | 2242 Bbc     |  |
| 1 days    | 2310 Bd     | 2315 Bd    | 2221 Aa    | 2240 AaBb | 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, α= 0.05).

Plants (Table 2). Infected leaves of Adl-Cross and of resistant F2s 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 F2s 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 F2s. 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 F2s.

Peroxidase activity increased in Adl-Cross and in the resistant F2s, 3.14 and 4.15 percent respectively (Table 2). However, Marvdasht
and susceptible F$_2$s 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 quinines, 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


تأثیر آلودگی WSMV بر روی تغییرات پیوسته‌سنجی در رقم گندم نان و نسل ۲ آنها

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

چکیده

ویروس موزاکیک رگ‌گاهی گندم باعث خسارت اقتصادی شدید محصول مناطق تولید گندم (Triticum aestivum L.) در جهان می‌شود. به منظور مطالعه و بررسی تغییرات عوامل پیوسته‌سنجی در WSMV، واکنش به آلودگی ناشی از ویروس موزاکیک رگ‌گاهی گندم (WSMV)، کاسه عدل و حساس (مرودشت)، آزمایش انجام شد. آزمایش فاکتوریل با دو فاکتور، شامل: زنوبی WSMV، کاسه عدل و حساس و به‌ونون مایزی (ماهیا)، و F۲ های مقاوم وMais و زن‌بی WSMV، کاسه عدل و حساس و به‌ونون مایزی (ماهیا) F۲ های مقاوم وMais و F۲ های حساس مایزی شده باعث کاهش پروتئین شده در رقم کاسه عدل و حساس F۲ های مقاوم وMais و F۲ های حساس مایزی شده از آن‌زیم پراکسیداز افزایش یافته ولی در رقم مرودشت و F۲ های حساس مایزی شده، فعالیت آن‌زیم پراکسیداز کاهش یافته. آن‌زیم پراکسیداز ممکن است در تولید ترکیبات موثر در مقاومت به ویروس موزاکیک رگ‌گاهی گندم نقش داشته باشد. روند افزایش مواد فلی که در زنوبی WSMV مقاوم و حساس در شرایط مایزی دان داشته که بطور کلی در ZnO های مقاوم ساخته شدند و میزان تجربه مواد فلی بیشتر از ZnO های حساس است. به نظر می‌رسد تغییرات میزان پروتئین و فعالیت آن‌زیم پراکسیداز و فلی کل در پایین به WSMV، به سل نان به، به اثر می‌رود و میزان این ترکیبات در یک زنوبی WSMV می‌تواند به عنوان معیار انتخاب در مراحل اولیه گروهی برای مقاومت به ویروس در نظر گرفته شود.

405