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Catalase, peroxidase and polyphenol oxidase activity of okra germplasm for resistant against jassids, *Amrasca biguttula biguttula* (Ishida)

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Abstract
Plants use a variety of Physio-chemical mechanisms to defend themselves from biotic stresses induced by insect pests. An investigation was undertaken to evaluate the response of oxidative enzymes, *viz.*, catalase, peroxidase and polyphenol oxidase in okra germplasm for resistance/susceptibility to leafhopper, *Amrasca biguttula biguttula* (Ishida) at All India Coordinated Research Project on Vegetable Crops, Odisha University of Agriculture and Technology and Central Horticultural Experiment Station, Bhubaneswar during *kharif*, 2018, summer, 2019 and *kharif*, 2019. Oxidative enzyme activity was estimated spectrophotometrically from leaf samples collected at peak jassid infestation period (49 – 63 days after sowing). The results indicated that the resistance germplasm BBSR-37, Pusa A-4, BBSR-36, BBSR- 57, BBSR-47 and BBSR-3 recorded higher enzymatic activities as compared to susceptible germplasm (Pusa Sawani and BBSR-53). The germplasm BBSR-4 and BBSR-09-6 were observed with moderate level of enzymatic activity. The results revealed that the enhanced activities of the enzymes may contribute to bio-protection of okra genotypes against leafhopper infestation.

Keywords: Catalase, leafhopper, okra, peroxidase, polyphenol oxidase, resistant

Introduction
Okra [*Abelmoschus esculentus* (L.) Moench], a member of the Malvaceae family, is a popular vegetable crop grown in tropical and sub-tropical regions of the world. It is good source of vitamin A, B, and C, as well as protein, carbohydrates, fats, iron, and iodine, and plays a vital role in human nutrition (Halder *et al*., 2016) [10].

From early stages to maturity, the okra crop is susceptible to insect pests; about 72 species of insects have been reported on okra (Srinivasa and Rajendran, 2003) [27]. The sucking pest complex of okra comprised of leafhopper (*Amrasca biguttula biguttula* Ishida), aphid (*Aphis gossypii* Glover), whitefly (*Bemisia tabaci* Gennadius) and mite (*Tetranychus cinnabarinus* Boisduval). The borer complex comprised of shoot and fruit borers *viz.*, *Earias vittella* (Fabricius), *Earias insulana* (Boisdual) and *Helicoverpa armigera* (Hubner) are known to cause severe damage to the crop (Rao and Rajendran, 2003) [21]. Several insect pests attack okra, but the jassid, *Amrasca biguttula biguttula* (Ishida) (Hemiptera: Cicadellidae), is one of the most dangerous, severely limiting its cultivation. (Dhandapani *et al*. 2003; Singh and Joshi 2004; Sandhi *et al*., 2017) [6, 23, 26].

The nymphs and adults suck the plant sap from the lower surface of the leaves, causing yellowing, browning, bronzing, cupping, withering, necrosis and premature leaf shedding. Hopper burn is a term for the phytotoxic damage induced by their infestation (Bindra and Mahal, 1981; Mahal *et al*., 1993; Mahal *et al*., 1994) [3, 16, 17]. Jassids have been responsible for reductions in okra yields ranging from 50.00 – 52.00% (Rawat and Sahu, 1973) [22], 40.00 – 56.00% (Krishnaiah, 1980) [13], 40.00 – 60.00% (Narke and Suryawanshi, 1987) [18] and 32.06 – 40.84% (Singh and Brar, 1994) [25]. The attack of leafhoppers has reduced the height and number of leaves by 49.80 and 45.10 percent, respectively (Rawat and Sahu, 1973) [22].

A successful pest management strategy is essential in order to overcome these pest problems. Host plant resistance (HPR) is a long-term cost effective and safe strategy for pest management, which is environmentally sustainable. Plants with a variety of biophysical and biochemical characteristics have resistance to a variety of insect pests. (Halder *et al*., 2006, Halder and Srinivasan, 2011) [11, 12]. Plants possess a large number of substances that serve primarily as defence mechanisms against natural enemies. (Devi *et al*., 2018) [5].

In integrated pest management, varietal resistance is a vital tool. A resistant variety aids in insect species suppression thus causing the least amount of disruption to the crop habitat, as well as reducing the need for toxic pesticides that pollute the environment (Sandhi *et al.*, 2017) [23]. A resistant variety can provide a base on which to formulate an integrated management system and may be most fruitful when used in combination with other methods (Sharma *et al.*, 2002; Kumar and Singh *et al*, 2002) [15, 24]. Currently, the information on biochemical mechanisms for resistance in okra in response to leafhopper is scarce. The aim of this study was to evaluate the enzymatic responses of different okra genotypes to identify whether they were resistant or susceptible to leafhoppers.

Materials and Methods

A total of fifty okra germplasm were screened under field conditions for resistance/susceptibility against jassids, *Amrasca biguttula biguttula* (Ishida) during *kharif*, 2018 and summer, 2019 at All India Coordinated Research Project on Vegetable Crops, Odisha University of Agriculture and Technology, Bhubaneswar. Based on two season data of jassid population and jassid injury index, the okra germplasm were categorized into resistance, moderately resistance, susceptible and highly susceptible groups. The field results of okra genotypes were further validated by studying their enzymatic activity.

The pot culture experiment was conducted at Central Horticultural Experiment Station (ICAR-IIHR), Bhubaneswar during *kharif* of 2019. The experiment was conducted in randomized block design with ten treatments and three replications. The treatments comprised of ten okra germplasm containing five resistant (BBSR-37, BBSR-36, BBSR-57, BBSR-47 and BBSR-3), a moderately resistant (BBSR-4), a moderately susceptible (BBSR-09-6) and a susceptible (BBSR-53) genotype, selected based on two year field trial (*Kharif*, 2018 and summer, 2019) with a resistant and a susceptible check (Pusa A-4 and Pusa Sawani, respectively).

Sowing was done on last week of September during *kharif* of 2019. Two seeds per polythene bag were sown and the bags were labelled properly. The crop was kept free from any insecticide application. The number of jassid nymphs and adults was counted in the top, middle, and bottom canopy of each tagged plant. The total number of jassids found on the adaxial and abaxial surfaces of the leaf was noted down. The observations were made at weekly intervals during the crop season in the early morning hours. The activity of oxidative enzymes catalase, peroxidase, and polyphenol oxidase were determined using standard procedures during the peak jassid infestation period (49–63 DAS).

Sample extraction for enzyme assays

The sample extraction for enzyme assay was done according to the method outlined by Alici and Arabaci (2016) [2]. The okra leaf samples were freshly collected from pot culture experiment and stored at -20° C. The samples were washed twice with distilled water. Ten gram of the leaf sample was cut quickly into thin slices. The leaf slices were homogenized with pre-chilled mortar and pestle in ice cold condition by adding 50 ml of 100 mM sodium phosphate buffer (pH 7.0) containing 1 mM ascorbic acid with 0.5% (w/v) polyvinylpyrrolidone for 5 minutes at 4 °C. The homogenate was filtered through three layers of cheesecloth. The filtrate was centrifuged at 5, 000 rpm for 15 minutes. The supernatant was collected and it was used for enzyme assay of catalase, peroxidase and polyphenol oxidase.

Estimation of catalase activity

The catalase (CAT) activity in okra leaf sample was estimated as per the procedure described by Aebi (1984) [1]; Alici and Arabaci (2016) [2]. The 3 ml reaction mixture was prepared in a test tube by dissolving 100 mM potassium phosphate buffer having pH 7.0 (1.9 ml), 30 mM H_2O_2 (1.0 ml) and 100 µl of okra sample extract. Just prior to the spectrophotometric readings, the sample extract was added to the reaction mixture. A test tube containing 100 mM potassium phosphate buffer with pH 7.0 (2 ml) and 30 mM H_2O_2 (1.0 ml) without okra sample extract was served as blank. The sample was added to the reaction mixture and mixed thoroughly, read the absorbance at 240 nm immediately at 0, 30, 60, 90, 120, 150 and 180 seconds. The catalase activity was determined spectrophotometrically at room temperature by monitoring the decrease in absorbance resulting from the decomposition of H_2O_2 at 240 nm. The enzyme activity was expressed in µM H_2O_2 (ε = 39.4 mM^{-1} cm^{-1}) oxidized min^{-1} mg^{-1} protein.

Estimation of peroxidase activity

The peroxidase (POD) activity of okra leaf sample was estimated according the procedure followed by Yadav *et al.* (2017) [29] using guaiacol as a substrate. The 3 ml reaction mixture was prepared by dissolving 100 mM potassium phosphate buffer with pH 7.0 (1.9 ml), 5 mM guaiacol (0.5 ml), 5 mM H_2O_2 (0.5 ml) and 100 µl of okra sample extract. A blank was prepared by mixing 100 mM potassium phosphate buffer having pH 7.0 (2 ml), 5 mM guaiacol (0.5 ml), 5 mM H_2O_2 (0.5 ml) without sample extract. The sample was added to the reaction mixture just prior take the observations. The reaction mixture was taken in cuvette, placed in the spectrometer at 470 nm and read the absorbance at 0, 30, 60, 90, 120, 150 and 180 seconds. The increase in the absorption caused by oxidation of guaiacol by H_2O_2 (ε = 26.6 mM^{-1} cm^{-1}) was measured at 470 nm spectrophotometrically.

Estimation of polyphenol oxidase activity

The polyphenol oxidase (PPO) activity was assayed as per the method adopted by Cosetang and Lee (1978) [4]; Oktay *et al.* (1995) [19]. The 3 ml reaction mixture contained 100 mM potassium phosphate buffer having pH 7.0 (2 ml), 5 mM catechol (0.5 ml) and 500 µl of okra sample extract (0.5 ml) in a test tube. A blank was prepared in a test tube by adding 100 mM potassium phosphate buffer with pH 7.0 (2.5 ml), 5 mM catechol (0.5 ml) without sample extract. The sample was added to reaction mixture just prior to the spectrophotometric readings and mixed thoroughly in a test tube. The reaction mixture was added to the cuvette using a micropipette and the spectrophotometric readings were recorded at 0, 30, 60, 90, 120, 150 and 180 seconds at 420 nm. The polyphenol oxidase activity was determined by measuring the increase in absorbance resulting from the oxidation of catechol (ε = 34.5 mM^{-1} cm^{-1}) at 420 nm spectrophotometrically.

The enzyme activity of catalase, peroxidase and polyphenol oxidase was calculated by using the following equation.

$$\text{Enzyme activity (µM/min/mg protein)} = \frac{\Delta \text{ O.D.}}{\text{Enzyme conc. (g) x mg/g Protein x } \varepsilon} \text{ x1000}$$

Where, Enzyme concentration (g) = Amount of enzyme in 3 ml reaction mixture, ε = Extinction coefficient

The data obtained on various oxidative enzymes of okra germplasm were analyzed by randomized block design procedure using OPSTAT software. F test was conducted to test the significance of variations in the treatments. The standard error mean [SE (m) ±] and critical difference (CD) at 5% level of significance were also calculated following the procedure given by Gomez and Gomez (1984) [8].

Result and Discussion

The data pertaining to catalase, peroxidase and polyphenol oxidase activity of okra germplasm against jassids during *kharif*, 2019 was depicted in Table 1.

Catalase activity

The catalase activity of selected okra germplasm during *kharif*, 2019 was ranged from 0.49 to 2.51 µM/min/g proteins. The data showed that the minimum catalase activity was observed on okra germplasm BBSR-53 (0.49 µM/min/g protein) which was *at par* with Pusa Sawani (0.53 µM/min/g protein), differed significantly with BBSR-09-6 (0.77 µM/min/g protein) and BBSR-4 (0.99 µM/min/g protein). The maximum catalase activity was observed on okra germplasm BBSR-37 (2.51 µM/min/g protein), which differed significantly with Pusa A-4 (2.22 µM/min/g protein) followed by BBSR-36 (2.10 µM/min/g protein). The moderate catalase activity was observed on okra germplasm BBSR-3, BBSR-47 and BBSR-57 with catalase activity of 1.52, 1.71 and 1.89 µM/min/g protein, respectively. These results are in agreement with the findings of Taggar *et al.* (2012) [28], who reported that the resistant genotypes recorded higher catalase activities at insect stress conditions as compared with non-stressed plants.

Table 1: Enzymatic activity of okra germplasm against jassids

Treatments	Germplasm	Catalase (µM/min/g protein)	Peroxidase (µM/min/g protein)	Polyphenol oxidase (µM/min/g protein)
T1	BBSR-37	2.51	5.82	2.95
T2	BBSR-36	2.10	4.90	2.60
T3	BBSR-57	1.89	3.97	2.30
T4	BBSR-47	1.71	3.61	1.82
T5	BBSR-3	1.52	3.43	1.87
T6	BBSR-4	0.99	2.29	1.21
T7	BBSR-09-6	0.77	1.69	0.71
T8	BBSR-53	0.49	0.92	0.36
T9	PUSA-A4 R	2.22	4.96	2.70
T10	PUSA SAWANI S	0.53	1.08	0.34
SE (m) ±		0.036	0.022	0.083
CD (5%)		0.11	0.06	0.25

R-Resistant check, S-Susceptible check

Peroxidase activity

The peroxidase activity of selected okra germplasm during *kharif*, 2019 was ranged between 0.92 and 5.82 µM/min/g proteins. The data revealed that the minimum peroxidase activity was observed on okra germplasm BBSR-53 (0.92 µM/min/g protein), which was *at par* with Pusa Sawani (1.08 µM/min/g protein), differed significantly with BBSR-09-6 (1.69 µM/min/g protein), followed by BBSR-4 (2.29 µM/min/g protein). The moderate level of peroxidase activity was observed on okra germplasm BBSR-3, BBSR-47 and BBSR-57 with peroxidase activity of 3.43, 3.61 and 3.97 µM/min/g protein, respectively. The maximum peroxidase activity was recorded on okra germplasm BBSR-37 (5.82 µM/min/g protein), which differed significantly with Pusa A-4 (4.96 µM/min/g protein), followed by BBSR-36 (4.90 µM/min/g protein). The peroxidase activity was higher in resistant genotypes as compared to susceptible genotypes (Kadu, 2018; Gurve, 2016; Dowd and Lagrimini, 2006; Taggar *et al.*, 2012) [7, 9, 12, 28].

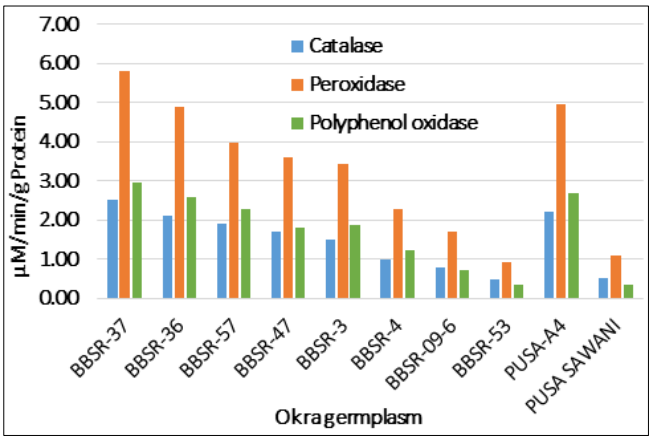

Fig 1: Enzymatic activity of okra germplasm

Polyphenol oxidase activity

The polyphenol oxidase activity of selected okra germplasm during *kharif*, 2019 was ranged from 0.34 to 2.95 µM/min/g proteins. The minimum polyphenol oxidase activity was observed on okra germplasm Pusa Sawani with enzyme activity of 0.34 µM/min/g protein, which was *at par* with BBSR-53 (0.0.36 µM/min/g protein) and differed significantly with BBSR-09-6 (0.71 µM/min/g protein) and BBSR-4 (1.21 µM/min/g protein). The maximum polyphenol oxidase activity was recorded on okra germplasm BBSR-37 (2.95 µM/min/g protein), which differed significantly with Pusa A-4 (2.70 µM/min/g protein) and BBSR-36 (2.60 µM/min/g protein). The moderate polyphenol oxidase activity was observed on okra germplasm BBSR-47, BBSR-3 and BBSR-57 with enzyme activity of 1.82, 1.87 and 2.30 µM/min/g protein, respectively. The polyphenol oxidase activity was higher in resistant genotypes as compared to susceptible genotypes (Gurve, 2016; Ranmalbhai, 2014) [9, 20].

Conclusion

The study concluded that resistant germplasm observed highest catalase, peroxidase and polyphenol oxidase activities as compared to susceptible germplasm. The okra germplasm BBSR-37, BBSR-36, Pusa A-4, BBSR-57 and BBSR-3 were found to be resistant to leafhopper, with BBSR-4 being moderately resistant, BBSR-09-6 susceptible besides Pusa Sawani and BBSR-53 being highly susceptible.

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Author's contribution

Yogesh Kumar H D: Data collection, analysis, interpretation and manuscript preparation; Jayaraj Padhi: Proper guidance, drafting the manuscript and article correction; Meenu Kumari: Providing laboratory facilities; Gouri Shankar Sahu:

Providing experimental field and okra germplasm; Ladu Kishore Rath: Critical revision of the article.

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