International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2021; 9(1): 625-631

Received: 01-10-2020
Accepted: 06-11-2020

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Adaptive strategies to minimise iron limiting stress under climate change scenario: A study pertinent to iron stress response of two soybean (*Glycine max* (L.) Merr.) Genotypes

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DOI: https://doi.org/10.22271/chemi.2021.v9.i1i.11296

Abstract
The response of soybean genotypes to iron deficiency stress under elevated CO_2 and temperature condition could be guided by three critical rhizospheric processes *viz*. proton (H^+) extrusion, root exudation and ferric chelate reductase (FCR) activity. In the present study, the iron efficient and responsive (FeER) genotype recorded an impressive performance over iron inefficient and responsive (FeIR) genotype in counteracting iron deficiency stress but experienced modest stress caused by the combined interaction between the genotype (G), environment (E) and HCO_3^- ion (B). The antagonistic interaction between Fe^{2+} with HCO_3^- ion resulted in greater iron stress. Plants grown in the absence of bicarbonate have significantly higher total chlorophyll content (1.79±0.04 mg g^{-1}, mean ± SE) than plants grown in presence of bicarbonate (1.48 ± 0.06 mg g^{-1}). To warfare the constraints in Fe availability, especially under more stressed e-[CO_2+T] environmental condition in the presence of bicarbonate ion, the root system of iron efficient genotype of soybean (FeER) exuded out significantly higher amount of low molecular weight organic acids. Furthermore, the presence of bicarbonate ion in the nutrient solution exacerbated the iron deficiency stress and consequently resulted in higher proton extrusion (~1.2 fold increase), lower ferric chelate reductase activity (~1.3 fold decrease) and higher organic acid exudation (up to ~1.9 fold increase in malic acid). The intra-specific variability between contrasting genotypes and their response to elevated CO_2 and temperature could be exploited to remediate emerging Fe deficiency of soybean plants under climate change scenario.

Keywords: Soybean, climate change, Iron (Fe), iron deficiency chlorosis (IDC), elevated temperature, elevated CO_2

Introduction
Iron is an essential for the synthesis of chlorophyll, photosynthesis, respiration and other vital functions (Raj *et al*, 2019a, b; Briat *et al*., 2015) [1-3]. It is a chief constituent in Fe–S cluster containing enzymes (Jeong and Connolly, 2009; Jeong and Guerinot, 2009) [4, 5]. Soybean is an important oil seed crop, very sensitive to iron deficiency stress (Vasconcelos and Grusak, 2014) [6]. The limited availability of iron is one among the major critical factors that limit soybean yield (Liang et al., 2010, 2013) [7, 8]. Iron deficiency chlorosis (IDC) is an abiotic stress caused by the limited availability of iron, observed primarily on calcareous soils of arid and semi-arid agro-ecosystems, which impacts over 30% of the world's crop land (Mori, 1999) [9]. Plants have evolved their own mechanisms for iron acquisition (Kobayashi *et al.*, 2019; Römheld and Marschner, 1986) [10, 11]. With the exception of cereals and grasses, all plants acquire iron by strategy I mechanism (Vert *et al*., 2002) [12]. Conversely, the graminaceous plants acquire iron by strategy II (Siderophore-mediated) mechanism (Inoue *et al*., 2004) [13]. Soybean being a dicotyledonous plant, acquire iron by Strategy I mechanism, wherein the plants induce an Iron-Stress Response (ISR) under conditions of iron deficiency stress, which involves increase in acidification of the rhizosphere, release of reductants and organic acids in the root soil interface (Zocchi *et al.*, 2007) [14]. Iron uptake by the plant roots results from a series of complex interactions between plant and soil within the rhizosphere (Briat *et al*., 2015) [3]. Solid phase modulation of iron in soils, the chemical speciation of iron in solution,

the redox status, the synthetic and natural chelates in transport processes that occur near roots, interaction of iron with other soil constituents are all soil-dependent factors that determine the bioavailability of iron (Lindsay, 1995) [15].

Climate change is a factual reality that we are witnessing in every corner of the world (Pachauri *et al.,* 2014) [16]. It was projected by the Intergovernmental Panel on Climate Chnge (IPCC) that, by the end of this century, the concentration of ambient atmospheric CO_2 would expected to rise from present 400 µmol mol^{-1} to 540-958 µmol mol^{-1} coupled with an average increase in surface air temperature by 3 to 4 ºC (Stocker *et al.,* 2014) [17], which would directly or indirectly results in undesirable impact on crop production (Tausz *et al.*, 2013; Ainsworth and Ort, 2010; McMichael, 2011) [18-20]. The average ambient atmospheric concentration is increasing at an alarming rate of 2 µmol mol^{-1} (Shiogama *et al*., 2016) [21].

Regulation of Fe stress response under climate change scenario and the underlying mechanisms are not well understood. Intra-specific variability in growth and yield response to elevated CO_2 has been reported for wheat (Ziska, 2008) [22], rice (Gillespie *et al.*, 2011; Shimono, 2011; Shimono *et al*., 2010; Baker, 2004) [23-26] and soybean (Sicher *et al.,* 2010; Ziska and Bunce, 2000) [27, 28] *etc*. Till today, the most effective strategy for combating iron deficiency chlorosis is adoption of iron-efficient genotypes (Wang *et al.,* 2008; Semenov *et al.,* 2009) [29, 30]. Yet, little is known about potential changes in the response of soybean plants, especially when grown on calcareous soil, to the iron limiting stress under climate change scenario. Understanding the intra-specific variability between contrasting genotypes and their response to elevated CO_2 and temperature is a key prerequisite to remediate emerging Fe deficiency of plants on calcareous soils. Selection of promising and better performing cultivars of a crop will be an important determinant for adaptation in the face of a changing climate (Ainsworth and Ort, 2010; Semenov *et al.,* 2009) [19, 30]. Thus, we conducted a hydroponic experiment to understand iron stress response of two contrasting soybean genotypes under the simulated climate change scenario using plant growth chambers. For this purpose, Fe efficient and responsive (FeER) and Fe inefficient and responsive (FeIR) genotypes of soybean were used and raised on calcareous Vertisol under elevated CO_2 and temperature conditions at the National Phytotron Facility of the Institute. The objectives of the experiments were to assess the adaptive strategies of soybean genotype to minimise iron limiting stress to the combined climate change factors (CO_2 and temperature), which could be useful in exploiting the intra-specific variations to the stress tolerance for future breeding programmes.

Materials and Methods

Plant material: Two contrasting genotypes of soybean *viz*. NRC-45 and IC-18374, which differ in iron stress response, were used as iron efficient and responsive (FeER) and iron inefficient and responsive (FeIR) category, respectively. The genotypes were identified from an earlier screening experiment of fifty genetically diverse soybean genotypes (Raj *et al*, 2019a) [1]. The genotypes were collected from the germplasm maintained at Soybean Breeding Laboratory of the Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi, India.

Experimentation: Seeds of soybean were surface sterilized with 0.1% $HgCl_2$ (w/v), washed thoroughly with double distilled water, which were then germinated on white sand. After separating out the cotyledons, five days old seedlings were kept individually in 250 mL Erlenmeyer flasks containing 100 mL of one-fifth strength Hoagland solution with sufficient Fe concentration. Only healthy and uniform seedlings were transferred to the culture solution. Modified Hoagland solution (Hoagland and Arnon, 1950) [31] was used in the experiment with the nutrient composition: KNO_3 (16,000 µM), $Ca(NO_3)_2.4H_2O$ (6000 µM), $NH_4H_2PO_4$ (4000 µM), $MgSO_4.7H_2O$ (2000 µM), KCl (50 µM), H_3BO_3 (25 µM), Fe-EDTA (25 µM), $MnSO_4.4H_2O$ (2 µM), $ZnSO_4$ (2µM), $Na_2MoO_4.2H_2O$ (0.5 µM) and $CuSO_4.5H_2O$ (0.5 µM). The plants were then grown for 24 days in full strength aerated Hoagland solution. Three known sources of variation (each at two levels) namely (i) genotype (FeER *vs.* FeIR), (ii) environment (a-[CO_2+T] (400±10 µmol mol^{-1}, 30 ºC/22 ºC) and e-[CO_2+T] (610±10 µmol mol^{-1}, 34 ºC/26 ºC)) and (iii) bicarbonate ion (-HCO_3^- *vs*. +HCO_3^- containing 0 and 20 mM $NaHCO_3$, respectively) were used as the fixed factors in this experiment. Three replications were maintained for each treatment combinations. The entire experiment was carried out in plant growth chambers of the National Phytotron Facility, New Delhi, India with 12 h photoperiod, 850 mmol m^{-2} s^{-1} photon flux density and 85% relative humidity.

Chlorophyll content: Chlorophyll content was measured in the recently matured fully expanded compound leaves at 24 days after planting (Hiscox and Israelstam, 1979) [32]. Accurately weighed 100 mg of clean pieces of fresh leaf sample, immersed in 10 mL of dimethyl sulphoxide (DMSO) solution and incubated at 70 ^{0}C for 4 h. An aliqot of 1 mL was diluted to 5 mL with pure DMSO and the absorbance of the samples were measured at 645 and 663 nm using double beam spectrophotometer with pure DMSO as the blank.

Iron concentration in shoot: Powdered plant samples were pre-digested with concentrated nitric acid followed by di-acid digestion (HNO_3: $HClO_4$ in the ratio 3:1). The volume of digested samples was made up to 50 mL for total iron analysis and the concentration was measured using atomic absorption spectrophotometer (Jackson, 1973) [33].

Total dry matter production and root/shoot ratio: After 24th days of hydroponic culture, plants were taken out; root and shoot were separated out, dried in shade for 24 h and thereafter at 70±2 ºC in hot air oven, till consecutive measurement gave constant weights. The root/shoot ratio was obtained by dividing the dry weight of root with that of shoot.

Proton (H^+) extrusion capacity: Extrusion of protons from roots was determined in hydroponic plants by the method described (Kabir *et al*., 2012) [34]. After 24 days of hydroponic culture, both the soybean genotypes were transferred to small vials containing 50 mL of nutrient solution. Adequate quantity of 50 mM KOH was added in order to increase the pH to 8.0, whereas in control no KOH was added which has pH 6.0. The pH was measured with digital pH meter and was maintained in subsequent days by addition of 0.1 M HCl or 0.1 M KOH. Proton (H^+)-efflux was estimated by measuring the titrated amount of acid or base to restore pH to its starting point.

Ferric chelate reductase (FC-R) activity: Apical root segments (6-8 mm long) of 1 g fresh weight were rinsed with 0.2 mM $CaSO_4$ solution for 5 min and incubated in 50 mL fresh nutrient solution supplemented with 0.3 mM

bathophenanthroline disulfonate (BPDS) (Sigma-Aldrich, St. Louis, MO, USA) and 100 µM Fe(III)-EDTA. The pH of the assay solution was adjusted to 5.5 with 5mm morpholine ethane sulfonic acid (MES)-NaOH. The beakers were fully covered with aluminium foil to avoid direct exposure to light. Upon reaction, BPDS forms a water soluble and stable red coloured complex with Fe^{2+} and only a weak complex with Fe^{3+}. After 2-6 h incubation at 23±1 ^{0}C for 1 h (with periodic swirling once in every 10-15 min), aliquots drawn from each sample and absorbance was measured at 535 nm with Hitachi U-3210 spectrophotometer. From the standard calibration curve, FC-R activity was measured and expressed in µmol Fe^{2+} reduced g^{-1} h^{-1} root FW (Chaney *et al.*, 1972) [35].

Low molecular weight organic acid exudation by root: Plants with four fully expanded trifoliate leaves (24 days after transplanting) were taken out of hydroponics system, and the roots were rinsed with double distilled water. Root exudates were collected using 50 mL of 0.05 mM $CaCl_2$ solution in 100 mL conical flask (Dong *et al.*, 2004) [36] and were kept in the respective growth chambers. After 4h, root exudates were collected from each of the flask, eluted through Amberlite resin IR 120 (H) filled in a glass column (10 cm 9 1.8 cm) and filtered through 0.4 lm filter before injecting into high pressure liquid chromatography (Agilent Technologies, 1200 Infinity Series, Santa Clara, CA, USA). The reverse phase column Hamilton PRP-1 was used and 0.1% Orthophosphoric acid as the mobile phase with a flow rate of 1 mL per min, at wavelength 210 nm using VWD (variable wavelength detector). Run time was 25 min for individual samples. For peak identification and quantification, calibration was performed using serial dilutions of standards of oxalic, citric, malic, and tartaric acids and was expressed in µmol g^{-1} root fresh weight.

Statistical analyses: Statistical analyses for differences among each dependent variables were compared against the fixed factors such as environmental conditions (a-[CO_2+T] *vs.* e-[CO_2+T]), genotype (FeER *vs.* FeIR), bicarbonate (HCO_3^-) ion (-HCO_3^- *vs.* +HCO_3^-) and their interactions were performed using General Linear Models programme of SAS 9.2. Statistical significance between treatment combinations were performed using p value associated with the pre-planned comparison of Least Square (LS) means using SAS 9.2 (SAS Institute Inc. 2009. SAS OnlineDoc®9.2**.** Cary, NC: SAS Institute Inc.).

Results and Discussions

Plant growth under more stressful growing environment e-[CO_2+T] in the presence of bicarbonate ion resulted in significant reduction in total chlorophyll content, chl a and chl b content in leaves (Table 1). In respect to total chlorophyll content, on an average, plants grown in the absence of bicarbonate have significant higher total chlorophyll content (1.79±0.04 mg g^{-1}, mean ± SE) than plants grown in presence of bicarbonate (1.48 ± 0.06 mg g^{-1}), which is attributed to the bicarbonate effect (B effect $p< 0.0001$).

Table 1: Effect of environmental condition (E), genotype (G) and bicarbonate (B) on chlorophyll content, iron concentration in shoot, dry matter production and root/shoot ratio of soybean plants grown in hydroponic culture for 24 days

Environ. condition (E)	Genotype (G) and bicarbonate (B)	Total Chlorophyll (mg g^{-1})	Chl a (mg g^{-1})	Chl b (mg g^{-1})	Fe conc. in shoot (mg kg^{-1})	Total dry matter (g $plant^{-1}$)	Root/Shoot ratio
a-[CO_2+T]	FeER (-HCO_3^-)	1.94±0.02^{a}	1.45±0.01^{a}	0.51±0.01^{a}	48.30±1.03^{a}	0.49±0.01^{a}	0.29±0.01^{d}
	FeIR (-HCO_3^-)	1.70±0.01^{bc}	1.34±0.01^{c}	0.40±0.02b^{c}	45.80±0.93^{b}	0.41±0.00^{bc}	0.28±0.02^{d}
	FeER (+HCO_3^-)	1.70±0.02^{bc}	1.32±0.02^{c}	0.38±0.01^{c}	39.56±0.86^{c}	0.36±0.01^{c}	0.50±0.01^{bc}
	FeIR (+HCO_3^-)	1.48±0.01^{d}	1.25±0.01^{d}	0.23±0.01d^{e}	30.33±1.08^{de}	0.26±0.01^{d}	0.37±0.03^{c}
e-[CO_2+T]	FeER (-HCO_3^-)	1.89±0.02^{b}	1.43±0.01^{b}	0.44±0.02^{b}	44.60±0.88^{b}	0.47±0.00^{b}	0.38±0.02^{c}
	FeIR (-HCO_3^-)	1.64±0.01^{c}	1.30±0.01^{cd}	0.30±0.02^{cd}	31.50±1.09^{d}	0.28±0.00^{d}	0.40±0.04^{c}
	FeER (+HCO_3^-)	1.54±0.01^{cd}	1.29±0.03^{cd}	0.25±0.02^{d}	35.45±0.78^{cd}	0.33±0.01^{c}	0.65± 0.03^{a}
	FeIR (+HCO_3^-)	1.18±0.01^{e}	1.01±0.01^{e}	0.17±0.02^{e}	23.22±1.11^{de}	0.27±0.01^{e}	0.59±0.03^{b}

Mean ± standard error of three replications are presented; different letters indicates significant difference between plant species at $P<0.05$

Table 2: P value table of different factors *viz.* environmental condition (E), genotype (G) and bicarbonate (B) on chlorophyll content, iron concentration in shoot, dry matter production and root/shoot ratio of soybean plants grown in hydroponic culture for 24 days

Parameters	Genotype (G)	Environ. condition (E)	Bicarbonate (B)	G x E	G x B	E x B	G x E x B
Total Chl.	<0.0001***	<0.0001***	<0.0001***	0.0007**	0.0223*	<0.0001***	0.0021**
Chl a	<0.0001***	<0.0001***	<0.0001***	<0.0001***	0.0083**	<0.0001***	<0.0001***
Chl b	<0.0001***	<0.0001***	<0.0001***	0.3969^{ns}	0.6692^{ns}	0.0775^{ns}	0.0448*
Fe conc.in shoot	<0.0001***	<0.0001***	<0.0001***	0.0002***	0.0498*	0.0259*	0.0142*
Total dry matter	<0.0001***	<0.0001***	<0.0001***	0.0133*	0.0005***	<0.0001***	<0.0001***
Root/Shoot ratio	0.0180*	<0.0001***	<0.0001***	0.1894^{ns}	0.0103*	0.0377*	0.5379^{ns}

P values of three way ANOVA; Significance levels $^{***}P< 0.001$; $^{**}P<0.01$; $^{*}P<0.05$; ^{ns} not significant at P>0.05

A similar trend for bicarbonate effect was noticed on the contents of chlorophyll a (mean±SE values of 1.38±0.02 mg g^{-1} *vs.* 1.22±0.02 mg g^{-1}) and chlorophyll b (mean±SE values of 0.41±0.02 mg g^{-1} *vs.* 0.26±0.02 mg g^{-1}). Genotype effect (G effect), environmental effect (E effect) and their interaction with bicarbonate (G x E x B) have significant influence on total chlorophyll content, chlorophyll a and chlorophyll b content in soybean plant (Table 2). Previous reports revealed that iron limiting condition disturb both chloroplast structure and rate of photosynthesis, which ultimately results in reduced dry matter production (Raj *et al.*, 2020) [37]

Across the treatment combinations, iron concentration in shoot varied between 23.22 ± 1.11 mg kg^{-1} (FeIR grown in the presence of bicarbonate ion under e-[CO_2+T]) to 48.30±1.03 mg kg^{-1} (FeER grown in the absence of bicarbonate ion under a-[CO_2+T]). Genotype, environment and the presence of bicarbonate have significant influence on shoot iron concentration $p< 0.0001$ (Table 2). Further, G x E (p=0.0002), G x B (p=0.0498), G x E x B (p=0.0142) interactions were have a significant influence on shoot iron concentration. Bicarbonate has significant effect on total dry matter production with the values 0.34±0.02 g $plant^{-1}$ (average

response all treatment combinations receiving +HCO_3^-) *vs.* 0.53±0.03 g plant^{-1} (average response all treatment combinations receiving -HCO_3^-); B effect $p< 0.0001$) as well as root/shoot ratio with average response values 0.41± 0.02 (+HCO_3^-) *vs.* 0.31±0.01 (-HCO_3^-); $p< 0.0001$). Among the treatment combinations, the total dry matter production dropped by ~1.8 times from 0.49±0.01 g plant^{-1} (FeER grown in the presence of bicarbonate ion under a-[CO_2+T]) to 0.27±0.01 g plant^{-1} (FeIR grown in the presence of bicarbonate ion under e-[CO_2+T]) (Table 1). We observed that the interaction between the factors *viz.* G x E (p=0.0133), G x B (p=0.0005), G x E x B (p<0.0001) on total dry matter production were significant (Table 2). Conversely, significant increase in root/shoot ratio was also observed under the more stressed condition, from 0.28±0.02 (FeIR grown in absence of HCO_3^- ion under a-[CO_2+T]) to 0.65±0.03 (FeER grown under e-[CO_2+T] in presence of bicarbonate ion). The more stressful growing conditions of e-[CO_2+T] together with HCO_3^- ion increased the root/shoot ratio implying greater biomass partitioning to the root system so as to exploit greater volume. The root systems are designed to explore water and nutrients from the the soil mass, profused early growth with higher root biomass, can contribute to better mineral nutrition to the crop plants (Tausz *et al.*, 2017) [38]. Iron limiting stress induce lower chlorophyll content which in turn resulted in reduced biomass production (Raj *et al.*, 2019a) [1].

We have also studied the effect of genotype, environment, bicarbonate and their interactions on exudation of proton, oxalic, citric, malic and tartaric acids by the roots. Statistical tests within HCO_3^- treatments (+HCO_3^- *vs.* -HCO_3^-) revealed that environment (E effect), genotype (G effect) and their interaction (G x E) were significant (Figure 1, 2 and ANOVA result in Table 3). Among the treatment combinations, presence of bicarbonate in the media (B effect) exacerbated iron deficiency stress and consequently resulted in higher proton extrusion (~1.2 fold increase), lower ferric chelate reductase activity (~1.3 fold decrease) and low molecular weight organic acid exudation (upto ~1.9 fold increase in malic acid). The bicarbonate effect was found to increase, on an average, the proton extrusion (mean±SE values of 1.90±0.09 *vs.* 2.25±0.11), exudation of oxalic acid (20.37±2.10 *vs.* 22.61±2.73 µmol g^{-1} fresh weigh (fw) root), citric acid (15.97±2.26 *vs.* 22.36±2.95 µmol g^{-1} fw root), malic acid (5.59±1.28 *vs.* 10.22±1.45 µmol g^{-1} fw root) and tartaric acid (3.60±0.33 *vs.* 3.70±0.33 µmol g^{-1} fw root). In contrast, the FCR activity was decreased by bicarbonate effect (0.61±0.07 *vs.* 0.48±0.03 µmol Fe (II) g^{-1} h^{-1}). Within each set of treatment of [CO_2+T] and HCO_3^- ion, iron stress response mechanisms were more pronounced in FeER than FeIR genotype. Further, with the exception of exudation of tartaric acid, the G x E interaction, G x B interaction, G x E x B interactions were significant for other iron stress response in soybean plants (Table 3). When the plants were grown under less stressful condition of a-[CO_2+T] in the absence of HCO_3^- ion, higher chlorophyll content, Fe concentration in the shoot and total dry matter production were observed (Table 1). To warfare the constraints in Fe availability, especially under more stressed e-[CO_2+T] environmental condition in the presence of bicarbonate ion, the root system of iron efficient genotype of soybean (FeER) exuded out significantly higher amount of low molecular weight organic acids (Fig. 2).

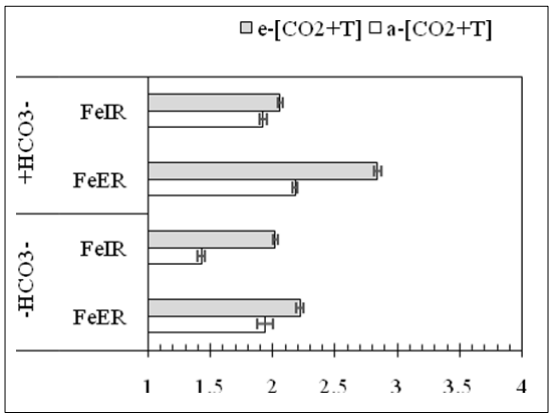

Fig 1: Effect of different factors *viz.* environmental condition (E), genotype (G), bicarbonate ion (B) and their interaction on proton extrusion by the roots of soybean plants grown in hydroponic culture for 24 days

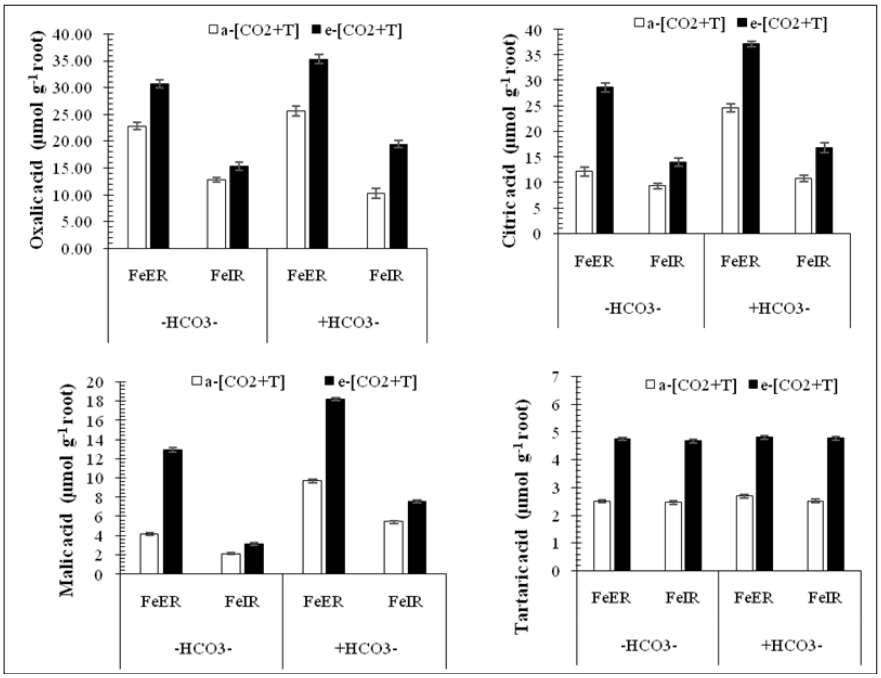

Fig 2: Effect of different factors *viz.* environmental condition (E), genotype (G), bicarbonate ion (B) and their interaction on citric, oxalic, malic and tartaric acid exudation by the roots of soybean plants grown in hydroponic culture for 24 days

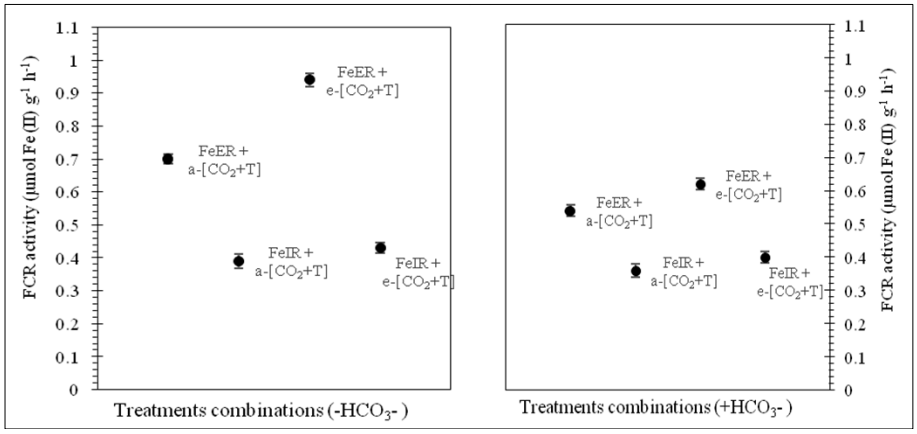

Fig 3: Effect of different factors *viz.* environmental condition (E), genotype (G) bicarbonate ion (B) and their interaction on ferric chelate reductase (FCR) activity by the roots of soybean plants grown in hydroponic culture for 24 days

Ferric chelate reduction was the rate-limiting step for iron acquisition, which was found to be influenced by the effect of genotype, environment, bicarbonate as well as their interactions (Fig. 3 and Table 3). Moreover, ferric chelate reductase activity was significantly reduced by the influence of HCO_3^- ion (B effect $p < 0.0001$; Figure 3 and Table 3). The findings corroborated well with the report of inhibitory effect of HCO_3^- ion on Fe (III) reducing capacity of roots was previously documented in crop plants (Schroeder *et al.*, 2013; Romera *et al*., 1997) [39, 40].

Table 3: P value table of different factors *viz.* environmental condition (E), genotype (G) and bicarbonate (B) on proton extrusion, low molecular weight organic acid exudation and FCR activity of soybean plants grown in hydroponic culture for 24 days

Parameters	Genotype (G)	Environmental condition (E)	Bicarbonate (B)	G x E	G x B	E x B	G x E x B
H^+-extrusion	$<0.0001^{***}$	$<0.0001^{***}$	$<0.0001^{***}$	0.0381*	0.0027**	0.4622^{ns}	<0.0001
Oxalic acid	$<0.0001^{***}$	$<0.0001^{***}$	0.0011^{**}	0.0205^{*}	0.0216^{*}	0.0019^{**}	0.0459^{*}
Citric acid	$<0.0001^{***}$	$<0.0001^{***}$	$<0.0001^{***}$	$<0.0001^{***}$	$<0.0001^{***}$	0.2237^{ns}	0.0333^{*}
Malic acid	$<0.0001^{***}$	$<0.0001^{***}$	$<0.0001^{***}$	$<0.0001^{***}$	$<0.0001^{***}$	0.0421^{*}	0.0068^{**}
Tartaric acid	0.0722^{ns}	$<0.0001^{***}$	0.0371^{*}	0.5303^{ns}	0.6885^{ns}	0.6069^{ns}	0.3362^{ns}
FCR activity	$<0.0001^{***}$	$<0.0001^{***}$	$<0.0001^{***}$	0.0003^{***}	$<0.0001^{***}$	0.0069^{**}	0.0069^{**}

P values of three way ANOVA; Significance levels $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$; ^{ns} not significant at P>0.0

Bicarbonate ion (HCO_3^-) inhibits the activity of ferric chelate reductase enzyme in peach roots under iron limiting stress (Molassiotis *et al.*, 2006) [41]. Besides, it was also confirmed that HCO_3^- offset the expression of genes related with ferric reductase (FRO) and iron transporter (IRT1), as well as the activity of the corresponding enzymes, FC-R in roots of several species such as arabidopsis, cucumber, tomato and pea (Lucena *et al.*, 2007) [42]. Bicarbonate ion, through its ability to buffer the pH, counteract the proton exuded by the membrane proton pumps, thereby prevent acidification of the medium and eventually restrain generation of transmembrane electrochemical gradient (Mengel, 1994; Mengel *et al.*, 1994) [43, 44]. Since FC-R activity is pH dependent (Susin *et al.*, 1996) [45], the HCO_3^- induced rise in the poplast pH may suppress reduction of Fe^{3+} to Fe^{2+} (Gharsalli and Hajji, 2002) [46], and which might resulted in reduced iron uptake into the symplasm.

Iron stress response prospects in soybean under elevated CO_2 and temperature condition depend on three critical factors that vary between genotypes: the compulsions of increased proton extrusion, root exudation, and ferric chelate reductase activity by the candidate genotypes. FeER recorded an impressive performance, as compared to FeIR, in counteracting iron deficiency stress, but went through modest stress to ward off the challenge exerted by the combined interaction between the genotype, environment and HCO_3^- ion. The FeER was always forced to reckon and adapted strategies to cope up with the nutrient limiting stress by enhanced proton and low molecular weight organic acid exudation together with a higher ferric chelate reductase activity.

Conclusion

The genotype FeER recorded an impressive performance, as compared to FeIR, in counteracting iron deficiency stress, and underwent modest stress to ward off the challenge put forth by the combined interaction between the genotype, environment and HCO_3^- ion. Exposure of soybean plants to the more intense stress resulted in lower chlorophyll content, lower dry matter production and higher root/shoot ratio. The FeER always forced to reckon and adapted strategies to cope up with the nutrient limiting stress by means of enhanced proton and low molecular weight organic acid exudation coupled with higher ferric chelate reductase (FCR) activity. Iron stress response prospects by soybean genotypes to the elevated CO_2 and temperature condition rely on three critical factors: the compulsions of increased proton extrusion, increased root exudation, and altered ferric chelate reductase activity that varied between the candidate genotypes. The better adaptive strategies by nutrient efficient soybean genotypes under iron limiting stress provide opportunities to structure the future breeding prospects under climate change scenario.

Conflict of interest: No conflict of interest in the present study

Acknowledgement

The authors are grateful to International Plant Nutrition Institute (IPNI), USA and University Grant Commission (UGC), India for the necessary supports. We are grateful to Indian Council of Agricultural Research for providing institutional support.

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