



Activity of defensive enzymes in chilli germplasm in relation to their reaction to chilli thrips, *Scirtothrips dorsalis* Hood

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ABSTRACT: Activity of defensive enzymes in the leaves of seven chilli germplasm collections of which two were resistant and five were moderately resistant to chilli thrips, *Scirtothrips dorsalis* Hood was studied in comparison to susceptible germplasm at Bhubaneswar, Odisha, India. The resistant chilli germplasm viz., BC-7-2-1, BC-25 and moderately resistant chilli germplasm viz., BC-27-2-2, BC-21, BC-79-1, Utkal Abha and BC-406 had 5.84-8.23 $\mu\text{M min}^{-1} \text{g}^{-1}$ peroxidase, 0.1-0.2 $\mu\text{M min}^{-1} \text{g}^{-1}$ poly phenol oxidase and 7.39-14.16 $\mu\text{M min}^{-1} \text{g}^{-1}$ catalase in the leaf sample respectively, as against 1.42-4.42 $\mu\text{M min}^{-1} \text{g}^{-1}$, 0.03-0.08 $\mu\text{M min}^{-1} \text{g}^{-1}$ and 3.18-6.36 $\mu\text{M min}^{-1} \text{g}^{-1}$ protein in the leaves of susceptible chilli germplasm (LCA-620, BC-78-1-2 and BC-24-1) and highly susceptible check chilli germplasm (Byadagi kaddi), respectively. A significantly inverse relation existed between the activity of defensive enzymes viz., peroxidase (-0.984**), polyphenol oxidase (-0.965**), catalase (-0.965**) and the incidence of *S. dorsalis*. The multiple linear regression analysis revealed that all these defensive enzymes together influenced the population of *S. dorsalis* to an extent of 97.81 per cent.

INTRODUCTION

Chilli, *Capsicum annum* L. (Family: Solanaceae) is the most common and extensively cultivated spice cum vegetable crop in the tropics and subtropics. In addition to its food value, chili also have important role in the pharmaceutical or medical field, particularly because of the capsaicinoid content in fruit which has been used for the treatment of pain and inflammation associated with various diseases such as rheumatoid arthritis, diabetic neuropathy, postmasectomy syndrome pain, cluster headaches, herpes zoster, and others (Lim, 2013; Srivastava, 2013). Nutritionally, chilli fruits are the rich source of vitamin- A, B, C and E. Capsaicin an alkaloid responsible for pungency in chillies has medicinal properties and it prevent heart attack by dilating the blood vessels (Gill, 1988) and anticancer properties (USDA, 2016).

In spite of concerted efforts at various levels, the productivity of chilli in India is stagnant over several years although, the crop has got great export potential besides huge domestic requirement. The attack of an array of insect pests to the crop right from the nursery stage till harvesting is considered as one of the major biotic constraints especially in tropical and sub-tropical countries due to conducive climate. Among several insect pests, chilli thrips or yellow tea thrips, *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae), a highly

polyphagous pest native to either Southeast Asia (Seal *et al.*, 2006) or in the Indian sub-continent (Kumar *et al.*, 2013) poses a considerable threat to production of chilli in southern and eastern Asia, Africa, and Oceania (Butani, 1976, Ananthakrishnan, 1993 and Kumar *et al.*, 2011).

The biochemical constituents of any crop or variety exert a definite influence over the pest species either by luring or deterring or by supporting or inhibiting growth and development through synergism or antibiosis (Ananthakrishnan, 1996). Biochemical parameters such as sugars, phenols, tannins, proteins, amino acids and several secondary plant metabolites imparting either a resistance or susceptible reaction in the host has been reported in many crops. Thus, better understanding of the biochemical basis of plant defence mechanisms in chilli resistant germplasm is highly imperative. One of the prominent plant responses to insect herbivore attack is the induction and accumulation of oxidative enzymes viz., catalase, peroxidase, phenylalanine ammonia-lyase and polyphenol oxidase which are the important biochemical markers in pest resistant plants (Green and Ryan, 1972; War *et al.*, 2012 and Sha *et al.*, 2015). Comparison of enzymatic responses of resistant vs. susceptible chilli germplasm with a purpose to decipher mechanisms that will facilitate the breeding programme formed the major aspect of the present investigation.

MATERIALS AND METHODS

Studies on enzymatic activities in resistant and susceptible chilli germplasm to *S.dorsalis* were carried out in the Department of Entomology, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha during 2019-20.

Incidence of *S. dorsalis* in selected germplasm:

Twelve selected chilli germplasm viz., BC-25, BC-79-1, BC-27-2-2, Utkal Abha, BC-21, BC-406, BC-28, LCA-620, BC-78-1-2, BC-24-1 along with resistant check BC-7-2-1 and susceptible check Byadagi kaddi were raised under insect free conditions in pot tray and transplanted at six weeks after germination. Three plants per genotype were planted in 10 x 12 inches poly bag with three replications in a randomized block design. Plants were spaced 60 cm between rows and 45 cm between plants in a row during summer 2019-20. Observations on population of nymphs and adults of *S. dorsalis* were recorded on three leaves of chilli at top, middle and bottom canopy from three plants at 14DAT, 21 DAT, 28 DAT, 35 DAT, 42 DAT, 56 DAT, 63 DAT, 70 DAT, 77 DAT, 84 DAT, 91 DAT and 98 DAT (days after transplanting). The population was counted visually by using a magnifying lens in early morning hours (Bhede *et al.*, 2008).

Enzyme activity assay in selected chilli germplasm to *S. dorsalis*

Preparation of enzymatic extract: Enzymatic activity of peroxidase, polyphenol oxidase and catalase of twelve selected germplasm was assessed during 2019-20 in order to ascertain the bases of resistance. The standard laboratory procedures adopted are briefly described in the following paragraphs. In this study for preparing the enzyme extract, leaf samples were collected from pot culture experiment at 60 DAT. Enzyme extract for peroxidase, polyphenol oxidase and catalase was prepared by the weighed amount of 2g of sample homogenized at 0-4^o by using pre chilled mortar and pestle with 10ml extraction buffer (0.1 M phosphate buffer pH 7.0) containing 1mM ascorbic acid and 0.5% polyvinyl pyrrolidone. The homogenate was filtrated through three layers of cheese cloth and filtrate was centrifuged at 10000 rpm for 20 minutes. The supernatant was used for enzymatic assay (Malick and Singh, 1980).

Peroxidase: Activity of peroxidase was assessed by following the procedure of Castillo *et al.*, (1984).

Polyphenol oxidase: Activity of polyphenol oxidase was assessed as per the procedure suggested by Augustin *et al.* (1985).

Catalase : Activity of catalase was assessed following the procedure of Barber (1980).

RESULTS AND DISCUSSION

Population of *S. dorsalis* in selected chilli

germplasm: The results of analysis of mean pool data of 14DAT, 21 DAT, 28 DAT, 35 DAT, 42 DAT, 56 DAT, 63 DAT, 70 DAT, 77 DAT, 84 DAT, 91 DAT and 98 DAT on population of *S. dorsalis* in different chilli germplasm revealed significantly lowest mean population of *S. dorsalis* in the resistant germplasm BC-7-2-1(resistant check) (0.70) which was at par with other resistant germplasm BC-25 (0.72). Lower population of *S. dorsalis* ranging from 1.18 to 1.31 per leaf was observed in the five moderately resistant germplasm viz., BC-27-2-2 (1.18), BC-21 (1.22), BC-79-1 (1.25), Utkal Abha (1.29) and BC-406 (1.31). The susceptible check Byadagi kaddi recorded the highest population of *S. dorsalis* (2.46/leaf) which was at par with the other susceptible germplasm BC-24-1 (2.38/leaf) (Table 1).

Enzyme activity in resistant and susceptible chilli germplasm

Peroxidase (POD): The activity of peroxidase was highest in the resistant check germplasm BC-7-2-1 (8.23 $\mu\text{M min}^{-1} \text{g}^{-1}$ protein) which was closely followed by the other resistant germplasm BC-25 (7.19 $\mu\text{M min}^{-1} \text{g}^{-1}$ protein) (Table 1). The moderately resistant germplasm viz., BC-27-2-2, BC-21, BC-79-1, Utkal Abha and BC-406 showed higher peroxidase activity values between 5.84 $\mu\text{M min}^{-1} \text{g}^{-1}$ protein (BC-406) and 6.82 $\mu\text{M min}^{-1} \text{g}^{-1}$ protein (BC-27-2-2). The activity of peroxidase was lowest in the susceptible check germplasm Byadagi kaddi (1.42 $\mu\text{M min}^{-1} \text{g}^{-1}$ protein) which was closely followed by the highly susceptible germplasm BC-24-1 (2.27 $\mu\text{M min}^{-1} \text{g}^{-1}$ protein). In other susceptible germplasm the activity of peroxidase ranged between 3.32 $\mu\text{M min}^{-1} \text{g}^{-1}$ protein (BC-78-1-2) to 4.42 $\mu\text{M min}^{-1} \text{g}^{-1}$ protein (BC-28).

Peroxidases are the glycoproteins with ubiquitous distribution in the plant kingdom. These enzymes are involved in various physiological functions viz., lignification, suberization, phenol oxidation, wound healing, protection against insect attack and regulation of cell elongation (Bruce and West, 1989). Its activity is known to increase with herbivore damage in many crop plants (Chaman *et al.*, 2001 and Allison and Schultz, 2004). Enhanced peroxidase activity allows the plant to detoxicate the peroxides which reduce the tissue damage (Hildebrand *et al.*, 1986). Information on relationship of peroxidase activity with the incidence of *S. dorsalis* in chilli is rather scarce in published literature, except

Table 1. Estimation of enzymes in leaves of selected chilli germplasm along with the incidence of *S. dorsalis* during 2019-20

| Germplasm | Mean population of <i>S. dorsalis</i> (Nos./leaf) | Peroxidase ($\mu\text{M min}^{-1} \text{g}^{-1}$ protein) | Poly phenol oxidase ($\mu\text{M min}^{-1} \text{g}^{-1}$ protein) | Catalase ($\mu\text{M min}^{-1} \text{g}^{-1}$ protein) | Category |
|-------------------------------|---|--|---|--|----------|
| BC-25 | 0.72 (0.85) | 7.19 | 0.19 | 13.51 | R |
| BC-27-2-2 | 1.18 (1.09) | 6.82 | 0.16 | 11.55 | MR |
| BC-21 | 1.22 (1.11) | 6.39 | 0.15 | 10.24 | MR |
| BC-79-1 | 1.25 (1.12) | 6.25 | 0.13 | 9.74 | MR |
| Utkal Abha | 1.29 (1.14) | 6.18 | 0.11 | 8.62 | MR |
| BC-406 | 1.31 (1.15) | 5.84 | 0.10 | 7.39 | MR |
| BC-28 | 1.86 (1.36) | 4.42 | 0.08 | 6.36 | S |
| LCA-620 | 1.90 (1.38) | 3.83 | 0.06 | 5.53 | S |
| BC-78-1-2 | 1.93 (1.39) | 3.32 | 0.07 | 4.4 | S |
| BC-24-1 | 2.38 (1.54) | 2.27 | 0.04 | 3.33 | HS |
| BC-7-2-1(RC) | 0.70 (0.84) | 8.23 | 0.20 | 14.16 | R |
| Byadagi kaddi (SC) | 2.46 (1.57) | 1.42 | 0.03 | 3.18 | HS |
| SE(m) \pm | 0.102 | 0.058 | 0.005 | 0.101 | |
| CD (5%) | 0.30 | 0.17 | 0.02 | 0.29 | |

Table 2. Correlation coefficient (r) of incidence of *S. dorsalis* with enzyme activity of chilli germplasm

| Incidence of <i>S. dorsalis</i> | Activity of enzymes | | |
|---|--|---|--|
| | Peroxidase ($\mu\text{M min}^{-1} \text{g}^{-1}$ protein) | Poly phenol oxidase ($\mu\text{M min}^{-1} \text{g}^{-1}$ protein) | Catalase ($\mu\text{M min}^{-1} \text{g}^{-1}$ protein) |
| Population of <i>S. dorsalis</i> (No./leaf) | -0.984** | -0.965** | -0.965** |

** Correlation is significant at the 0.01 level * Correlation is significant at the 0.05 level

Table 3. Multiple linear regression equations depicting the influence of enzyme activity on incidence of *S. dorsalis* in chilli germplasm

| Incidence of <i>S. dorsalis</i> | Regression Models | Coefficient of determination (R^2) |
|---|---|--|
| Population of <i>S. dorsalis</i> (No./leaf) | $Y1 = 8.6710 - 0.0709 * X1 - 1.0071 * X2 - 0.0179 * X3$ | 97.81 |

Where, Y1 = Population of *S. dorsalis*, X1=Peroxidase, X2=Poly phenol oxidase, X3=Catalase

the report of Meena *et al.* (2008) who found a higher intensity of peroxidase activity in diseased (leaf curl virus) chilli leaf as compared to that of healthy leaf. Increased peroxidase activity might be due to increased phenol concentration which acts as a cofactor of peroxidase, thus influenced the resistance in chilli. The present results get ample support from the findings of earlier researchers *viz.*, Dowd and Lagrimini (2006) and Gulsen *et al.* (2010) who reported the higher activity of peroxidase an important defensive enzyme in plants implicating a broad range resistance mechanism to various insect pests.

Poly phenol oxidase (PPO): The activity of poly phenol oxidase in the leaves of the selected chilli germplasm varied from 0.03 to 0.20 $\mu\text{M min}^{-1} \text{g}^{-1}$ protein, the lowest activity being in susceptible check germplasm Byadagi kaddi and highest in the resistant check germplasm BC-7-2-1 (Table 1). Higher poly phenol oxidase activity values (0.10 $\mu\text{M min}^{-1} \text{g}^{-1}$ protein to 0.20 $\mu\text{M min}^{-1} \text{g}^{-1}$ protein) was registered in the resistant and moderately resistant germplasm *viz.*, BC-7-2-1, BC-25, BC-27-2-2, BC-21, BC-79-1, Utkal Abha and BC-406. The susceptible and highly susceptible germplasm exhibited lower poly phenol oxidase values between 0.03 $\mu\text{M min}^{-1} \text{g}^{-1}$ protein (Byadagi kaddi) (susceptible check) to 0.08 $\mu\text{M min}^{-1} \text{g}^{-1}$ protein (BC-28).

Poly phenol oxidases are the heme-containing monomeric glycoproteins located in the chloroplasts which are involved in the plant defence system (Saiedian *et al.*, 2007). This enzyme was responsible for phenol buildup because it oxidised O-dihydroxy phenol by which it lowers the availability of proteins, control feeding and growth of pests (Meena *et al.*, 2008; Zhang *et al.*, 2008 and He *et al.*, 2011). The activity of polyphenol oxidase as an important anti-herbivore factors was significantly increased resulting in a substantial decrease in the abundant herbivores, including insects (Thaler *et al.*, 2001). The present findings on activity of polyphenol oxidase in the leaves of selected chilli germplasm are in accordance to the report of Mondal *et al.* (2013) who found that the resistant chilli genotype CUCH-4 recorded the greatest oxidase enzyme activity than the sensitive genotype CUCH-23. A positive correlation between host plant resistance and the amount of phenols and increased activity of peroxidase and polyphenoloxidase has earlier been recorded in chilli (Jabeen *et al.*, 2009 and Chandan *et al.*, 2016).

Catalase (CAT): The activity of catalase in the leaves of twelve test chilli germplasm ranged from 3.18 $\mu\text{M min}^{-1} \text{g}^{-1}$ protein to 14.16 $\mu\text{M min}^{-1} \text{g}^{-1}$ protein (Table 1). The resistant check germplasm BC-7-2-1 had the highest

catalase activity of 14.16 $\mu\text{M min}^{-1} \text{g}^{-1}$ protein which was closely followed by the other resistant germplasm BC-25 (13.51 $\mu\text{M min}^{-1} \text{g}^{-1}$ protein). The moderately resistant germplasm *viz.*, BC-27-2-2, BC-21, BC-79-1, Utkal Abha and BC-406 had comparatively higher catalase activity value of 11.55, 10.24, 9.74, 8.62 and 7.39 $\mu\text{M min}^{-1} \text{g}^{-1}$ protein respectively, than susceptible and highly susceptible germplasm where activity of catalase ranged between 3.18 $\mu\text{M min}^{-1} \text{g}^{-1}$ protein to 6.36 $\mu\text{M min}^{-1} \text{g}^{-1}$ protein. Lowest activity of catalase was recorded in the susceptible check Byadagi kaddi (3.18 $\mu\text{M min}^{-1} \text{g}^{-1}$ protein) followed by highly susceptible germplasm BC-24-1 (6.36 $\mu\text{M min}^{-1} \text{g}^{-1}$ protein).

Catalase, a major H_2O_2 -scavenging anti oxidant enzyme is involved in the cell wall resistance of plants and it also acts as a signal for the induction of defence genes (Chen *et al.*, 1993). Involvement of catalase activity in the plant defence against the sucking insects has been reported by Hanaka *et al.* (2018). Information on relationship of catalase activity with the incidence of *S. dorsalis* in chilli could not be traced out in literature. However, the fluctuation in catalase activity due to insect feeding has been documented in other crops (Heng-Moss *et al.*, 2004 and Khattab, 2007). Dillwith *et al.* (1991) reported higher activity of catalase in the alfa alfa plants resistant to spotted alfa alfa aphid than the susceptible plants. Black gram genotypes resistant to white fly, *Bemisia tabaci* (Gennadius) exhibited high activities of peroxidase and catalase (Taggar *et al.*, 2012). The results of the present studies are consistent with the findings of those previous workers.

Relationship of enzyme activity of chilli germplasm with incidence of *S. dorsalis*

Results on correlation studies between population of *S. dorsalis* and various biochemical parameters of chilli germplasm revealed that the population of *S. dorsalis* showed significant negative correlation with activity of enzymes *viz.*, peroxidase (-0.984**), polyphenol oxidase (-0.965**), catalase (-0.965**) and the population of *S. dorsalis* (Table 2). The multiple linear regression analysis indicated that various defensive enzymes of chilli germplasm *viz.*, peroxidase ($X_1=0.0709$), polyphenol oxidase ($X_2=1.0071$) and catalase ($X_3=0.0179$) together influenced the population of *S. dorsalis* to an extent of 97.81 per cent (Table 3).

CONCLUSION

The results of the study on induced mechanism of defense in chilli revealed that the resistant and moderately resistant germplasm exhibited higher activity of defense related enzymes *viz.*, peroxidase, polyphenol oxidase and

catalase in the leaf sample. The resistant germplasm *viz.*, BC-7-2-1, BC-25 and moderately resistant germplasm *viz.*, BC-27-2-2, BC-21, BC-79-1, Utkal Abha and BC-406 had 5.84-8.23 $\mu\text{M min}^{-1} \text{g}^{-1}$ peroxidase, 0.1-0.2 $\mu\text{M min}^{-1} \text{g}^{-1}$ poly phenol oxidase and 7.39-14.16 $\mu\text{M min}^{-1} \text{g}^{-1}$ catalase in the leaf sample respectively, as against 1.42-4.42 $\mu\text{M min}^{-1} \text{g}^{-1}$, 0.03-0.08 $\mu\text{M min}^{-1} \text{g}^{-1}$ and 3.18-6.36 $\mu\text{M min}^{-1} \text{g}^{-1}$ protein in the leaves of susceptible and highly susceptible check germplasm, respectively. However, the defense mechanisms by which these enzymes are accumulated in chilli remain to be explored in future studies.

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