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Role of biochemical constituents and minerals against cotton leaf curl disease in cotton

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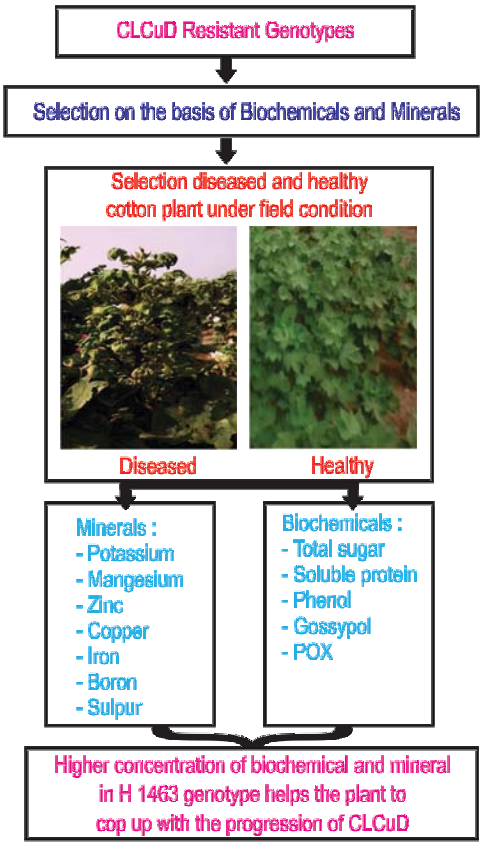
Abstract

Aim : Cotton Leaf Curl Disease (CLCuD) is among the most devastating disease infecting the cotton plants. The plants have developed both inherent, consecutive and induced defence mechanism against the disease like pre-existing physical and chemical barriers, such as inducible defence responses that interfere with pathogen establishment. Therefore, the present study was conducted to examine the status of biochemical constituents and minerals with their imparting defensive role against CLCuD.

Methodology : The four cotton genotypes H 1478, H 1098i, H 1156 and H 1463 were screened for cotton leaf curl disease on the consecutive and induced defensive role basis of polyphenoloxidase, peroxidase, biochemical constituents and minerals. The genotypes were grown under field condition at Cotton Research Farm, Chaudhary Charan Singh Haryana Agricultural University, Hisar. Leaf samples of healthy and diseased cotton plants were collected and analyzed for biochemical constituents, minerals and enzymes activities.

Results : The amount of phenol, gossypol and soluble protein were significantly higher in healthy plant as compared to diseased plant in all genotypes, whereas sugar content differed non-significantly and inconsistently among the genotypes. The polyphenoloxidase and peroxidase activities were also found significantly higher in healthy plant in all genotypes and correlate with high amount of phenolic compounds. Potassium, manganese, zinc and sulphur were significantly less in diseased plant of all genotypes. Inconsistent pattern was observed among genotypes in response to CLCuD for copper content. It differed significantly between healthy and diseased plants only in H 1156 and H 1463 genotypes.

Interpretation : The high content of potassium, manganese, sulphur and phenolic compounds in healthy plant of H 1463 genotypes leads to path for plant protection by means of their direct and indirect role against plant pathogen.



Introduction

Leaf curl is one of the most common and destructive disease of upland cotton in Indian subcontinent, presently restricted to northern cotton growing areas of around 15 lakh ha in Haryana, Punjab and Rajasthan. The disease was first reported in India on *Gossypium barbadense* at Indian Agricultural Research Institute, New Delhi in 1989, subsequently it appeared in patches during 1993 around Sriganganagar district of Rajasthan and Ferozpur district of Punjab adjoining to Pakistan border on *G. hirsutum* and spread to entire north India in a short span of 4-5 years (Monga, 2014). The disease is caused by a complex of monopartite begomoviruses and a small symptom modulating, single stranded satellite DNA, β component transmitted by whitefly *Bemisia tabaci* (Akhtar *et al*., 2013). The begomovirus beta satellite complexes that cause cotton leaf curl disease in Asia and Africa continent are distinct. The complex reported in India and Pakistan during the 1990s consisted of multiple begomovirus species (often occurring as multiple infections more than one virus per plant) supporting a disease-specific beta satellite, as well as an alpha satellite (Tahir *et al*., 2011). Till date, at least nine begomovirus species (CLCuVs) infecting cotton have been reported in Indian subcontinent. Cotton leaf curl burewala virus (CLCuBuV) infected plants are usually stunted and bushy. Younger leaves of infected plants can show downward cupping, followed by either upward or downward curling of leaf margins, swelling and darkening of veins, which frequently develop into cup shaped and leaf like out growths called 'enations'. Leaves from infected plants become thickened and more brittle than those from healthy plants. Severely infected leaves can show rolling and a reduction in size followed by malformation of leaf petioles, branches and main stem (Akhtar *et al*., 2008). The use of resistant varieties is the safest, economical and effective option to manage, but unfortunately introgressed host plant resistant was rapidly overcome by the resistant breeding strain of CLCuBuV during 2005 and all the available cultivated genotypes from *G. hirsutum* are susceptible (Akhtar *et al*., 2010).

The development of more CLCuD-resistant cotton is necessary to alleviate future threats to cotton in Indian subcontinent. Disease resistance in plants is associated with activation of a wide array of defence responses that slow down or halt infection at certain stages of the host-pathogen interaction. Plants have evolved various pre-existing physical and chemical barriers (Mandhania *et al*., 2015), such as inducible defence responses that interfere with pathogen establishment (Jones and Dangl, 2006; Zhao *et al*., 2008; Vanitha *et al*., 2009). The exact status regarding the response of CLCuV to biochemical constituents and minerals of cotton plant is not clear, which is the dire need of present situation. With this background, the present study was carried out to gain more information on the possible changes of biochemical constituents and minerals during systemic virus infection in healthy plants of cotton genotypes.

Materials and Methods

Four cotton genotypes, H 1478, H 1098i, H 1156 and H 1463 with CLCuV diseased and healthy plants were selected, grown in research field area, Cotton Section, Department of Genetics and Plant Breeding during year 2013-14. The leaves samples were collected from fourth leaf from top on the main stem. The presence of CLCuV was confirmed after isolation of total genomic DNA from these leaves by cetyl trimethylammonium bromide (CTAB) method (Doyle and Doyle, 1990) and subjected to PCR for the amplification of cotton leaf curl virus using specific primers (Briddon *et al*., 2002; Amrao *et al*., 2010).

Dried crushed (500 mg) leaf samples were mixed with 30 ml 80% (v/v) hot ethanol and centrifuged at 7000 rpm for 10 min and the supernatant were collected. Total soluble sugar content was determined following the method of phenol sulphuric acid (Dubois, 1956), total phenolic compounds were determined by Folin-Ciocalten's calorimetric method (Bray and Thorpe, 1954) and gossypol was estimated by pholesoglucinol reagent (Bell, 1967). For protein estimation, 500 mg leaves were ground in cold extraction buffer and samples was centrifuged at 15,000 x g for 10 min at 4°C, supernatant was separated and measured by dye binding assay, as described by Bradford (1976).

Polyphenoloxidase activity was measured according to the method described by Ying and Zhang (2008). The enzyme activity was determined spectrophotometrically using catechol as substrate. The changes in absorbance at 420 nm for 5 min was measured with UV-VIS spectrophotometer. One unit of polyphenoloxidase activity was defined as the amount of enzyme that produces one µmol of o-dopaquinone per minute.

Peroxidase activity was assayed following the method of Shannon *et. al.* (1966) with minor modification. The leaves were homogenized in a medium containing of 0.1 M Tris HCl buffer (pH 7.6), 0.1 M EDTA. The assay solution (4 ml) contained 3.620 ml of 0.1M phosphate buffer (pH 7.0), 0.2 mL 0.1% O-dianisidine, 0.1 ml of 0.2% H_2O_2 and 0.08 ml enzyme extract. The reaction was initiated by adding H_2O_2. The increase in absorbance was recorded after every minute. One unit POX activity was defined as the amount of enzyme that produce one µmol of product per minute.

Heavy metals like manganese, zinc, copper, boron and sulphur in leaf samples were estimated with an atomic absorption spectrometer Analyst 100 (Perkin Elmer, Beaconsfield, Germany), wherever potassium content was determined with a flame photometer (Elico CL 378, India). Statistical analyses were performed by two-way analysis of variance (ANOVA) using OP STAT (online version) and comparison between the mean values were made by least significant difference at 0.05 probability level.

Results and Discussion

Total phenol, gossypol and protein content differed significantly between healthy and diseased cotton plants. The activity of peroxidase enzyme in all genotypes was significantly higher in healthy plant, whereas healthy plant of H 1478 (1.13 µmol g^{-1} f. wt.) and H 1098i (1.17 µmol g^{-1} f. wt.) did not show significant differences to respective diseased plant 1.12 and 1.12 µmol g^{-1} f. wt. for polyphenoloxidase enzyme. The higher level of these biochemical constituent particularly phenol and gossypol may contribute towards the defence of plant against viral disease. Siddique *et al*. (2014) and Rohini *et al*. (2011) reported higher accumulation of phenolic compound after infection. The highest phenol (Fig. 1a) and gossypol (Fig. 1b) content (1.40 and 0.48%) was found in healthy plants of H 1478 genotype, followed by H 1463 genotype. Similar results was reported by Markakis *et al*. (2010) who observed negative association between phenolic compound and *Verticillium dahlia*, pathogen of Verticillium wilt in olive tree. These results are in line with the results reported on other plant-pathogenic fungal, bacterial and viral interactions, which showed that certain common phenols and phenolic substances are toxic to pathogens, which have long been considered as important defence related compounds whose levels are naturally high in the resistant varieties of many crops (Gogoi *et al*., 2001) and accumulate in plants after infection, especially in resistant varieties (Agrios, 1997; Rohni *et al*., 2011).

The results in Fig.1c represent inconsistent and non-significant sugar content in healthy and diseased plant. Healthy plant of H 1478 genotype contained high amount of sugar (6.26%), whereas least sugar (2.14%) was observed in healthy plants of H 1463 genotype. Higher content of sugar contribute towards higher rate of infection. Higher disease incidence in high sugar containing genotypes as well as less content of phenolic compounds was also reported by Abdullah and Singh (2004) and Mandhania *et al*. (2016) in cotton genotypes. Involvement of protein components in plant disease resistance has been documented in many plant pathogenic interactions (Tornero *et al*., 2002; Carvalho *et al*., 2006). Significant difference was observed for soluble protein among healthy and diseased plant leaves, but within genotypes it was non-significant (Fig. 1d). In the present study, protein content was found to decreas significantly in CLCuV-infected plants. A possible explanation for significant decrease in total soluble protein contents in CLCuV diseased plants may be due to poor upregulation of plant defence mechanism. Similar results have been reported in maize, tomato, grapevine, and apple infected with mollicutes (Musetti, 2010).

Significantly higher peroxidase (Fig.1e) and polyphenoloxidase (Fig. 1f) activities (13.02 and 1.36 µmol g^{-1} f. wt.) were also found higher in H 1463 and H 1156 genotypes (12.95 and 1.34 µmol g^{-1} f. wt.), respectively. The enzymes peroxidase and polyphenoloxidase play an important role in plant protection against the disease incidence by involving in the lignifications, wound healing, regulation of cell wall, oxidation of phenolic to more free radicals that can react with biological molecules, thus creating an unfavourable environment for pathogen development (Maksinov *et al*., 2014; Mohamed *et al*., 2012). Similar results were reported in tomato infected with tomato mosaic tobamovirus (Madhusudan *et al*., 2009) and cucumber infected with cucumber mosaic virus (Riedle-Bauer, 2000). Significantly higher enzyme activity of polyphenoloxidase and phenylalanine ammonia lyase as well as higher concentrations of chlorogenic acid and total soluble phenols in potato was studied by Ngadze *et al*. (2012) upon inoculation with bacterial suspension. The resistance in varieties was correlated with high polyphenoloxidase and phenylalanine ammonia lyase enzyme activity, as well as increased concentrations of chlorogenic acid and total soluble phenols.

The incidence of disease attack leads to accumulation and depletion of different nutrients in both healthy and diseased plant leaves. Potassium, sulphur, zinc and manganese content were found significantly more in healthy plant leaves as compared to infected plant leaves. A considerably lower concentration of potassium in diseased leaves of all genotypes, laid out in Fig. 2a, can be explained by the fact that potassium is mobile nutrient (Mangel and Kirkby, 1987; Marschner, 1995) and under normal condition it readily moves from older to younger leaves through phloem. Since infected leaves of all genotypes were severely affected and curled and occurrence of substantial/ partial blockage of vascular system of these leaves may hamper the flow of potassium. It has also been reported in scanning electron microscope studies by Iqbal *et al*. (2006). In contrast, the healthy plant leaves were not curled and normal, thus almost maintained the potassium state. Diseased plants had lesser manganese and sulphur content. Fig. 2b shows that the manganese content decreased upto 40% in three genotypes, except H 1098i genotype where it showed maximum decrease (15.83%) in sulphur content (Fig. 2d). Healthy plant leaves was found to contain non-significantly lesser boron (Fig. 2e) content as compared to diseased plant leaves. As shown in Fig. 2c, inconsistent pattern was observed among the genotypes in response to CLCuD for copper content and it differed significantly between healthy and diseased plants only in H 1156 and H 1463 genotypes. The role of manganese, copper and zinc is poorly understood. The high amount of these nutrient in healthier plant may contribute their role toward provide tolerance against cell wall integrity. Copper and manganese particularly play an important function in the activation of metabolic synthesis of lignin (Porcheron *et al*., 2013), and thus in controlling pathogen. Manganese also contribute important role in establishment of pathogen resistant by inhibiting cell wall degrading enzyme (Dordas, 2008).

Significantly reduced zinc content was observed in diseased plant leaves of all genotypes than healthy plant leaves (Fig. 2f). Reduced zinc content leads to lesser green plant hence

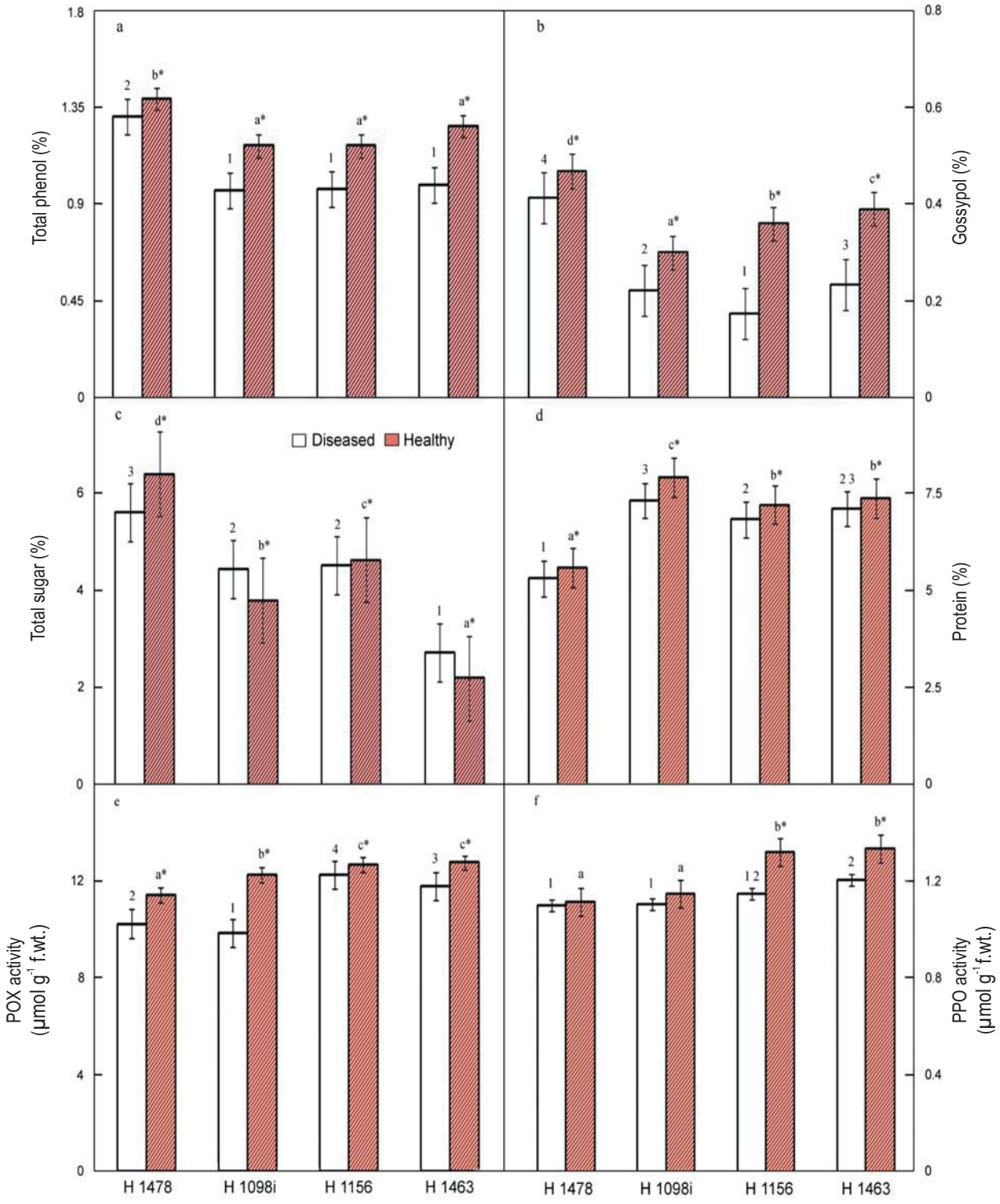


Fig. 1 : Percentage values of (a) Total phenol (b) Total gossypol (c) Total sugar (d) Total soluble protein (e) Peroxidase activity (f) Polyphenoloxidase activity in healthy and respective diseased plants leaves; Figures followed by same alphabet and same numeric do not differ statistically; Figures followed by * differ statistically within genotype

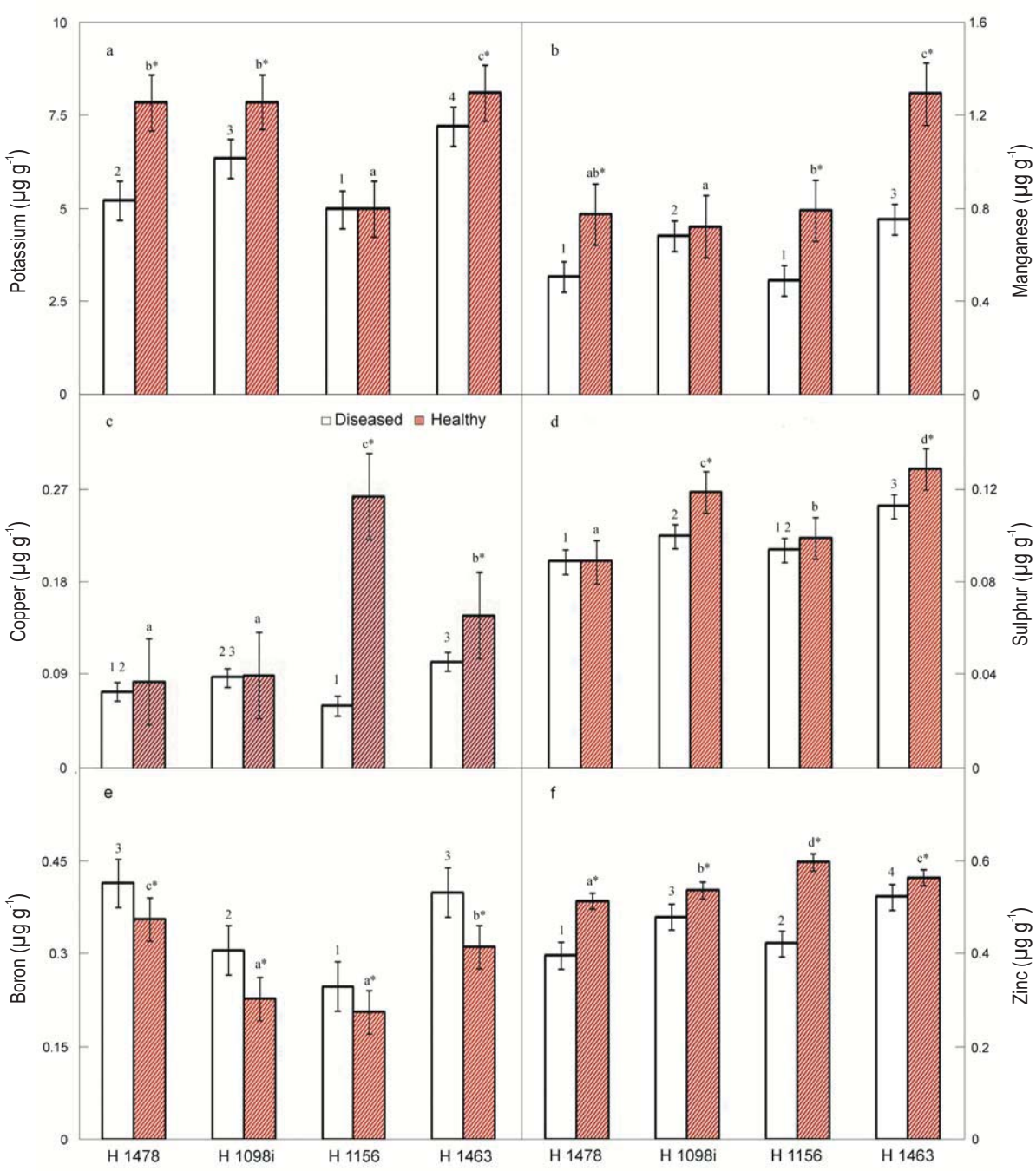


Fig. 1 : Microgram per gram values of (a) Potassium (b) Manganese (c) Copper (d) Sulphur (e) Boron (f) Zinc content in healthy and respective diseased plant leaves; Figures followed by same alphabet and same numeric do not differ statistically; Figures followed by * differ statistically within genotype

had lesser chlorophyll content (Data not shown). The possible explanation is that zinc is an immobile element in the plant tissues and directly constitutes chlorophyll. In healthy plants, zinc maintain the integrity of plasma membrane as zinc ions are known to be a strong inhibitor of NADPH oxidase resulting in lesser production of oxyradical (Pinton *et al.,* 1994, Porcheron *et al*., 2013).

The elevated response of biochemical constituents and mineral content were observed among the cotton genotype as a result of CLCuV infestation. The H 1463 genotype had higher content of potassium, manganese and sulphur but low sugar content. The PPO and POX activities were also found higher in H 1463 genotype. The higher concentration of biochemical, mineral and pathogenesis related protein helps the plant to cope up with

the progression of CLCuD in H 1463 genotype.

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