African Journal of Basic & Applied Sciences 6 (3): 65-75, 2014
ISSN 2079-2034

DOI: 10.5829/idosi.ajbas.2014.6.3.85130

# Biochemical and Enzymatic Response of *Cajanus cajan* Plant Towards the Biotic Stress Induced by *Fusarium oxysporum udum*

*Sunitha Mary and R. B. Subramanian*

B. R. Doshi School of Biosciences, Sardar Patel Maidan, Vadtal Road Post Box No. 39,
Sardar Patel University, Vallabh Vidyanagar-388120, India

**Abstract:** Pigeon pea (*Cajanus cajan* (L) Millsp) is one of the major grain legume crops of the tropics and subtropics, but biotic stresses (*Fusarium* wilt) of pigeon pea is a major threat for the sustainable crop production. An incompatible interaction between the host and the pathogen results in resistance whereas a compatible interaction leads to the establishment of disease. Infection of a plant by a pathogen initiates changes in the structural, physiological and molecular level. Seven cultivars of *Cajanus cajan* were screened to differentiate the cultivars as resistant and susceptible. Of the seven, ICPL 87119 was found to be a resistant variety and T 1515 was found to be susceptible on the basis of disease severity index. At the physiological level changes in the biochemical parameters in both the varieties (control as well as inoculated) were observed. Due to infection total soluble proteins, chlorophyll content and phenolics were found to be comparatively higher in the resistant variety than the susceptible variety. Assays of different defence related enzymes such as Peroxidase, Catalase, Phenylalanine ammonia lyase, β-1,3 glucanase and Pectin methyl esterase revealed lower activity in the susceptible variety in comparision to resistant variety.

**Key words:** Pigeon Pea · POX · CAT · B-1, 3-Glucanase · Phenylalanine-ammonia lyase · Pectin methyl esterase

## INTRODUCTION

Pigeon pea (*Cajanus cajan* (L) Millsp) is one of the major grain legume pulse crops of the tropical and subtropical regions of the world [1]. The genus *Cajanus* comprises 32 species most of which are found in India, Australia while one is native to West Africa. India is the largest producer of pigeon pea (3.5 Mha) followed by Myanmar (0.54 Mha) and Kenya (0.20 Mha) [2]. It plays an important role in the food security, balanced diet and alleviation of poverty because of its diverse usage as a food, fodder and fuel wood [3]. Several abiotic (e.g. drought, salinity and water logging) and biotic (e.g. diseases like *Fusarium* Wilt) stresses threatens pigeon pea production.

*Fusarium* wilt (FW) caused by *Fusarium oxysporum udum* is an important biotic constraint in pigeon pea production in the Indian subcontinent, which results in 16-47% crop loss [4]. The fungus enters the host vascular system at the root tips through wounds or invasion made by nematodes, leading to progressive chlorosis of leaves, branches, wilting and collapse of the root system [5]. In India alone, the loss due to the disease is estimated to be US$71 million and the percentage of disease incidence varies from 5.3 to 22.6% [6].

Plants have evolved a number of inducible defence mechanisms against pathogen attack. Some of the responses are constitutive and pathogen non-specific, but the majority of them are induced after recognition of the pathogen. Recognition results in the activation of a variety of defence responses, including rapid localized cell death (The hypersensitive response) [7] synthesis of pathogen related (PR) proteins and induction of systemic acquired resistance [8].

Some of the major elements of active defence of plant cells against pathogen attacks are changes in oxidation metabolism, which are thought to contribute to restricting pathogen ingress. These mechanisms include the

**Corresponding Author:** Sunitha Mary, B.R. Doshi School of Biosciences, Sardar Patel Maidan, Vadtal Road Post Box No. 39, Sardar Patel University, Vallabh Vidyanagar-388120, India, Tel: +91-2692-234412 Eext. 107.

production of reactive oxygen species (ROS) such as superoxide anion ($O_2^-$), hydrogen peroxide ($H_2O_2$), hydroxyl radical ($OH^-$) as well as production of phenolic compounds, induction of hydrolytic enzymes (e.g. chitinases and glucanases) [9], free radicals and quinines [10]. The ROS produced; participate in the damage of the attacking pathogen. There are number of enzymes involved in Reactive Oxygen Species (ROS) [11]. The systemic induction of an important component peroxidase a PR protein [12] is involved in cross-linkage of extension molecules and in the polymerization of hydroxycinnamyl alcohols to form lignin [13, 14]. Peroxidase (POD) is believed to play roles in auxin catabolism. Catalase (EC 1.11.1.6) is present as multiple isoforms in plants [15]. Catalase plays an important role in the protection of cells from toxic effects of hydrogen peroxide. Phenylalanine-ammonia lyase is another component which is responsible for the conversion of phenyl to trans-cinnamic acid a key intermediate in the pathway for the production of lignin and salicylic acid. Depending on the plant species Phenylalanine-ammonia lyase (PAL) may play role in either localized resistance or Systemic Acquired Resistance (SAR). It is believed that PAL activity is correlated with the synthesis of phenols in response to pathogen infection. Pectin, one of the main components is secreted in highly methyl esterified form and is dimethyl esterified in muro by pectin methyl esterase (PME). PMEs have been reported to play a role in resistance to fungal and bacterial pathogens [16]. The role of enzymes in plant-fungal interaction was summarized by Lebeda *et al*. [17].

The natural resistance of plants to diseases is based not only on preformed defences, but also on induced mechanisms. The induced mechanisms are associated with local changes at the site of pathogen infection, such as hypersensitive response (HR) which is one of the most forms of plant defences. The present study was undertaken to determine the changes in biochemical parameters that is concentration of chlorophyll, proteins, phenols in the resistant and susceptible variety due to *Fusarium* wilt.

## MATERIALS AND METHODS

**Pigeon Pea Cultivars/Plant Material:** The present study was carried out for two seasons from December 2010 to February 2011 and March 2011 to August 2011. Screening of the seven cultivars to differentiate the resistant and susceptible varieties was done by in vitro and in vivo bioassay methods. In vitro bioassay was done by leaf disc bioassay method. In vivo assay was done by direct sowing method for the seven varieties banas, icpl 87119, gt100, gt101, gt1 bdn2 and t 1515. Amongst the seven varieties icpl 87119 and t 1515 were taken for further studies.

**Sampling:** Treated plants were grown in soil inoculated with 10 ml of conidial suspension with a concentration of $1\times10^4$/ml while the control plants were grown in soil devoid of the fungal inoculums. The primary leaves and first trifoliate of same size and position were harvested from both treated and control plants from the day of emergence of the first leaflet for 7 days continuously and used for total protein, total phenol and chlorophyll content.

**Biochemical Evaluation:** Plant-pathogen interaction brings about a change in the biochemical parameters like protein, total phenol and chlorophyll content. Biochemical assays on total protein, total phenol and chlorophyll content were performed for two seasons to observe the pattern of the quantitative changes taking place in both resistant and susceptible variety during infection by *Fusarium*.

**Estimation of Total Protein Content:** Control and inoculated leaf samples were extracted with cold 0.1 M phosphate buffer at pH 7.0 and filtered. The residue was re-extracted, filtered and combined. The filtrate was centrifuged at 15,000 g at 4°C. The protein concentration was measured using colorimetric procedures of Lowry *et al*. (1951) [19] using bovine serum albumin as a reference standard.

**Estimation of Chlorophyll Pigment:** Chlorophyll pigments were extracted from healthy and infected leaves in 80% acetone according to Mahadevan and Sridhar (1982) [20]. The absorbance was recorded at 663 and 645nm in a spectrophotometer.

**Estimation of Phenolic Content:** The phenol content was estimated using Folin-Ciocalteau reagent. One gram of plant material was homogenized in 10 volumes of 80% ethanol. The homogenate was centrifuged and the supernatant was evaporated in a water bath. The residue was dissolved in a known volume of distilled water. Different alliquotes were taken into test tube. The volume in each tube was adjusted to 5 ml with water. To each tube 0.5 ml of Folin-Ciocalteau reagent was added. After 3 minutes 2 ml of 20% $Na_2CO_3$ solution was added after

incubation in boiling water bath for exactly one minute and absorbance at 660 nm was measured. Catechol was used as a standard. The concentration of phenols was calculated and expressed as mg phenols/100g material. (Folin and Ciocalteau, 1927) [21]

**Enzymes Assay:**

**Assay of Peroxidase (EC.1.11.1.7):** The reaction mixture consisted of 1.0 ml of 0.01 M O-Dianisidine, 2.4 ml of distilled water, 1.0 ml of 0.1 M Phosphate buffer and 0.5 ml of 20 mM $H_2O_2$. The reaction was initiated by 0.2 ml of enzyme extract and the mixture was incubated at room temperature. After 5 minutes reaction was terminated by application of 1.0 ml of 2N $H_2SO_4$ and absorbance was taken at 430 nm in a spectrophotometer. At the start of enzyme reaction, the absorbance of the control mixture containing 1.0ml of 0.01 M O-Dianisidine, 1.0 ml of 0.01 M Phosphate buffer and 0.5 ml of 20 mM $H_2O_2$, 0.2 ml of enzyme extract and 1.0 ml 2 N $H_2SO_4$ was set to zero in a spectrophotometer. Peroxidase (PO) activity was expressed as change in the absorbance of the reaction mixture unit/$min^{-1}g^{-1}ml^{-1}$ of fresh weight.

**Assay of Phenylalanine-ammonia Lyase (EC.4.3.1.5):** PAL activity was assayed according to the method of Malik and Singh (1980) [22]. With minor modification the reaction was initiated by adding to it 0.5ml of 0.1 M phenylalanine in 0.1 M Sodium borate buffer pH 8.8, 3 ml of 0.1 M sodium borate buffer pH 8.8 and 0.1 ml of enzyme aliquot. The reaction mixture was incubated at 37°C for 2 hrs. Change in absorbance was described as n moles of cinnamic acid released /hr/gm fresh wt.

**Assay of B-1,3 Glucanase (EC.3.2.1.39):** β-1,3 Glucanase activity was assayed by the laminarin Dinitrosalicylate method. 1 gram of pigeon pea leaf was homogenized with 3ml of sodium acetate buffer (0.05 M), pH 5.0 at 4°C using a chilled pestle and mortar. The extract was then centrifuged at 10,000 g for 15 min at 4°C and the supernatant was used as crude enzyme extract. The crude enzyme extract (62.5 µl) was added to an equal volume of laminarin (4%) and incubated at 40°C for 10 min. The reaction was stopped by adding 375 µl of dinitrosalicylic acid reagent and boiled for 5 min on a boiling water bath. The resulting colored solution was diluted with 4.5 ml of water vortexed and absorbance at 500 nm was determined. The blank was the crude enzyme preparation mixed with laminarin with zero time incubation. The enzyme activity was expressed as µmol $min^{-1}$ $ml^{-1}$.

**Assay of Catalase (EC.1.11.1.6):** Catalase was assayed by the method of Mahadevan and Sridhar 1986 [23] The reaction mixture contained 2.7 ml of 0.1 M phosphate buffer (pH 6.5) at 4°C and 0.1 ml of 0.2 M hydrogen peroxide and 0.2 ml of crude enzyme extract. The absorbance was taken at 230 nm in a spectrophotometer at 15 sec interval for 2 min.

**Assay of Pectin Methyl Esterase (PME) (EC 3.1.1.11):** PME (EC 3.1.1.11) was assayed according to Hagerman and Austen (1986) [24]. A known quantity of material was homogenized in 15 ml of of 8.8% NaCl at 4°C. The extract was centrifuged at 20,000g for 10 min. The supernatant was adjusted to pH 7.5 with NaOH and used for assay. The reaction mixture contained 2 ml of pectin and 0.15 ml bromothymol blue and 0.83 ml of water. The mixture is incubated at 25°C with circulating water bath. Initial absorbance is determined at 620 nm against water blank. The reaction is started by adding 100 µl of enzyme solution and the rate of decrease is measured at 20, 40, 60 and 80 sec interval. The activity of enzyme was calculated from the linear part of the curve by subtracting the initial absorbance value obtained at step 2. The enzyme activity was expressed as unit/min/gm material.

## RESULTS

**Total Protein:** There was a steady increase in the protein content reaching to the maximum on the 4th day with both control (33.933 mg/gm fresh wt) and inoculated (39.17 mg/gm fresh wt) in the resistant variety (ICPL 87119) showing maximum activity whereas in the susceptible variety the increase in the protein content was seen on the 6th day with control showing (21.6433 mg/gm fresh wt) and inoculated showing (24.467 mg/gm fresh wt). (Fig 1 a)

In the second season also the protein content was high in the resistant variety on the 3rd day with ICPL control showing (35.022 mg/gm fresh wt) and inoculated showing (40.66667 mg/gm fresh wt). The susceptible variety showed highest amount on the 6th day with control showing (20.17 mg/gm fresh wt) and inoculated showing (25.782 mg/gm fresh wt) in quantity. The protein content followed a similar quantitative increase in both the seasons (Fig 1 b).

**Total Chlorophyll:** During the first season the chlorophyll content was maximum in the resistant variety (ICPL) on the 3rd day of the inoculation in both control (0.85 mg/g tissue) and inoculated (1.43 mg/g tissue), while

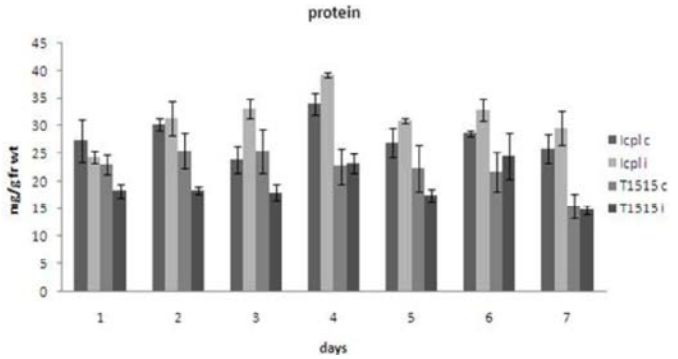


Fig. 1 a: Changes in total soluble protein content on pigeon pea cultivars during February. Values represents mean± S.D. n=3 Icpl c-Icpl control, Icpl i-icpl inoculated, T 1515 c-T 1515 control, T1515 i-T 1515 inoculated

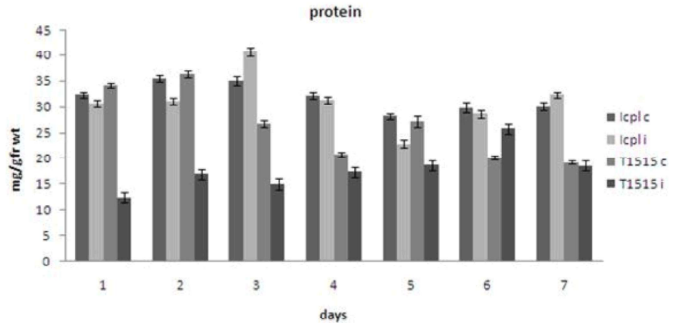


Fig. 1 b: Changes in total soluble protein content on pigeon pea cultivars during June. Values represents mean± S.D. n=3 Icpl c-Icpl control, Icpl i-Icpl inoculated, T 1515 c-T 1515 control, T1515 i-T 1515 inoculated

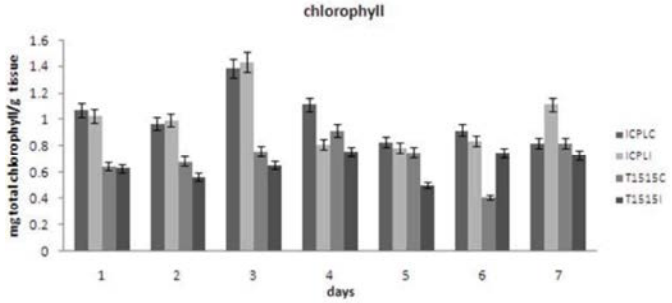


Fig. 2 a: Changes in total chlorophyll content on pigeon pea cultivars during February. Values represents mean± S.D. n=3 Icpl c-Icpl control, Icpl i-Icpl inoculated, T 1515 c-T 1515 control, T1515 i-T 1515 inoculated

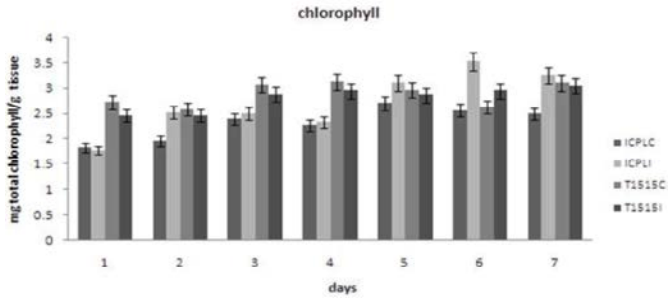


Fig. 2 b: Changes in total chlorophyll content on pigeon pea cultivars during June. Values represents mean± S.D. n=3 Icpl c-Icpl control, Icpl i-Icpl inoculated, T 1515 c-T 1515 control, T1515 i-T 1515 inoculated

the susceptible variety (T 1515) had maximum chlorophyll content on the 4th day in both control (0.91 mg/g tissue) and inoculated (0.75 mg/g tissue) respectively (Fig 2 a).

In the second season it was seen to be highest on the 6th day. The chlorophyll content was 2.5 fold times more in the resistant variety (ICPL) with control showing 2.56 mg/g tissue and inoculated showing 3.52 mg/g tissue (Fig 2 b).

**Total Phenolics:** In the first season, the phenol content in the resistant variety was highest on the third day. The total phenol content of the control resistant variety was 18.9 mg/100g and of the inoculated 19.9 mg/100g. The susceptible variety showed maximum concentration on the 2nd day with control showing 12.8 mg/100gm and inoculated showing 12.5mg/100gm. The quantity of total phenols gradually decreased in the following days in both the varieties with the susceptible variety showing a lower quantity than the resistant variety on all days. (Fig 3 a)

During the second season, highest total phenolics content was observed in the resistant variety (ICPL) on the 2nd day with control showing 18.3 mg/100g and inoculated showing 25.1 mg/100g. In the susceptible variety (T 1515), the control showed 12.1 mg/100g and inoculated 14.1 mg/100g. The phenol content was also seen to increase a 1.25 fold times more in the second season(Fig 3 b).

**Peroxidase Activity:** The maximum peroxidase activity was observed on the 4th day with control of the resistant variety showing 1.91 unit/gm activity and inoculated showing 2.29 unit/gm activity. The susceptible variety had shown higher induction on the 3rd day, but the amount of enzyme activity was 1.5 unit/gm in the control and 2.11 unit/gm in the inoculated that is lower than the resistant variety (Fig 4 a).

Second season had the enzyme activity 1.7 fold times more than the first season. In the second season the peroxidase activity was maximum for the resistant variety on the 5th day with control showing 3.175533 unit/gm activity and the inoculated showing 4.0413 unit/gm activity. The susceptible variety was seen to induce higher activity on the 2nd day itself, with control showing 2.569 unit/gm and inoculated showing 2.109667 unit/gm activity. (Fig 4 b)

**Phenylalanine-ammonia Lyase Activity:** Phenylalanine-ammonia lyase activity was maximum induced in the resistant variety on the 1st day with control showing (10.075 unit/hr/gm) activity and inoculated showing (8.166667 unit/hr/gm) activity. The susceptible variety had higher induction on the 2nd day with control showing (5.66667 unit/hr/gm) activity and inoculated showing (6.9333 unit/hr/gm) activity (Fig 5 a).

In the second season, in the resistant PAL activity was maximum seen on the 4thday with control showing (8.975 unit/hr/gm) activity and the inoculated showing (11.95 unit/hr/gm) activity. The PAL activity increased 1.4 fold times more in the second season. The susceptible variety had maximum induction on the 2nd day itself with control showing (5.766667 unit/hr/gm) activity and inoculated showing (6.9333 unit/hr/gm) activity. (Fig 5 b)

**β-1,3 Glucanase Activity:** β-1,3 glucanase was maximum induced on the 3rd day with control showing (4.45 unit/min/gm) activity and inoculated showing (4.245 unit/min/gm) activity. The susceptible variety had higher induction on the 2nd day with control showing (3.1975 unit/min/gm) activity and inoculated showing (2.9675 unit/min/gm) activity. (Fig 6 a)

In the second season the resistant variety had (1.34 unit/min/gm) activity in control and (1.6635 unit/min/gm) activity while the susceptible variety had in control (0.365unit/min/gm) and inoculated with (0.245 unit/min/gm) activity. The activity of β-1, 3 glucanase increased 2.5 fold times more in the second season (Fig 6 b).

**Catalase Activity:** The catalase activity was maximum on the 5th day with control showing (0.15 unit/min/gm) activity and the inoculated showing (0.2 unit/min/gm) activity. The susceptible variety had maximum induction on the 6th day with control showing (0.1388 unit/min/gm) activity and inoculated showing (0.15625 unit/min/gm) activity (Fig 7 a).

In the second season the catalase activity was maximum on the 5th day with control showing (0.088 unit/min/gm) activity and inoculated showing (0.087 unit/min/gm) activity. The susceptible variety had induction of (0.079 unit/min/gm) in control and (0.067 unit/min/gm) activity in inoculated. In the second season the enzyme activity decreased 2.5 fold times than the first season (Fig 7 b).

**Pectin Methyl Esterase Activity:** The PME was induced on the 5th day with control showing (0.15 unit/min/g) activity and inoculated showing (0.201 unit/min/g) activity. In the susceptible variety the PME was induced on the second day with control showing (0.12unit/min/g) and inoculated showing (0.135 unit/min/g) activity (Fig 8 a).

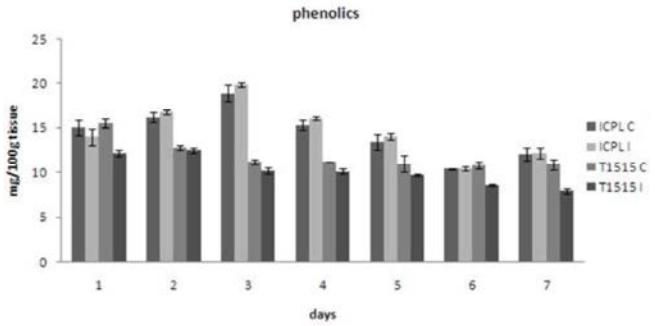


Fig. 3 a: Changes in total phenolics content on pigeon pea cultivars during February. Values represents mean± S.D. n=3 Icpl c-Icpl control, Icpl i-Icpl inoculated, T 1515 c-T 1515 control, T 1515 i-T 1515 inoculated

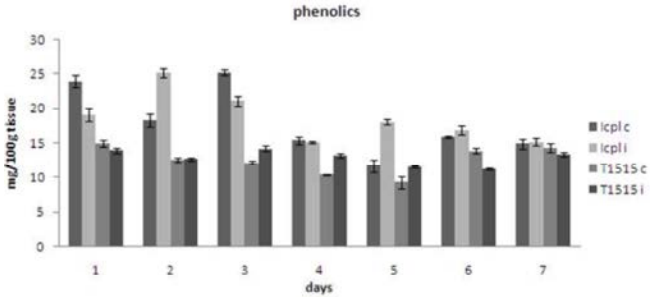


Fig. 3 b: Changes in total phenolics content on pigeon pea cultivars during June. Values represents mean± S.D. n=3 Icpl c-Icpl control, Icpl i-Icpl inoculated, T 1515 c-T 1515 control, T1515 i-T 1515 inoculated

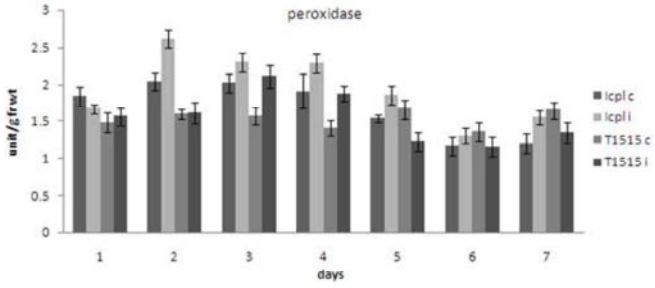


Fig. 4 a: Change in peroxidase activity on pigeon pea cultivars during February. Values represents mean± S.D. n=3 Icpl c-Icpl control, Icpl i-Icpl inoculated, T 1515 c-T 1515 control, T 1515 i-T 1515 inoculated

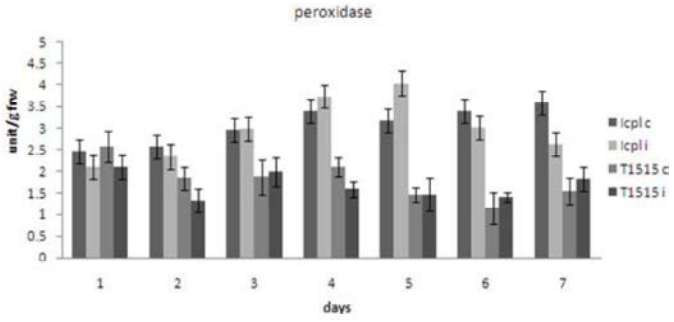


Fig. 4 b: Change in peroxidase activity on pigeon pea cultivars during June. Values represents mean± S.D. n=3 Icpl c-Icpl control, Icpl i-Icpl inoculated, T 1515 c-T 1515control, T 1515i-T 1515 inoculated

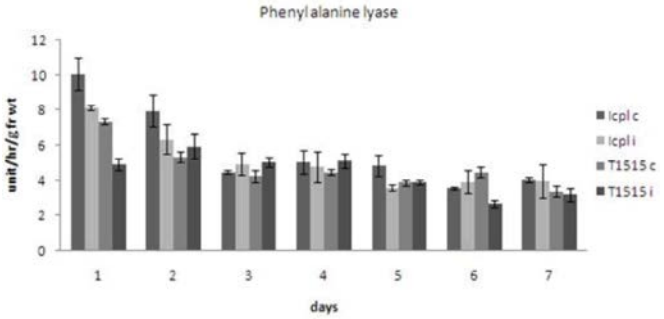


Fig. 5 a: Change in Phenylalanine ammonia-lyase activity on pigeon pea cultivars during February. Values represents mean± S.D. n=3 Icpl c-Icpl control, Icpl i-Icpl inoculated, T 1515 c-T 1515 control, T1515 i-T 1515 inoculated

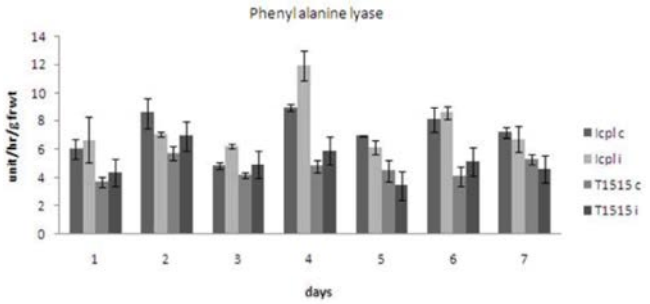


Fig. 5 b: Change in phenylalanine ammonia lyase activity on pigeon pea cultivars during June. Values represents mean± S.D. n=3 Icpl c-Icpl control, Icpl i-Icpl inoculated, T 1515 c-T 1515 control, T 1515 i-T 1515 inoculated

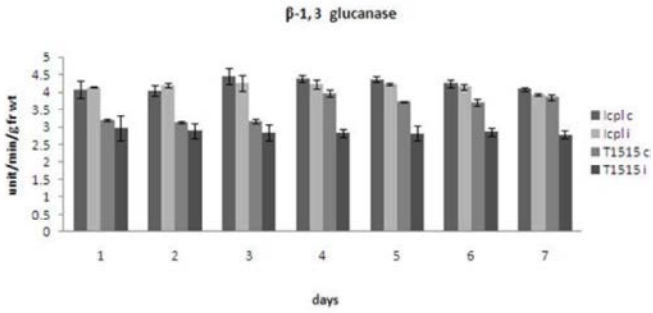


Fig. 6 a: Change in β-1,3 glucanase activity on pigeon pea cultivars during February. Values represents mean± S.D. n=3 Icpl c-Icpl control, Icpl i-Icpl inoculated, T 1515 c-T 1515 control, T 1515 i-T 1515 inoculated

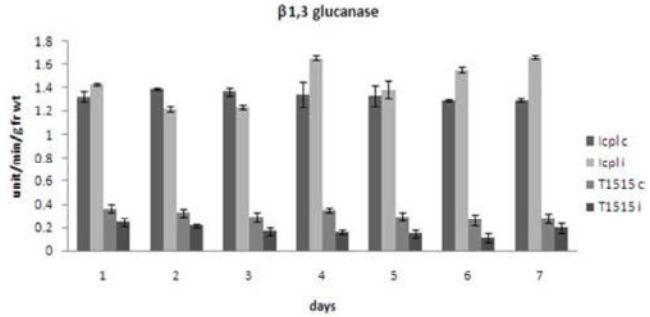


Fig. 6 b: Change in β-1,3 glucanase activity on pigeon pea cultivars during June. Values represents mean± S.D. n=3 Icpl c-Icpl control, Icpl i-Icpl inoculated, T 1515 c-T 1515 control, T 1515 i-T 1515 inoculated

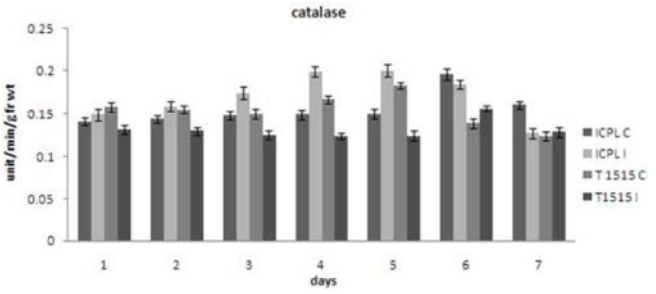


Fig. 7 a: Change in Catalase activity on pigeon pea cultivars during February. Values represents mean± S.D. n=3 Icpl c-Icpl control, Icpl i-Icpl inoculated, T 1515 c-T 1515 control, T 1515 i-T 1515 inoculated

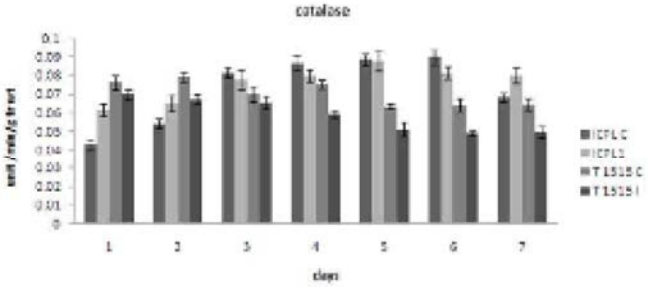


Fig. 7 b: Change in Catalase activity on pigeon pea cultivars during June. Values represents mean± S.D. n=3 Icpl c-Icpl control, Icpl i-Icpl inoculated, T 1515 c-T 1515 control, T1515 i-T 1515 inoculated

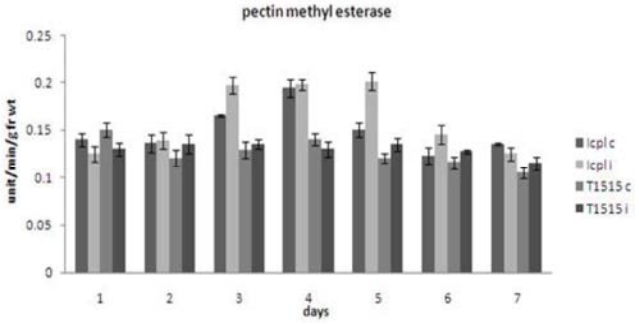


Fig. 8 a: Change in Pectin Methyl esterase activity on pigeon pea cultivars during February. Values represents mean± S.D. n=3 Icpl c-Icpl control, Icpl i-Icpl inoculated, T 1515 c-T 1515 control, T 1515 i-T 1515 inoculated

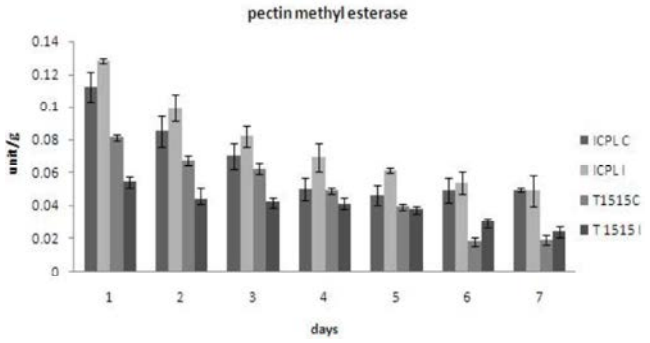


Fig. 8 b: Change in Pectin Methyl esterase activity on pigeon pea cultivars during February. Values represents mean± S.D. n=3 Icpl c-Icpl control, Icpl i-Icpl inoculated, T 1515c-T 1515control, T 1515 i-T 1515 inoculated

In the second season the PME activity was highest with control showing (0.085 unit/min/g) activity and the inoculated showing (0.099 unit/min/gm) activity in the resistant variety. In the susceptible variety the resistant variety showed high enzyme activity on the 1st day with control showing (0.081 unit/min/g) activity and inoculated showing (0.054 unit/min/g) activity. The PME activity decreased two fold times in the second season (Fig 8 b).

## DISCUSSION

Throughout the seven days of study the protein, chlorophyll and phenolic content in both control and inoculated in the resistant variety increased. There was not much change in the protein content in both the seasons. The chlorophyll content increased to 2.5 fold times and the phenolic content also increased to 1.25 fold times in the second season. The enzyme peroxidase was seen to increase 1.7 fold times, PAL 1.4 fold times, β-1, 3 glucanase 2.5 fold times in the second season, whereas the catalase activity decreased 2.5 fold times and the PME activity decreased to 2 fold times.

The change in the protein content in the resistant variety could be due to the change in the metabolic activity due to the plant pathogen interaction. The protein content showed a decrease in the susceptible variety compared to the resistant variety. Changes in protein occur when the pathogen penetrates the host cells resulting in disturbances in protein and related metabolisms [25]. The significant decrease in the protein content as a result of pathogen infection may be due to some activities related to a hypersensitive response [26].When a foliar pathogen establishes infection inside host tissues, the chlorophyll content is usually decreased; this is accompanied by yellowing of the infected leaves [27]. The chlorophyll pigments in Sorghum leaves decreased significantly due to infection of *Drechslera sorghicola* and continued with the progress of disease. Various plant pathogens are known to produce toxic metabolites, which may destroy the chloroplast resulting into decrease of chlorophyll pigments [28, 29].

The increase in total phenolics observed in the present study have been reported by others using different plant-pathogen interactions [30, 31]. The accumulation of phenolic compounds in infected host tissues may be related to their release from glycosidic esters by the enzymatic activity of host or pathogen [32], enhanced synthesis by host through the shikimic acid pathway [33] or due to migration of phenols from non-infected tissues.

Peroxidase enzymes are reported to regulate protein synthesis and the enzymes of the phenyl propanoid cycle in host. Similar changes in the levels of oxidative enzymes due to fungal infection have been reported by several workers [34]. The alteration in the oxidative enzyme level in sorghum infected with *Drechslera sorghicola* may be due to injury of the host tissues by fungal hyphae leading to the oxidation of phenolic compounds, however, the amount of oxidation products is insufficient to stop the invasion of mycelium in the leaf tissues.

PR-proteins are host-coded proteins induced by different types of pathogens and abiotic stresses [35, 36] reported that induction of systemic resistance by *P. fluorescens* was correlated with the accumulation of β-1, 3-glucanase and chitinase. In pea, seed treatment with *P. fluorescens* isolate induced the accumulation of hydrolytic enzymes such as chitinases and β-1,3-glucanase at the site of penetration of fungal hyphae of *F. oxysporum* f. sp. *pisi*. These enzymes act upon the fungal cell wall resulting in degradation and loss of inner contents of cells [37]. The enzymatic degradation of the fungal cell wall may release non-specific elicitors [38, 39]; which in turn elicits various defense reactions. PAL is a key enzyme in the production of phenolics and phytoalexins in cucumber [40]. Methyl esterification of pectin may correlate with a lesser accessibility to pectin-degrading enzymes and therefore with an increased resistance to pathogens [41, 42].

Certain PRs that are synthesized de novo upon SAR induction have antifungal activity. Alternatively the plant may become sensitized to activate appropriate defense mechanisms faster and more strongly upon infection with a challenging pathogen. These mechanisms also operate at lower frequency, intensity, or at a later stage during pathogen attack.

## CONCLUSION

From the above results it can be concluded that in the resistant variety biochemical parameters like protein, total phenol and chlorophyll content increased irrespective of the seasons. Also the enzymes induced were more in the resistant variety than the susceptible variety. It was also

observed that the resistant variety showed more speed in the increase of the biochemical changes and the induction of the enzymes, than the susceptible variety.

## ACKNOWLEDGEMENTS

The work was supported by grant sponsored by BASF INDIA Ltd. Navi Mumbai.

## REFERENCES

1. Varshney, R.K., D.A. Hoesington, H.D. Upadhyaya, P.M. Gaur, S.N. Nigam, K. Saxena, V. Vadez, N.K. Shetty, S. Bhatia and R. Aruna, 2007. Molecular genetics and breeding of grain legume crops for the semi-arid tropics, Genomics-assisted crop improvement. Springer, pp: 207-241.
2. Faostat, F., 2006, FAO statistical databases: Food and Agric. Org*an. of the* United Nations, Rome.
3. Rao, S.C., S.W. Coleman and H.S. Mayeux, 2002. Forage production and nutritive value of selected pigeon pea ecotypes in the southern great plains. Crop Sci., 42: 1259-1263.
4. Prasad, P., N.E. Reddy, R.J. Anandam and G.L. Reddy, 2003. Isozymes variability among *Fusarium udum* resistant cultivars of pigeon pea (*Cajanus cajan* (L) Millsp). Acta Physiol Plant, 25: 221-228.
5. Butler, E.J., 1906. The wilt disease of pigeon pea and pepper. Agricult J. India, 1: 25-26 .
6. Kannaiyan, J., Y.L. Nene, M.V. Reddy, J.G. Ryan and T.N. Raju, 1984. Prevalence of pigeon pea diseases and associated crop losses in Asia, Africa and the Americas. Trop. Pest Manag, 30: 62-72.
7. Hammond-Kosack, K.E. and J. Jones, 1996. Resistance gene-dependent plant defense responses. The Plant Cell, 8: 1773.
8. Schneider, M., P. Schweizer, P. Meuwly and J. Métraux, 1996. Systemic acquired resistance in plants. Int. Rev. Cytol., 168: 303-3409.
9. Dixon, R.A. and C.J. Lamb, 1990. Molecular communication in interactions between plants and microbial pathogens. Annu. Rev. Plant Biol., 41: 339-367.
10. Peng, M. and J. Kuc, 1992. Peroxidase-generated hydrogen peroxide as a source of antifungal activity in vitro and on tobacco leaf disks. Phytopathol, 82: 696-699.
11. Parmar, P. and R.B. Subramanian, 2012. Biochemical alteration induced in tomato (*Lycopersicum esculentum*) in response to *Fusarium oxysporum* F.Sp. *Lycopersici.* AJBAS, 4: 186-191.
12. Xue, L., P.M. Charest and S. Jabaji-Hare, 1998. Systemic induction of peroxidases, β-1,3 glucanases, chitinases and resistance in bean plants by binucleate Rhizoctonia species, Phytopathol, 88: 359-365.
13. Irving, H.R. and J.A. Kuc, 1990. Local and systemic induction of peroxidase, chitinase and resistance in cucumber plants by $K_2HPO_4$. Physiol Mol Plant Pathol, 37: 355-366.
14. Dalisay, R.F. and J.A. Kuc, 1995. Persistence of induced resistance and enhanced peroxidase and chitinase activities in cucumber plants. Physiol Mol Plant Pathol, 47: 315-327.
15. Scandalios, J.G., C. Foyer and P. Mullineaux, 1994. Regulation and properties of plant catalases: Causes of photooxidative stress and amelioration of defense systems in plants, pp: 275-315.
16. Wietholter, N., S. Graebner, M. Mierau, A.J. Mort and B.M. Moerschbache, 2003. Differences in the methyl ester distribution of homogalacturonans from near-isogenic wheat lines resistant and susceptible to the wheat stem rust fungus. Mol. Plant-microbe Interact, 16: 945-952.
17. Lebeda, A., L. Luhová, M. Sedlarova and D. Jancova, 2001. The role of enzymes in plant-fungal pathogens interactions. Z.Pfianzenkr Pflanzenschutz, 108: 89-111.
18. Lowry, O.H., N.J. Rosenbrough, A.L. Farr and R.J. Randall, 1951."Protein measurement with the Folin Phenol Reagent" J. Biol. Chem., 193: 265-275.
19. Mahadevan, A. and R. Sridhar, 1982. Methods in physiological plant pathology. II. Ed. Sivakami Publ., pp: 157-159.
20. Folin, C. and V. Ciocalteu, 1927. Tyrosine and tryptophan determination in protein. J. Biol. Chem., 73: 627-650.
21. Malik, E.P. and M.B. Singh, 1980. Plant Enzymology and Hittoenzymology (1st Edn.) Kalyani Publishers: New Delhi, pp: 286.
22. Mahadevan, A. and R. Sridhar, 1986. Methods in Physiological Plant Pathology, 3rd Edn. Sivakami Publications, Madras. India. pp: 316.
23. Hagerman, A.E. and P.J. Austin, 1986. Continuous spectrophotometric assay for plant pectin methyl esterase. J. Agric. Food Chem., 34: 440-444.

24. Uritani, I., 1976. Protein metabolism in Encyclopedia of Plant Physiology. R.Heitefuss and P.M.Williams. Springer, 4: 509-525.
25. Chandra, A. and R.K. Bhatt, 1998. Biochemical and physiological response to salicylic acid in relation to the systemic acquired resistance. Photosynthetica, 35: 255-258.
26. Farkas, G.L. and Z. Kiraaly, 1962. Role of phenolic compounds in the physiology of plant diseases and disease resistance. Journal of Phytopathol, 44: 105-150.
27. Fulton, N.D., K. Bollenbacher and G.E. Templeton, 1965. A toxic metabolite from *Alternaria tenuis* that inhibit Chlorophyll production. Phytopathol, 55: 49-51.
28. Peru, R.W. and C.B. Main, 1970. Chlorosis of tobacco induced by alternariol produced by *A tenius. Phytopathology,* 60: 1570-1573.
29. Chattopadhyay, S. and A.K. Bera, 1980. Phenols and polyphenol oxidase activity in rice leaves infected with *Helminthosporium oryzae*. Phytopathol. Z, 98: 59-63.
30. Henderson, S.J. and J. Friend, 1979. Increase in PAL and Lignin-like Compounds as Race-specific Resistance Responses of Potato Tubers to Phytophthora infestans. Phytopathol. Z. 94: 323-334.
31. Noveroske, R.L., E. Williams and J. Kuc, 1964. β-Glycosidase and phenoloxidase in apple leaves and their possible relation to resistance to *Venturia inaequalis*. Phytopathology, 54: 98-103.
32. Neish, A.C., 1964. Major pathways of biosynthesis of phenols: Biochemistry of Phenolic Compounds. Academic Press, New York, pp: 295-359.
33. Brune, W. and Van, Lelyveld, 1982. Biochemical comparition of leaves of five avocado cultivars and its possible association with susceptibility to *Phytophthora cinnamomi* root rot. Phytopathol. Z. 104: 273-254
34. Van Loon, L.C., 1997. Induced resistance in plants and the role of pathogenesis-related proteins. Eur J Plant Pathol, 103: 753-765.
35. Maurhofer, M., C. Hase, P. Meuwly, J.P. Metraux and G. Defago, 1994. Induction of systemic resistance of tobacco to tobacco necrosis virus by the root-colonizing *Pseudomonas fluorescens* strain CHAO: Influence of the gacA gene and of pyoverdine production. Phytopathol, 84: 139-146.
36. Benhamou, N., R.R. Belanger and T.C. Paulitz, 1996. Induction of differential host responses by *Pseudomonas fluorescens* in Ri T-DNA-transformed pea roots after challenge with *Fusarium oxysporum* f. sp. pisi and *Pythium ultimum*. Phytopathol, 86: 1174-1185.
37. Ren, Y.Y. and C.A. West, 1992. Elicitation of diterpene biosynthesis in rice (*Oryza sativa* L.) by chitin, Plant Physiol, 99: 1169-1178.
38. Hammerschimdt, R., 1999. Induced disease resistance. Physiol. Mol. Plant P, 55: 77-84.
39. Daayf, F.R., R. Bel-Rhlid and R.R. Bélanger, 1997. Methyl Ester of p-Coumaric Acid: A Phytoalexin-Like Compound from Long English Cucumber Leaves. J. Chem, 23: 1517-1526.
40. McMillan. G.P. and M.C.M. Perombelon, 1993. "Purification and characterization of a high pl pectin methyl esterase isoenzyme and its inhibitor from tubers of *Solanum tuberosum* subsp. tuberosum cv. Katahdin." Physiol. Mol. Plant P, 46(5): 413-427.
41. Marty, P., B. Jouan, Y. Bertheau, B. Vian and R. Goldberg, 1997. "Charge density in stem cell walls of *Solanum tuberosum* genotypes and susceptibility to blackleg." Phytochem, 8: 1435-1441.
42. Boudart, G., C. Latiffe, G.P. Barthe, D. Frasez and M.T. Esquerre-Tugaye, 1998. Differential elicitation of defense responses by pectic fragments in bean seedlings. Planta, 206: 86-94.