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Short Communication

RESPONSE OF TOTAL PHENOLS AND PEROXIDASE ACTIVITY IN CHILLI EXPOSED TO PEPPER LEAF CURL VIRUS DISEASE

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Chilli (*Capsicum annuum* L.) is cultivated for variety of purposes. Pepper leaf curl disease is one of the major production constraints in tropical and sub-tropical areas of the world. The disease causes up to 100 per cent yield reduction. Once plants infected with pepper leaf curl virus (PepLCV), can not be recovered by chemical control or any other management practices. Hence, development and use of resistant varieties is the best way to keep the disease under control. Understanding plant defence mechanisms is necessary to develop approaches to overcome the damages caused by the diseases. Before initiating resistance breeding programme, thorough knowledge on the biochemical basis of resistance to (PepLCV) would help in formulating effective breeding programme. The present study reports difference in biochemical factors and enzyme activities among two pepper genotypes (Kashi Anmol and BS-35) against PepLCV disease.

Plant phenolics are secondary metabolites that constitute one of the most common and widespread groups of substances in plants. Phenolics biogenetically arise from the shikimate-phenylpropanoids-flavonoids pathways (Lattanzio *et al*., 2006). Plants need phenolic compounds for pigmentation, growth, resistance to pathogens and for many other functions. Therefore, they represent adaptive characters that have been subjected to natural selection during evolution. Plants evolved chemical defense mechanisms against their predators unlike animals because they cannot rely on physical mobility to escape their predators. Phenolic compounds have long been correlated with the resistance of plants to infective agents (Link and Walker, 1933). Keeping in view of above facts, the present work plan was formulated to elucidate how pepper leaf curl disease influence the level of total phenol together with the change in peroxidase activity in chilli genotypes.

Two genotypes of *Capsicum* species, one resistant to PepLCV *viz*., BS-35 (an interspecific derivatives of *C. chinense* and *C. frutescens*, Kumar *et al*., 2010) and another susceptible, KA-2 (Kashi Anmol; *C. annuum*), were included in this study. Twenty plants of both genotypes were grown in net-house and after 20 days of transplanting 10 plants of both genotypes were inoculated with viruliferous whiteflies following Kumar *et al*. (2006). Three weeks post inoculation, leaf samples were collected from an inoculated and an uninoculated plant of both the genotypes. Phytochemical analysis of diseased and healthy samples was carried out to observe the effect of the virus/disease on the level of total phenol and peroxidase in the leaves of two genotypes. Disease severity data and morphological data, such as, plant height and total fruit number in once over harvest were recorded. Disease severity was elucidated by calculating the coefficient of infection (CI) (Kumar *et al*., 2006).

The total phenol content of leaf samples of pepper plants were analysed according to the Folin–Ciocalteu method as described by Cliffe *et al*. (1994). In brief, leaf sample extracts were well mixed with 2.5 ml of distilled water, 0.5 ml of the Folin–Ciocalteu stock reagent and 1.0 ml of Na_2CO_3 reagent (75 g/l) was added to the mixture and incubated at room temperature for 30 min. The mixture absorbance was spectrophotometrically measured at wavelength 650 nm. The total phenol content was expressed in micrograms of catechol equivalents per gram of leaf extract. To determine the change in peroxidase activity, 200 mg of each samples, collected pre- and post-inoculation of both genotype, were homogenized in 1.5 ml chilled 50 mM sodium phosphate buffer (pH 7.0) as described by Malik *et al*. (1980). The homogenate was centrifuged at 15000 rpm for 20 min at 4 °C. Assay mixture in a total volume of 3.0 ml

contained 50 mM sodium phosphate buffer (pH 7.0), 4 mM H_2O_2, 20 mM guaiacol and 200 μl enzyme extract. The decomposition of H_2O_2 was followed at 436 nm by decrease in absorbance per minute in order to calculate the peroxidase activity.

The result showed that upon infection through artificial inoculation of viruliferous whiteflies, the level of total phenol (28.5 mg/100 g) was reduced by 28 percent in the susceptible genotype (KA-2) as compared to uninoculated conditions (39.6 mg/100 g) (Table 1). However, in case of BS-35 (resistant genotype), the amount of total phenol was increased by 18 percent under artificial inoculated condition (48.1 mg/100 g) as compared to uninoculated stage (56.8 mg/100 g) (Fig. 1). The results for the susceptible genotype (KA-2) are in contrast to the findings of Anand *et al*. (2009), who reported that total phenols, activities of peroxidase and other pathogenesis-related enzymes increased in green and ripe fruits upon inoculation with *Colletotrichum capsici* and *Alternaria alternata* compared to the corresponding healthy plants. However, they also reported that phenolic content decreased frm 4th day after inoculation which might be due to the oxidative polymerization of phnolics into melanin in necrotic tissues or incorporation of phenols into lignin (Thompson, 1964). The resistant genotype (BS-35) did not show any symptom of leaf curl virus upon artificial inoculation (CI = 0.0), however the total phenolic content was increased. In case of susceptible genotypes (KA-2), severe curling of leaves was recorded (CI = 73.6) upon inoculation along with decrease in total phenolic content. Thus, the results indicate a direct correlation between total phenol content and resistance against PepLCV. The altered level of total phenolic content in leaf curl virus infected plants have been reported earlier (Prasath *et al.*, 2008). Stage dependant alteration in phenols has also been reported by Jabeen *et al*. (2009).

The peroxidase activity was reduced by 37 percent in the susceptible genotype, KA-2 (0.18 activity/minute/g sample) as compared to uninoculated conditions (0.29 activity/minute/g sample). On the other hand, in case of BS-35, the amount of peroxidase activity was increased by 25 percent under artificially inoculated condition (0.40 activity/minute/g sample) as compared to uninoculated stage (0.32 activity/minute/g sample) (Fig.2). Thus, the activity of the peroxidase expressed a direct impact for resistance in the host, which could be due to the conversion of the enzymes into quinones, which are toxic to the pathogen (Bhavani *et al.*, 2001).

The increased peroxidase and polyphenol oxidase activity and changes in the phenolic constituents immediately after infection are normal responses of a

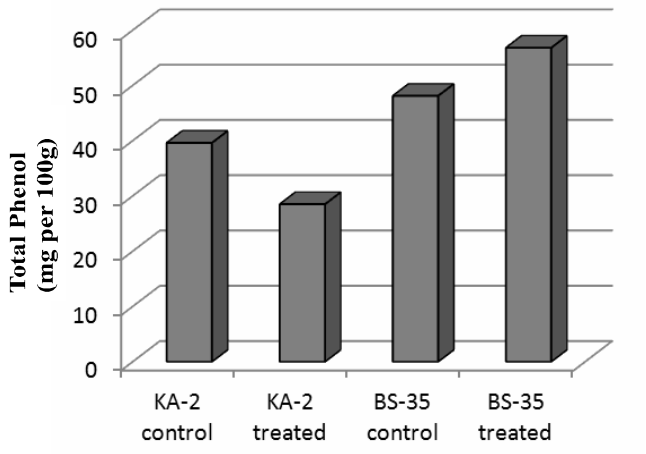

Fig. 1 Change in the total phenolic content in resistant and susceptible genotypes upon pepper leaf curl virus infection

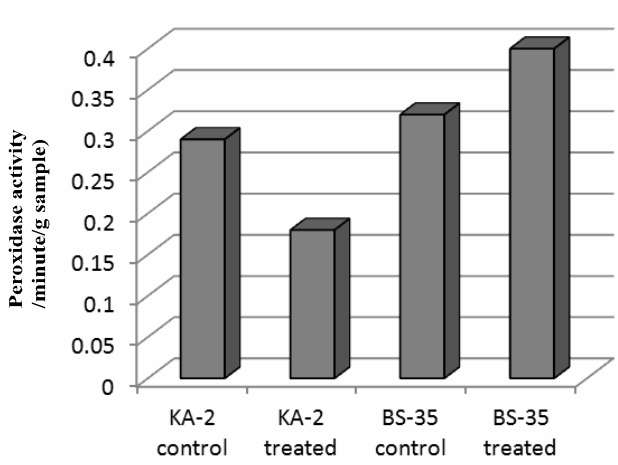

Fig. 2. Change in the peroxidase activity in resistant and susceptible genotypes upon pepper leaf curl virus infection

Table 1. Total phenolics and peroxidase activity in chilli genotypes under pepper leaf curl virus screening

Genotypes	CI	Total Phenol (mg per 100g)	Peroxidase (activity/min/g sample)	Plant Height (cm)	Number of fruits
KA-2 control	0.0	39.6 ± 0.83	0.29 ± 0.12	55 ± 2.24	310 ± 5.16
KA-2 treated	73.6	28.5 ± 0.83	0.18 ± 0.12	25 ± 2.24	15 ± 5.16
BS-35 control	0.0	48.1 ± 0.83	0.32 ± 0.12	120 ± 2.24	338 ± 5.16
BS-35 treated	5.3	56.8 ± 0.83	0.40 ± 0.12	110 ± 2.24	295 ± 5.16

host plant in putting up initial defense as observed and reported by Harbourne (1964). This mechanism breaks down in susceptible genotypes as found in chilli infected with *Fusarium* wilt probably due to lower synthesis of phenolic enzymes and substrates; however, it persists in the resistant hosts (Jabeen *et al*., 2009).

The plants of KA-2 were severely affected, which is depicted from very high coefficient of infection (CI = 73.6). While on the other hand, there were no symptoms (CI = 0.0) recorded in BS-35 plants. The plant height of the KA-2 after infection was adversely affected (25 cm) as compared to control plant (55 cm) at red ripe fruit stage. In case of BS-35, no significant decrease in height was observed. In severely infected plants of KA-2, total number of fruits at the time of picking was decreased drastically (15) as compared to healthy untreated plants (310 g). The decrease in plant height and fruit number might be due to less food accumulation for proper growth and development of plants upon infection with PepLCV disease. However, no significant decrease was observed in artificially inoculated BS-35 plants (295) as compared to uninoculated plants (338) which is evident from the resistance response of the plants through phenols and peroxidase. The results are in agreement of the finding of BS-35 as a resistance source against PepLCV by Kumar *et al*. (2006).

Studies on the changes in total phenol and peroxidase among resistant and susceptible genotypes during disease reaction indicated that there was wide variation in the enzyme activity among different conditions. Under stress conditions, including viral infection, stimulation and increased activity of phenolics and peroxidases plays a pivotal role in defense mechanism. It is suggested that phenolic compounds and peroxidases are involved in chemical defense mechanisms which control the development of PepLCV disease in chillies. Further, the roles of phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), catalase (CAT) and other pathogenesis-related proteins may be investigated for elucidating the biochemical basis of PepLCV resistance.

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