PROCEEDINGS OF THE INTERNATIONAL CONFERENCE ON PLANT PHYSIOLOGY 2014

Enhancing Strategic Plant Physiological Research and Technologies for Sustainable Resources

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Indonesian Coffee & Cocoa Research Institute

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Antiodixant Defence System in Iron Deficient Groundnut Plants

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Introduction

Iron is one of the essential micro-nutrients required by the plants. It plays an important role in the synthesis of chlorophyll, carbohydrate production, cell-respiration, chemical reduction of nitrate, sulphate and N assimilation, component of cytochrome oxidase, ferredoxin protein and several enzyme systems. Iron deficiency symptoms appear on younger leaves indicating yellowish inter-veinal areas of leaves (commonly referred to as iron chlorosis). In general, plants are prone to iron deficiency in alkaline, calcareous soils, coarse textured soils, eroded soils, low organic matter soils and cold weathered areas soils except flooded rice field soils. In India more than one-third of the soil is calcareous and spread mostly in the low rainfall areas of the western (Gujarat, Maharashtra, Rajasthan and Karnatka) and central (M.P. U.P.) parts of the country where groundnut is a major crop. Therefore iron chlorosis is causing considerable yield reductions. The crops sensitive to iron chlorosis are groundnut, citrus, field bean, grapes, soybean, vegetables and ornamentals.

Groundnut is grown during kharif (July-October) as well as during rabi-summer (Feb-May) seasons. In calcerous soils of Saurashtra, Gujarat more than 60% groundnut fileds showed chlorosis during rabi-summer season compared with less than 20% during kharif season. During Kharif, groundnut crop is grown as rainfed while during rabi-summer season crop, most of the farmers give frequent irrigation mainly due to the high evapo-transpiration demand of the crop. As a result, majority of the groundnut fields show chlorosis and remain chlorotic throughout the cropping season. Plant Fe deficiency has economic significance because crop quality and yields can be severely compromised and the use of expensive corrective methods is often required (Alvare-Fernandez et al., 2004). Despite the ubiquitous presence of Fe in the earth's crust, the low solubility of Fe compounds in many soils prevents plant Fe uptake and induces development of Fe deficiency symptoms. Crop genotypes differ greatly in their response to iron availability (iron stress) in the soil and have been designated as iron-inefficient (susceptible to iron chlorosis) and iron-efficient cultivars (Brown and Jolley, 1989; Singh and Chaudhari, 1993). Iron efficient genotypes have already been identified for many crop plants, although reports on a limited number of groundnut genotypes are available, an extensive and systematic investigation has not been undertaken for the identification of genotypes tolerant to lime induced iron chlorosis for use in breeding programmes.

A deficiency in mineral nutrients is generally not considered as a stress factor for plants. In this context, the study of Fe deficiency is particularly appealing. First, Fe deficiency is a worldwide problem, enormously detrimental to plant production. Secondly, Fe is a transition metal of pivotal importance in reactions involving active oxygen (Halliwell and Gutteridge, 1989) and a constituent of antioxidant enzymes such as catalase, peroxidases and SOD. On the other hand, there is little information about the relationship between iron-deficiency and the onset of oxidative stress status (Iturbe-Ormaetxe et al., 1995) despite both the world-wide problems represented by scarce iron bioavailability in the soil and the well known double role which iron plays within the cell metabolism. Thus, in this work we have investigated effect of Fe deficiency on oxidative stress in groundnut plants. In view of this, the present research activity was planned to study the correlation between Fe and enzyme activities in groundnut (*Arachis hypogea*) by comparing the antioxidant enzymes of Fe-sufficient (Fe+) and Fe-deficient (Fe-) leaves.

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Materials and Methods

Plant materials and treatments

Eight Fe-efficient and eight Fe-inefficient groundnut genotypes, as listed in Table 1, were sown in twelve nutrient blocks of size 4x5m each in a randomized block design (RBD) with two replicates. In each replicate 60 plants of each genotype were grown in adjacent rows each 5 m long. The row to row and seed to seed spacing were 45 cm and 8 cm respectively with Fe-efficient and inefficient genotypes in separate nutrient blocks. The plants were supplied with iron free nutrient solution at required intervals. At the vegetative stage, 20–25 d after sowing, Fe was added in the form of nutrient solution with $FeSO_4$ through rooting medium. The treatments included T_1 as control where no nutrients were applied; T_2 was nutrient supply with Fe source (Fe+) and T_3 nutrient supply without Fe source (Fe-). The third mature leaf from top was taken for sampling after three days of iron treatments.

Table 1. List of groundnut genotypes.

Sr No	Fe inefficient	Sr No.	Fe efficient
1	NRCG 7472	9	ICGV 86590
2	NRCG 162	10	ICGV 86031
3	CO2	11	CSMG 9510
4	NRCG 7599	12	GG 7
5	TIRUPATI 4	13	GIRNAR 2
6	MH 1	14	CSMG 84-1
7	JL 220	15	ICGV 000348
8	VRI 3	16	KADIRI 9

Enzyme extraction: Fresh leaf samples from control and treated plants were ground with liquid nitrogen, and suspended in 0.1 M phosphate buffer, pH 7.5 containing 0.5 mM EDTA. The Brie was passed through four layers of cheese cloth and the filtrate was centrifuged at 15000 rpm for 20 min at 4°C and resulting supernatant was used for enzyme assays. Then respective enzyme assays were performed according to the following methods:

Peroxidase (EC 1.11.1.7): The enzyme activity was estimated by the method of Bergmeyer (1974). To begin with enzyme assay, the reaction mixture containing 0.5 mL enzyme extract, 100 mM phosphate buffer, pH 6.1, 12 mM H_2O_2 and 96 mM guaiacol was mixed. Final volume was made up to 3.0 ml with distilled water. Absorbance due to formation of tetra-guaiacol was recorded at 470 nm. The enzyme activity was expressed as units $min^{-1}mg^{-1}$ protein.

Catalase (EC 1.11.1.6): Catalase activity was estimated by the UV method of Aebi (1983). The reaction mixture contained 50 µL enzyme extract, 12.5 mM H_2O_2 and 50 mM potassium phosphate buffer. Decrease in absorbance was recorded at 240 nm for 30s. The enzyme activity was expressed as µ moles H_2O_2 decomposed $min^{-1}mg^{-1}$ protein by using the H_2O_2 extinction coefficient 36 $\mu M^{-1}cm^{-1}$.

Superoxide dismutase (EC 1.15.1.1): The activity of superoxide dismutase was estimated by the method of Giannopolitis and Ries (1977). The reaction mixture contained 0, 0.2, 0.3, 0.5 and 1.0 mL enzyme extract in different sets. To each set, 200 mM methionine, 2.25 mM nitroblue tetrazolium, 3 mM EDTA and 1.5 M Na_2CO_3 were added. Total reaction volume was brought to 3 mL adjusting the pH to 10.2. At the end, 2 µM riboflavin was added. The tubes were shaken and placed 30 cm from the light source consisting of two 15-W fluorescent lamps. The reaction was allowed to run for 15 min and then stopped by switching off the lights. The tubes were immediately covered with black cloth. The absorbance was recorded at 560 nm. A non-irradiated reaction mixture which did not develop a colour, served as control.

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However, in the presence of SOD, the reaction was inhibited and the amount of inhibition was used to quantify the enzyme. $LogA_{560}$ was plotted as a function of volume of enzyme extract used in the reaction mixture. From the resultant graph, the volume of enzyme extract corresponding to 50 % inhibition of the photochemical reaction was obtained and considered as one enzyme unit. The enzyme activity was expressed as units $min^{-1}mg^{-1}$ protein.

Glutathione reductase (EC 1.11.1.9): Glutathione reductase activity was estimated by the method of Goldberg and Spooner (1983). To 0.1 mL enzyme extract, 200 mM K-phosphate buffer (pH 7.5), 0.015 mM EDTA and 20 mM oxidised glutathione were added. After 5 min, 2.0 mM NADH was added and mixed thoroughly. The absorbance was recorded at 412 nm at intervals of 5 s. The enzyme activity was expressed as nmole NADH oxidized $min^{-1}mg^{-1}$ protein.

Results and Discussion

In leaves supplied with iron source, the catalase and peroxidase activities were higher which decreased by almost 50% with elimination of Fe from nutrients (Fe-), whereas the effect on glutathione reductase was not significant. On the other hand, superoxide dismutase activity was increased by 20-35 % among different genotypes under Fe deficiency in Fe-inefficient genotypes. The genotypes e.g. NRCG 162, MH1, VRI3 etc, which are unable to utilize the available iron efficiently(Fe-inefficient) are having less activities of catalase, peroxidase in the absence of Fe whereas higher activity in presence of Fe. From the results, it is clear that the reactive oxygen scavenging (ROS) enzyme activities are less in the inefficient genotypes showing that the plants are lacking iron and thus showing Fe deficiency symptoms.

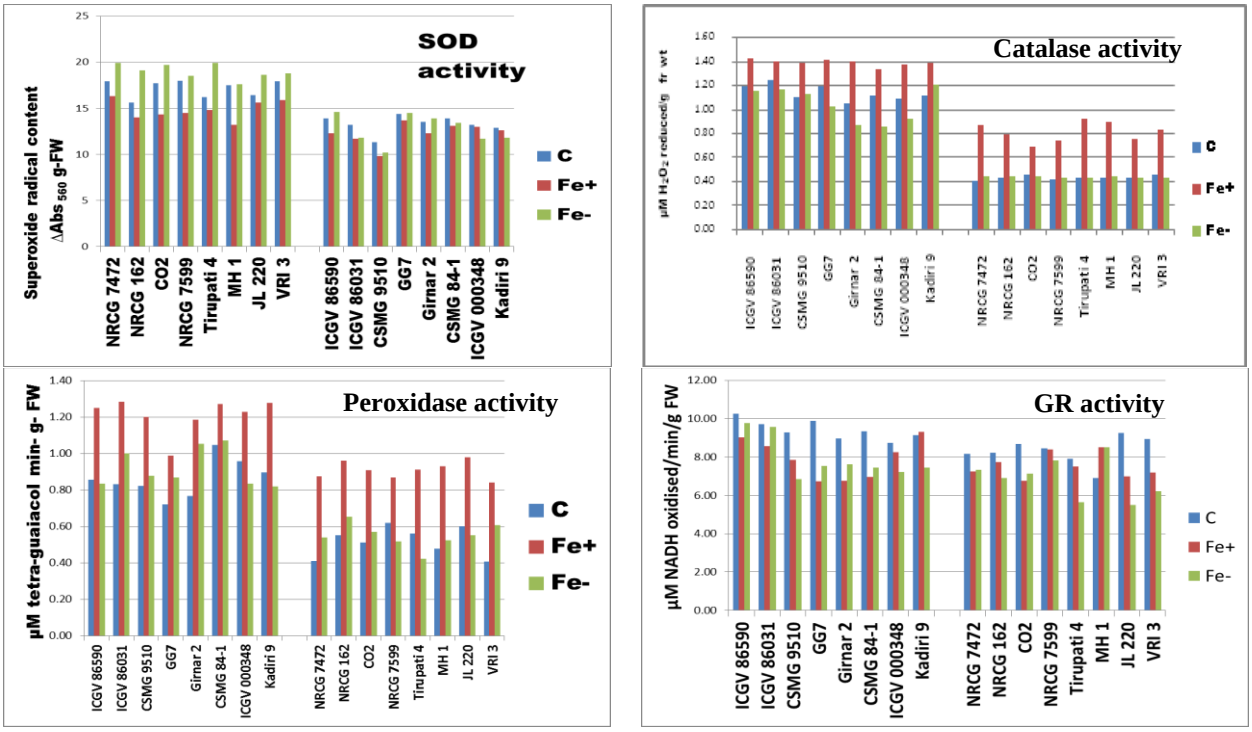

Figure 1. The activities of superoxide dismutase, catalase, peroxidase and glutathione reductase in Fe-efficient and inefficient groundnut (*A. hypogea*) genotypes in presence (Fe^{+}) and in absence of ion (Fe^{-}).

SOD is a major scavenger of O^{2-} and its enzymatic action results in the formation of H_2O_2 and O_2. Peroxidase decomposes H_2O_2, by oxidation of co-substrates whereas catalase breaks down H_2O_2 into water and molecular oxygen (Mittler, 2002). The capacity to scavenge ROS and to reduce their damaging effects on macromolecules appears to represent an important stress tolerance trait. In the present investigation, increased SOD activity following iron deficiency leads to increased H_2O_2 production but on the other hand, the decreased capacity to detoxify the enhanced H_2O_2 may be due to unsuccessful activation or reduced production of heme containing antioxidant enzymes like peroxidase and catalase. As an abiotic stress for plants, iron deficiency was shown to affect the expression and the activity of certain peroxidase isoenzymes and induces secondary oxidative stress in dicotyledonous species (Ranieri et al., 2001). Zaharieva et al., (2004) found in sugar beet roots that iron deficiency resulted in the decreased activity of APX and increased content of GSH. The activities of several antioxidant enzymes were greater than the controls in Mg-deficient bean leaves (Cakmak and Marschner, 1992) and Mn-deficient needles of Norway spruce trees which may reflect a response of plants to increased free radical production. It has been suggested that plants exposed to iron deficiency may be more sensitive to oxidative stress because iron is a constituent of enzymes associated with the cellular antioxidant system such as APX, CAT, peroxidase, and Fe-SOD (Kumar et al., 2010).

The relationship between decreased iron availability in the nutrient media and the possible onset of oxidative stress is becoming more evident. The decrease in activity of catalase and peroxidase may also be due to the fact that these two are heme containing enzymes and their activities are dependent on Fe. Thus the changes in activities of antioxidant enzymes are due to oxidative stress induced by Fe deficiency suggesting that groundnut plants come under Fe stress affecting their growth.

Conclusions

The results of the present study clearly showed that there were differential genotypic variations in activities of scavenging enzymes in groundnut cultivars grown under different iron nutrient conditions. The groundnut plants which were moderately tolerant to iron deficiency might have active ROS scavenging system, in addition to other tolerance mechanisms, to cope with stress. Therefore, plants with the ability to scavenge and/or control the level of cellular ROS may be useful in future to withstand nutrient deficiency conditions.

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