Indian Journal of Plant and Soil
Volume 3 Number 1, January - June 2016

Original Article

Calcium Ameliorates Bronzing in Rice (*Oryza Sativa* L.) Under Field Condition

Minsura Begum, Bhagawan Bharali

Author's Affiliation: Department of Crop Physiology, Assam Agricultural University.

Abstract

Amelioration of iron toxicity (i.e. Bronzing) by pulsed [Ca^{2+}] through root dip treatment (RDT) of transplanted Bahadur (iron susceptible) and Mahsuri (iron resistant) rice varieties were studied in field condition (initial soil pH 4.68, mean [Fe^{2+}] =954.98.ppm). An increase in [Ca^{2+}] in leaf was detected commensuration with [$CaCl_2$] » 100-1000ppm in RDT for 24h, which was higher in Bahadur > Mahsuri. A decline in soil pH (increased acidity) was observed along with the days after transplanting. So, available [Fe^{2+}] in soil decreased from 67% to 80% at 30-90 DAT. The [Fe^{2+}] in soil caused reduction in chlorophyll (≈54%). Increase in [$CaCl_2$] **>**100ppm in RDT lessened chlorophyll (≈21.77%) and leaf area (≈18.11%) at 90DAT of the crop. Over all, Bahadur was superior than Mahsuri in most of the physiological parameters viz., panicle no.m^{-2} (by 28.20%), no. of spikelet per panicle (by 23.76%), 1000 grain weight (by 24.78%), sink capacity (by 59.77%) and economic yield (by 43.09%). 100ppm $CaCl_2$ RDT brought positive relationship between yield and its attributes viz., spikelet, r =0.264, 0.657, test weight, r=0.315, 0.402, sink capacity, r=0.196, 0.491) in Bahadur and Mahsuri respectively at 30 days after transplanting under higher iron condition in field.

The paradigm of Bronzing in rice caused by higher [Fe^{2+}] in leaf derived from acid soil, and prophylactic role of Ca^{2+} ameliorating the disorder in field condition is discussed in this paper.

Keywords

Acid Soil; Bronzing; Calcium; Chlorophyll; Correlation Coefficient (R); Iron; Leaf Area; Root-Dip; Sink Capacity; Test Weight.

Introduction

Iron toxicity is commonly known as 'Bronzing' (i.e. development of yellowing of leaves, dark brown spots followed by radish brown colouration) in India. It is caused by excess of available iron (Fe^{2+}) mobilised passively from acid soils into the Donnan Free Space of plant cells (Brown and Wells, 1988). So, Bronzing might be one of the yield limiting factors in wetland rice (Bridgit et al., 1993). Bronzing has been a chronic problem leading to reduction of yield of kharif rice in Assam (Medhi et al., 1975). The iron contents in Bronzed plant is three times higher than in normal one (Verma, 1991) viz., 209-630 ppm at panicle initiation stage (Singh et al., 1992) and 209-630ppm at flowering stage (Mahapatra et al., 1985).

Plants exhibit yellowing of green leaves thirty days after transplanting at 400ppm of iron; however it recedes after seventy days after transplanting (DAT). Rice shows potassium deficiency symptom at 150-450ppm available iron in soil solution. Leaf chlorosis occurs at 350-450ppm, dark brown spots followed by Bronzing are observed at 450-780ppm, and a concentration of available iron between 800-1200ppm in soil becomes lethal to the plants (Borah and Borkakati, 1997). It's quite amazing that iron concentration as low as 45ppm causes Bronzing in rice (Baba, 1958), whereas a concentration as high as 500ppm may not show the Bronzing in rice (Takagai, 1958). However, in general, 650ppm iron on plant dry weight basis is considered as a tentative critical limit of iron for Bronzing in rice in the Himalayan region. Rice plants are characterised as deficient, low and sufficient with less than 0.1 per cent, 0.1-0.2 per cent and more than 0.2 per cent Ca^{2+} respectively (Tanaka and Yoshida, 1970). Calcium concentration

---

Corresponding Author: Bhagawan Bharali, Professor and
Head, Department of Crop Physiology,
Assam Agricultural University Jorhat-785013. Assam.
E-mail: bbharali33@rocketmail.com

in different plant parts increases with an increase in Ca^{2+} supply (Jain et al., 1997). The role of Ca^{2+} on amelioration of physiological disorders in rice caused by higher iron (400ppm $FeSO_4.7H_2O$) in acid soil under laboratory pot culture condition had been already reported by Sarma and Bharali (2015). However, information on physiological characteristics of kharif rice plants uploaded with Ca^{2+} by $CaCl_2$ root dip treatment (RDT), and transplanted upon natural acidic soil having higher iron concentration in field condition was lacking. Therefore, the further study was undertaken to ascertain the role of Ca^{2+} on amelioration of Bronzing in rice under field condition.

Materials and Methods

A field experiment during wet season (2003) was conducted in the ICR farm of Assam Agricultural University to examine the role of Ca^{2+} uploaded into rice plants through its RDT in $CaCl_2$ solutions. The experiment was laid in factorial RBD with four replications (Panse and Sukhatme, 1978). The cumulative rainfall and bright sunshine during the growing period were 1116.10mm and 900.1 hours respectively. The mean maximum temperature (32.94^{0}C) was found in July, and it gradually receded to a minimum of 11.9^{0}C in December at Jorhat (26.47oN latitude, 94.12oE longitude and 86.6 m altitude).

The certified seeds of two contrasting rice varieties viz., Mahsuri (iron resistant) and Bahadur (iron susceptible) were collected from the Regional Agricultural Research Station, AAU (Titabar). The experimental plot contained a maximum of 976.56 ppm available Fe^{2+} estimated by colorimetric method (Krishnamurty et al., 1970). N, P, and K in terms of Urea, Single Super Phosphate (SSP) and Muriate of potash (MoP) @40:20:20 Kgha^{-1} were applied in splits. Half of Urea, the whole of SSP and MoP were supplied as basal. The rest half of Urea was dissolved in distilled water and sprayed on the foliage at tillering satge of the crop (45DAT). Thirty days old seedlings of both the rice varieties were uprooted, washed with tap water gently to remove the soil particles adhered to root surfaces. The cleaned roots of the rice varieties were dipped in $CaCl_2$ (0, 100, 500, 1000ppm) solutions. A bulk of 900 seedlings of each variety was treated in 1.5 litres of each solution once for a period of 24h (Nayak et al., 1983) prior to transplanting.

Soil pH was determined using a digital pH meter at 0 (initial) 30, 60 and 90 DAT. A sample of 50g soil was taken in a beaker and then added 50ml of distilled water into it. The solution was stirred by a glass rod for uniform mixing of the soil particles in water. The pH meter was standardised using buffer solutions at pH 4.0 and pH 7.0. The electrode of the pH meter was rinsed with distilled water to avoid any contamination after dipping either in the buffer or the sample solutions. Available N (Perur et al., 1973), P (Jackson, 1973) and K (Jackson, 1973) were determined in soil samples initially. The exchangeable [Ca^{2+}] in root samples before RDT and before transplanting (i.e. at 0 DAT), and both exchangeable [Fe^{2+}] and [Ca^{2+}] in leaf samples at 30, 60 and 90 DAT were determined by EDTA method (Jackson, 1973). All the physiological parameters were measured based on five hills collected time to time from each plot at 30, 60 and 90 DAT. Total chlorophyll contents in green leaves were estimated using spectrophotometer (Arnon, 1949). The area of each green leaf was measured by portable laser leaf area meter (CID Inc.). Economic yield and yield attributes viz., panicle no. m^{-2}, total spikelet per panicle, 1000 grain weight (test weight), high density (HD) grains and sterility per cent (Bharali et al.,1995) were recorded at harvest. Sink capacity (Pancle no.m^{-2} x spikelet per panicle x individual grain weight) was calculated following the method of Venkateswar and Visperas (1981). Data were analysed by two way factorial ANOVA using GLIM Compute program (Version 3.77, update 1, Royal Statistical Society, London (Crawley, 1993).

Results and Discussion

The paradigm of Bronzing in kharif rice and its physiological interference by Ca^{2+} pulse in roots under higher iron condition in field have been focussed in the present investigation. Soil status of the experimental plot was assessed. The soil was acidic in reaction (pH 4.68). The available N, P and K contents in soil were 244.61, 67.20 and 20.65 kg ha^{-1} respectively. The mean [Fe^{2+}] content in the soil at the time of transplanting of rice seedlings was 954.98ppm. To support the crop growth, Urea, SSP and MoP were applied basally, and as foliar spray at 30 DAT to provide the crop nutrient sufficiently during its growth period. The iron condition in the native soil was higher as compared to the critical iron concentration (i.e. 4.5-4.5 ppm) in soil, which causes Branzing in rice (Baba, 1958, Singh, 1995). In fact, a high amount of [Fe^{2+}] was facilitated by the low acidic condition (pH <5.0) in the experimental plots. Thus, the experimental site was found suitable for the field investigation into Bronzing and its amelioration by exogenously supplied Ca^{2+}.

A marked difference in soil pH (Figure 1) was observed at 30, 60 and 90 DAT in comparison to the soil pH at 0 DAT (before transplanting). It was interesting to note that soil pH accelerated gradually commensuration with the advancement of the crop growth period. One of the causes of this increment in soil pH might be the constant availability of water (5-10 cm) by either natural precipitation or intermittent irrigation in the field. So, the time period of field submergence after transplanting of rice crop has tremendous effects on drop of redox potential of soil (Bhattachyrya and Baruah, 1995). In the present study, soil pH rose fairly and remained near neutrality at the later stages of the crop growth. The higher soil pH values (low acidity) with the concurrent increment in growth indicated manifestation of lesser iron toxicity in crop plants. However, the study lacks the measurement of redox potential and cation exchange capacity (CEC) related to the fluctuation of soil pH during the crop growth period.

The available $[Fe^{2+}]$ in soil samples decreased from 67% to 80% at 30-90DAT while compared with the initial one (Table 1). The transformation of available iron into insoluble hydroxides at higher pH might be one of the major causes of the deceleration of $[Fe^{2+}]$ in soil at later DAT (Halder and Mandal, 1987; Borthakur and Bora, 1992).

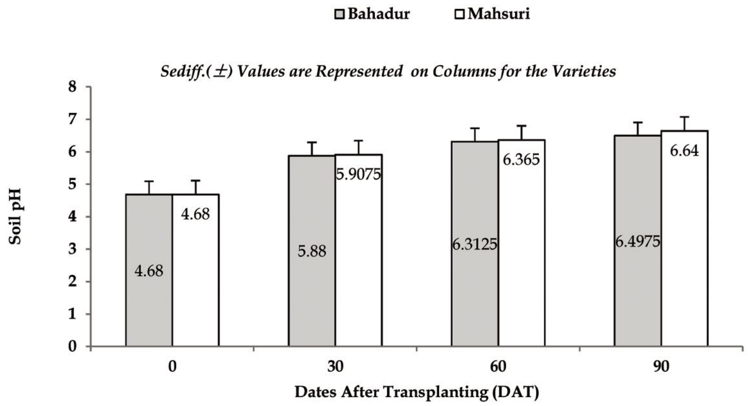

Fig. 1: Soil pH at DATs of rice plants under higher iron condition

Table 1: Available iron in growth medium at 0 (before transplanting), 30, 60 and 90 days after transplanting (DAT)

DAT	Available $[fe^{2+}]$ in soil (ppm)	Percentage change over initial period
0	1500	0
30	500	-66.67
60	400	-73.33
90	300	-80.00
Data are means of six samples at different DAT		

Data are Means of Six Samples at Different DAT

The background $[Fe^{2+}]$ at 0 DAT (before RDT) in plant leaves of Bahadur (425ppm) was 23.53% higher than in Mahsuri (325ppm). Bronzing disease appeared in the rice varieties transplanted onto the soil with higher native $[Fe^{2+}]$. Because, $[Fe^{2+}]$ in a range of 209-630ppm in plant may cause Bronzing symptoms in rice (Singh et al., 1992). In general, $[Fe^{2+}]$ in Bahadur was higher than in Mahsuri at 30 DAT. The dynamic nature of iron in leaf was recognised when the $[Fe^{2+}]$ increased (≈46%) at 30DAT, and decreased (≈60-90%) at 60 & 90DAT in comparison to the initial $[Fe^{2+}]$ in leaf. The rice varieties absorbed appreciable amount of Fe^{2+} at 30 DAT as the soil acidity at the stage was relatively higher (low pH) than at 60 and 90 DAT (Figure 2). A concentration of $[Fe^{2+}]$ >300ppm in leaf at flowering stage in rice was reported by Mahapatra et al. (1985). The greater decline in $[Fe^{2+}]$ in leaf might also be dependent on the oxidizing power of the rice roots (Fageria and Rabelo, 1987), which has not been explored in our study.

The mean $[Ca^{2+}]$ in plant roots before root deep treatment and transplanting was 100 fold higher in Bahadur (800ppm) than in Mahsuri (400ppm). The native $[Ca^{2+}]$ although found lower in plant, its concentration in leaf at all the periods of crop growth increased significantly with the increase in $[CaCl_2]$ in the root dip treatment. The susceptible variety Bahadur (2400, 2775, 3675, 4125 ppm) contained invariably higher $[Ca^{2+}]$ than in Mahsuri (2000, 2400, 3025, 3550 ppm) at 0, 30, 60 and 90 DAT respectively

(Figure 3).

The results suggest that rice plants probably are efficient in absorbing Ca^{2+} either from the incubation medium or the soil solution. Sarmah and Bharali (2015) also confirmed that there is substantial increase in $[Ca^{2+}]$ in roots after RDT pre treatment. The present study lacked the estimation of $[Ca^{2+}]$ in roots following RDT. However, the enrichment of $[Ca^{2+}]$ in leaves of the rice varieties had reflected its course of action in the physiological parameters as discussed in this paper.

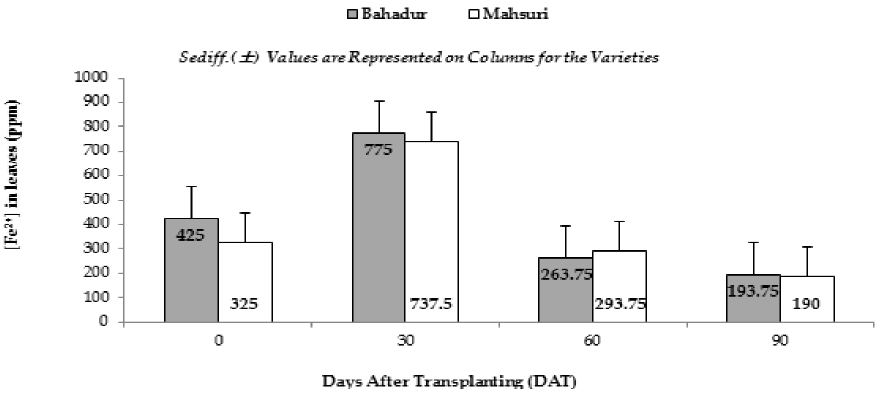

Fig. 2: Available $[Fe^{2+}]$ in rice leaves at different DAT

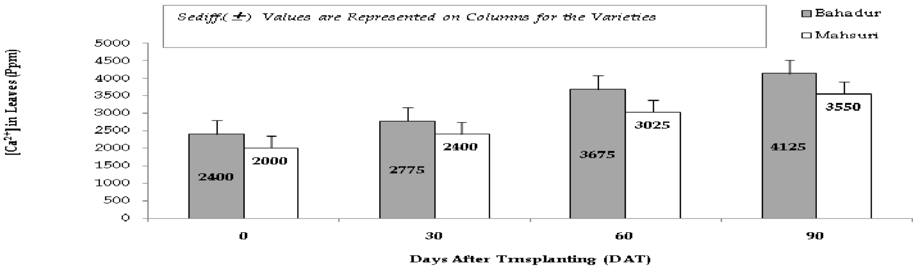

Fig. 3: Available $[Ca^{2+}]$ in rice leaves at different DAT

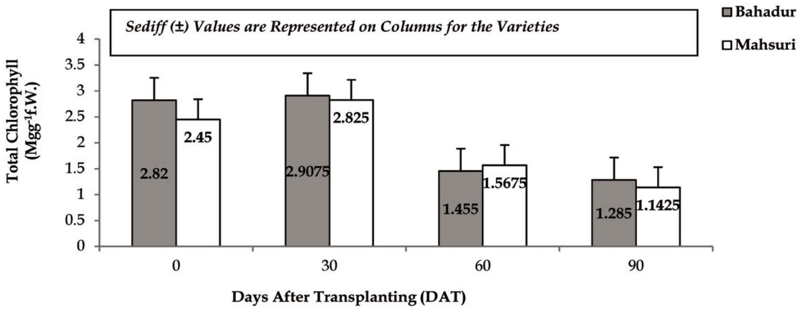

Fig. 4(a): Total chlorophyll in leaf at different DAT

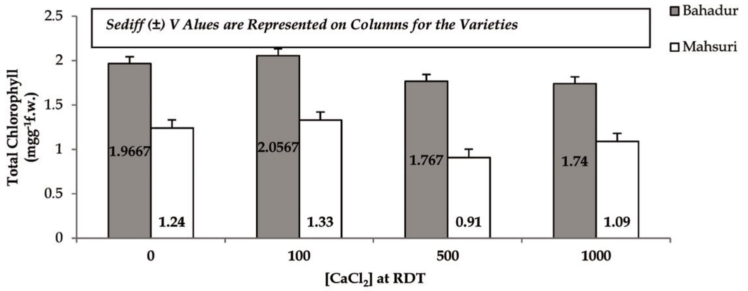

Fig. 4(b): Total chlorophyll in leaf following root dip treatment (RDT)

Chlorophyll content in plants at different dates after transplanting (Figure 4a) was higher in Bahadur (susceptible) than in Mahsuri (resistant). In general, decrease in chlorophyll contents was observed with the increase in [$CaCl_2$] in RDT (Figure 4b). There was increase in total chlorophyll contents in both the varieties at 60DAT, and it decreased at 90DAT commensuration with accelerated senescence. In the present work, there was evidence of Ca^{2+} enrichment in the leaves. But, instead of maintaining chlorophylls, it was probably involved in interfering iron toxicity. In many cases, Ca^{2+} deficiency in plants on acid soil leads to development of chlorosis (Shear, 1971). Increment in [Ca^{2+}] perhaps could not make up the Ca^{2+} requirement in the plants growing under higher iron condition. Excess [Ca^{2+}] in leaf may be another plausibility (Jain et al., 1997). Therefore, Ca^{2+} plays dual roles, and it must have a critical limit, excess of which exerts detrimental effects on plants.

Data portrayed in Table 2 revealed significant effects of $CaCl_2$ RDT on functional leaf area per hill at 30DAT only. Higher the concentration of $CaCl_2$ (i.e.1000ppm), more was the functional leaf area at 30DAT. Overall, Bhadur had 14.2% more functional leaf area than in Mahsuri at the stage. The variety Bahadur being susceptible had to acquire more amount of Ca^{2+} than Mahsuri at 60 and 90 DAT to cope up the higher iron situation. The number of tiller might have decreased due to tiller mortality and leaf number (data not reported for these parameters). So, functional leaf area also decreased at later (i.e. 90DAT) stage of the crop. Of course, the negative effects of higher iron on new tiller growth could not be eliminated. Functional leaf area declined either ways i.e. due to higher [Fe^{2+}] or higher [Ca^{2+}] in leaf as explained for chlorophyll synthesis in the foregoing discussion.

Higher iron concentration dissociates plasma membrane especially in root cells. Iron helps in catalytic conversion of H_2O_2 to oxygen radical responsible for breakdown of lipids (Price and Hendry, 1991). Since, the structural integrity of the membrane of lipid is disrupted (Fitter and Hay, 1981), permeability property is altered resulting in increased rate of solute leakage from the interior of the cell (protoplasm). Then, the cell no longer functions normally. Thus, it reduces the rate of nutrient absorption by root cells. The development of leaf area and shoot growth in conjunction with chlorophyll contents are retarded directly under higher iron condition. The existing work needs evidence on the nutritional aspects of the crop and measurement of membrane integrity as affected by higher iron in rice soils.

Calcium ion acted antagonistically with iron on Bahadur and Mahsuri plants. In the case of physiological observations, the response of plants to $CaCl_2$ was concentration limited. It had been previously described that Ca^{2+} is an important nutrient for root development and functioning (White and Broadly, 2003; Mengel and Kirby, 1978). Other way, Ca^{2+} protects membrane from free radical or peroxidative break down when only a large amount of Ca^{2+} binding is present in membranes. Ca^{2+} aids packing of lipids and brings about their aggregation (Ohishi and Ito, 1974). Bridging of membranes involving phosphate and $COOH^-$ groups, is brought about by Ca^{2+} to maintain its permeability (Nobel, 1974; Legge et al., 1982, Epstein, 1972, Bharali and Bates, 2004). Therefore, the mechanisms underlying the membrane stability by Ca^{2+} present in rice root and shoot tissues under higher iron condition in field condition remain as one of the major aspects for future investigation.

Table 2: Variation in functional leaf area (cm^2) per hill at 30, 60 and 90 days after transplanting (DAT) of rice plant under higher iron condition

RDT	30 DAT		60DAT		90DAT	
	Bahadur	**Mahsuri**	**Bahadur**	**Mahsuri**	**Bahadur**	**Mahsuri**
0ppm $CaCl^2$	406.2	273.4	3693	3113	1735	1171
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
100ppm $CaCl_2$	334.8	412.5	4506	3811	1469	1470
	(-21.33)	(33.72)	(18.04)	(18.32)	(-18.11)	(20.34)
500ppm $CaCl_2$	443.1	320.9	3878	30.57	4809	1431
	(8.33)	(14.80)	(4.77)	(-1.83)	(63.92)	(18.17)
1000ppm $CaCl_2$	473.5	418.5	2595	3247	1275	1398
	(14.21)	(34.67)	(-42.31)	(4.13)	(-36.08)	(16.24)
	SEDiff(±)	LSD(0.05)	SEDiff(±)	LSD(0.05)	SEDiff(±)	LSD(0.05)
Variety	40.77	86.90	356.39	n.s.	877.72	n.s.
$CaCl_2$	57.08	122.89	473.93	n.s.	1244.42	n.s.
Variety x $CaCl_2$	78.48	170.99	668.74	n.s.	1773.01	n.s.

Data are means of three replications, data in parenthesis are the per cent changes over control, n.s. : non significant

There were intrinsic varietal variation in terms of yield and yield attributing parameters in rice rather than direct effects of [$CaCl_2$] on the plants. Bahdur showed superiority in almost all yield attributes over

Mahsuri. In both Bahadur (16.12%) and Mahsuri (15.69%), higher spikelet number per panicle was found in 100ppm $CaCl_2$ treated plants as compared to control. Overall, Bahadur possessed 23.76% more total spikelet per panicle at harvest than in Mahsuri (Table 3a). Although there was no significant difference in test weight due to $CaCl_2$ treatments, Bahadur showed 24.78% increase in test weight than in Mahsuri (Table 3b). Similarly, Bahadur had 28.20% higher panicle no m^{-2} than in Mahsuri. Of course, the highest no. of panicle m^{-2} was recorded in 1000ppm $CaCl_2$ for both the varieties (Table 3c).

The root deep treatment with $CaCl_2$ showed significant changes in High Density (HD) grains (Table 4a), percentage sterility of the rice varieties (Table 4b), Sink capacity (Table 4c), and grain yield (Fig. 5). The maximum number of HD grains was found in Bahadur (76.23%) at 500ppm [$CaCl_2$] RDT, whereas, Mahsuri (81.77%) produced more HD grains in 100ppm [$CaCl_2$] RDT. Overall, Mahsuri produced 14.33% higher HD grains due to lower sterility per cent than in Bahadur. The highest sink capacity was found in Bahadur (1113.7g) and Mahsuri (460g) treated with 100ppm $CaCl_2$. Overall, Bahadur had 59.77% more sink capacity than in Mahsuri. Similarly, Mahsuri responded poorly to $CaCl_2$ RDT than Bahadur in terms of grain yield. Bahadur produced 43.09% higher grain yield than in Mahsuri in the study.

Table 3: Variation in (a) spikelet no. per panicle, (b) test weigh and (c) no. of panicle m^{-2} at harvest under the influence of [$CaCl_2$] root dip treatment (RDT) on rice plant under higher [Fe^{2+}] condition

RDT	(a) No. of spikelets per panicle		(b) Test weight (g)		(c) No. of panicle m^{-2} area	
	Bahadur	Mahsuri	Bahadur	Mahsuri	Bahadur	Mahsuri
0ppm $CaCl_2$	122.25	100.75	25.10	17.75	308.7	224.7
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
100ppm	145.75	119.50	22.73	17.90	323.4	220.4
$CaCl_2$	(16.12)	(15.69)	(-10.43)	(0.84)	(4.55)	(-1.95)
500ppm	144.00	100.25	24.35	18.78	298.2	212.1
$CaCl_2$	(15.10)	(-0.49)	(-3.080	(5.48)	(-3.52)	(-5.94)
1000ppm	138.50	99.25	23.70	17.67	344.4	258.3
$CaCl_2$	(11.73)	(-1.510	9-5.91)	(-0.45)	(10.37)	(13.01)
	SEDiff(±)	LSD(0.05)	SEDiff(±)	LSD(0.05)	SEDiff(±)	LSD(0.05)
Variety	8.05	17.47	0.49	1.04	21.65	n.s.
$CaCl_2$	11.27	n.s.	0.67	n.s.	30.91	n.s.
Variety x $CaCl_2$	16.49	n.s.	0.93	n.s.	46.26	n.s.

Data are means of three replications, data in parenthesis are the per cent changes over control, n.s. : non significant

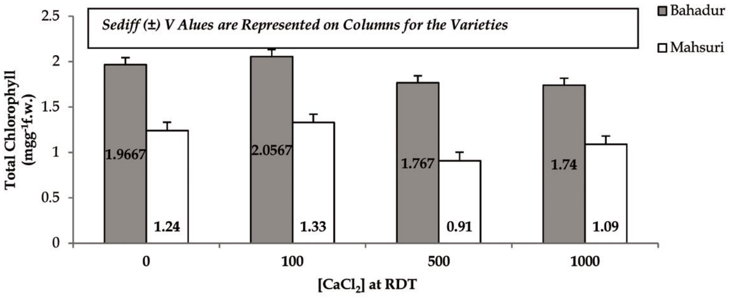

Fig. 5: Yield of rice varieties as influenced by $CaCl_2$ RDT under higher Fe^{2+} condition

Table 4: Variation in (a) HD garains, (b) grain sterility and and (c) Sink capacity at harvest under the influence of [$CaCl_2$] root dip (RDT) treatment on rice plant under higher [Fe^{2+}] condition

RDT	(a) HD garains (%)		(b) Grain sterility (%)		(c) Sink capacity (g)	
	Bahadur	Mahsuri	Bahadur	Mahsuri	Bahadur	Mahsuri
0ppm $CaCl_2$	71.30	75.43	28.70	24.57	959.0	400.3
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
100ppm	68.37	81.77	31.63	18.23	1113.7	460.5
$CaCl_2$	(-2.93)	(6.34)	(2.93)	(-6.34)	(13.89)	(13.07)
500ppm	76.23	81.05	23.77	18.95	1035.5	398.8
$CaCl_2$	(-4.93)	(5.62)	(-4.930	(-5.62)	(7.39)	(-0.38)
1000ppm	56.72	79.98	43.28	20.02	1108.2	439.3
$CaCl_2$	(-14.58)	(4.55)	(14.58)	(-4.55)	(13.46)	(8.88)
	SEDiff(±)	LSD(0.05)	SEDiff(±)	LSD(0.05)	SEDiff(±)	LSD(0.05)
Variety	3.26	6.95	3.73	7.94	175.31	175.31
$CaCl_2$	4.42	n.s.	5.29	n.s.	n.s.	n.s.
Variety x $CaCl_2$	5.83	n.s.	7.00	n.s.	n.s.	n.s.

Data are means of three replications, data in parenthesis are the per cent changes over control, n.s. : non significant

The yield attributes were suppressed in plants under higher iron conditions (Nath et al., 2001). Higher iron in rice plant affects panicle length, filled grains, total spikelets per panicle, test weight even in iron tolerant varieties (viz., Pankaj, TTB-101-14). Higher spikelet sterility in the iron sensitive varieties (viz., Khorma and Biraj) and ionic imbalance have also been reported elsewhere (Genon et al.1994; Baruah and Nath, 1996). A drastic reduction in yield of Bronzed rice (Medhi et al., 1975) is associated with lower amount of chlorophyll contents in leaf (Baba et al., 1964), reduction in leaf area (Tanaka and Navasero, 1966), interference in nutrient absorption and enzymatic inhibition of protein synthesis (Bauah and Nath 1996). Moreover, higher iron builds up respiratory inhibitor i.e. hydrogen sulphide in roots, and translocation of photosynthates to sink from source is slowed or held up by excess iron in plants (Mitsui, 1955).

In general, genotype resistant to iron toxicity shows accumulation of substantial amount of iron as phytopheritin (stored protein). In the present study, there was significant decrease in [Fe^{2+}] in leaf at 60 and 90 DAT, which aided to sustain yielding ability of the rice varieties. The RDT with $CaCl_2$ could have brought positive effects on Bahadur variety, which was always superior in terms of the integral yield-attributes like HD grains, and sink capacity. The yield components have contributions to HD grains or economic yield in rice varieties while the influence of $CaCl_2$ has been discounted (Sumiullah et al., 1991; Lone et al., 1999). Bahdur recorded forty three per cent more yield than in Mahsuri despite a non significant effect of $CaCl_2$ RDT. The yielding ability of a genotype is influenced by the profound biomass production and its partitioning to the sinks (Chauhan, 2000). Therefore, higher photosynthetic efficiency leading to HD grains has been suggested for improving grain yield in rice (Venkateswarlu et al., 1986). In the present study, calcium modulated response in the susceptible rice variety (Bahadur) was ubiquitous. It's because, Ca^{2+} sensors such as CaM (Ca^{2+}-modulated protein) activates NAD kinase in chloroplast stroma (Jarret et al., 1982; Mutto, 1983). The light induced conversion of NAD to NADP in photosystem-I is regulated by NAD kinase. Light stimulates Ca^{2+} uptake into the chloroplast, and Ca^{2+} activates NAD kinase to trigger NADPH production from NADP for the reductive Pentose Pathway. So, Ca^{2+} is involved in yield formation in crop plants.

A threshold concentration of $CaCl_2$ i.e. 100ppm was observed in yield attributing parameters in the present study. The native [Ca^{2+}] in the rice varieties were several fold higher than the optimum

Table 5: Relationship ('r' values) of grain yield with yield attributing characters as influenced by [Ca^{2+}] in plant leaves under higher iron condition in field

Grain yield / Leaf [Ca^{2+}] from	Available Fe^{2+} content at 30DAT		Available Ca^{2+} content at 30DAT	
	Bahadur	Mahsuri	Bahadur	Mahsuri
0ppm $CaCl_2$	-0.210	-0.324	0.186	-0.649
100ppm $CaCl_2$	0.389	0.397	0.739	-.0747
500ppm $CaCl_2$	-0.729	-0.742	-0.522	0.870
1000ppm $CaCl_2$	0.448	-0.199	0.692	0.802

Grain yield / Leaf [Ca^{2+}] from	Total chlorophyll at 30DAT		Functional leaf area at 30DAT	
	Bahadur	Mahsuri	Bahadur	Mahsuri
0ppm $CaCl_2$	-0.435	0.533	-0.347	-0.381
100ppm $CaCl_2$	-0.910	-0.527	-0.652	-0.011
500ppm $CaCl_2$	-0.849	0.275	-0.051	0.389
1000ppm $CaCl_2$	-0.865	0.774	0.944*	0.017

Grain yield / Leaf [Ca^{2+}] from	Panicle m^{-2} at harvest		Spikelets per panicle at harvest	
	Bahadur	Mahsuri	Bahadur	Mahsuri
0ppm $CaCl_2$	0.868	0.534	0.905	-0.070
100ppm $CaCl_2$	-0.351	-0.471	0.264	0.657
500ppm $CaCl_2$	0.997	0.760	-0.596	0.568
1000ppm $CaCl_2$	0.870	-0.337	-0.859	0.577

Grain yield / Leaf [Ca^{2+}] from	HD grains at harvest		Test weight		Sink Capacity	
	Bahadur	Mahsuri	Bahadur	Mahsuri	Bahadur	Mahsuri
0ppm $CaCl_2$	-0.857	-0.146	-0.620	0.600	0.862	0.457
100ppm $CaCl_2$	-0.179	0.860	0.315	0.402	0.196	0.491
500ppm $CaCl_2$	0.370	-0.343	-0.431	-0.825	0.276	0.840
1000ppm $CaCl_2$	0.942*	0.559	-0.879	-0.608	0.501	-0.481

*Significant at P(0.05)

concentration. Rasmussen (1983) pointed out that the response in plant parameters is proportional to the strength of Ca^{2+}-CaM. A concentration in the range of 1-10mm in cytosol is reported to be sufficient to achieve a response element (Brown and Newton, 1981; Tsien et al., 1982). The maintenance of such a mill molar concentration is controlled by several Ca^{2+} pumps (Higinbotham et al., 1967; Mecklon and Sim, 1981) present in plasma membrane, (Dieter and Marme, 1980), Endoplasmic reticulum (Buckhout, 1983), and tonoplast (Hartel et al., 1980). All these internal components keep the excess Ca^{2+} reserved. The stored Ca^{2+} can be released either through the action of external stimuli e.g. light (Fabiato, 1983) to maintain a required concentration in the cytoplasm under Ca^{2+} starvation. Therefore, identification and characterization of various enzymes controlled by Ca^{2+}-CaM in rice under higher iron condition could focus readily available information on molecular markers and disseminate knowledge on resistance mechanism of rice varieties to iron toxicity.

Correlation Studies

As soil pH was the lowest, and [Fe^{2+}] in leaf was the highest at 30DAT, a simple correlation was worked out for some of the physiological parameters at this stage (Table 5). In the investigation, Ca^{2+} contents in leaf at 30DAT, panicle no.m^{-2}, spikelet number per panicle, HD grains, test weight and sink capacity at harvest being positively correlated with yield at 100ppm $CaCl_2$ RDT. These parameters may be earmarked for their profound influence on yield development in rice under higher iron condition. A positive correlation between [Fe^{2+}] in leaf and grain yield was also observed concurrently. It means these parameters modulated by Ca^{2+}, are prolific in rice variety like Bahadur to perform under higher iron condition. Negative correlation coefficients were, too, observed among some of the yield attributes and yield in rice under higher iron condition. A dose of 100ppm [$CaCl_2$] was found to be threshold level in the root dip treatment for better performance of the rice varieties.

Conclusion

The present study tested the hypothesis that Ca^{2+} ameliorates Bronzing in rice crop. The iron susceptible Badadur exhibited more physiological response to [Ca^{2+}] derived from $CaCl_2$ RDT than iron resistant Mahsuri variety grown under field condition (Fe^{2+} » 954.98ppm at pH 4.68). However, the extent of Bronzing prevailed up to 30DAT, where [Fe^{2+}] was maximum in plant leaves. Thereafter, the effects of Bronzing were carried over to yield attributes and yield in rice. Bahadur possessed 50% higher native root [Ca^{2+}] than in Mahsuri. Bahadur also enriched more [Ca^{2+}] in leaf tissues than Mahsuri in commensuration with [$CaCl_2$] in RDT. So, $CaCl_2$ [100ppm] RDT improved yield along with the most yield attributes viz., spikelet number, HD grains, test weight, and sink capacity in the tested rice varieties. Thus, it's inferred that $CaCl_2$ root-dip treatment becomes a prophylactic measure for Bronzing in rice under higher iron condition in field.

Acknowledgements

The authors express their sincere gratefulness to Assam Agricultural University for furnishing all necessary field and laboratory facilities for accomplishing the investigation in the Department of Crop Physiology, Faculty of Agriculture, Jorhat.

References

1. Arnon, D.I., Coper enzyme in isolated polyphenol oxidase in *Beta vulgaries*, *Plant Physiol.* 1949; 24: 1-15.
2. Baba, I., Method of diagonising 'Akiochi' iron and sulphide toxicity in wet zone rice fields of Ceylon. *Trop.Agri.* 1958; 114: 231-236.
3. Baba, I., Inada, K. And Tajima, K., Mineral nutrition and the occurrence of physiological disease. In: *Mineral Nutrition of Rice Plant*, Johns Hopkins Press, USA. 1964; pp.173-195.
4. Bauah, K.K. and Nath, B.C.(1996) Changes in growth, iron uptake and metabolism of rice seedlings at excess iron in growth medium,. *Indian J. of Plant physiol.* (New series). 1996; 1: 114-118.
5. Bharali B. and Bates, J.W., Influences of extracellular calcium and iron on membrane sensitivity to bisulfite in the mosses *Pleurozium schreberi* and *Rhytidiadelphus triquetrus*. *Journal of Bryology. 2004;* 26: 53-59.
6. Bharali, B., Chandra, K. and Dey, S.C., Physiological evaluation of production efficiency of some rice (*Oryza sativa* L.) varieties for low light stress situation. *Indian Journal of Plant Physiology. 1995;* 38: 1: 158-159.
7. Bhattachyrya, D. and Baruah, R.C., Management of iron toxic soils with FYM and K for growing rice under submergence. *Proc. Sem. Agric. Sci. Soc.* NE, India. Nov.27-28 1995; Pp. 237-246.
8. Bora, D.K. and Borkakati, K, 'Soil Acidity' In: 'Managing Acid Soils for Sustainable Agriculture' *Review of Soil Rrsearch in Assam*, Ed. H.P. Borthakur, Dept. of Soil Science., AAU, Jorhat, 1997; 22-40.
9. Bridgit, T.K., Potty, N.N., Markutty, K.C. and Anilakumar, K., Anionic relation to iron in rice culture in lateritic soils. *Proceedings of Fifty Kerala Science Congress*, 28-30 Janyuary 1993; Kotyam.

pp.140-141.

10. Brown, D.H. and Wells, J.M. (1988). Sequential elution technique for determining the cellular locations of cations. In: *Methods in Bryology.* Ed. Glime, J.M. Nichinan, Japan. Hatori Botanical Laboratory.
11. Brown, E.G. and Newton, R.P., Cytosolic AMP and higher plants. *Phytochem.* 1981; 20: 2453-63.
12. Buckhout, T.J., ATP dependent Ca^{2+} transport in endoplasmic reticulum isolated from roots of *Lepidium sativum* L. *Planta.* 1983; 159: 84-90.
13. Chauhan, J.S.,Variability for high density grain index andhull weight ratio and their interrelationships with other grain traits in rice (*Oryza sativa* L.). *Indian J. plant Physiol.* 2000; 5(1)(NS); 7-12.
14. Crawley, M.J. (1993) *GLIM for Ecologists.* Blackwell Scientific Publications, London.
15. Dieter, P. and Marme, D., Calmodulin activation of plant microsomal Ca^{2+} uptake. *Proc. Natl. Aced. Sci. USA.* 1980; 77: 7311-7314.
16. Epstein, E. (1972) *Mineral Nutrients of Plants: Principles and Perspectives.* John Wiley and Sons, New York.
17. Fabiato, A., Calcium-induced release of calcium from the *sarcoplasmic reticulum. American J. Physiol.* 1983; 245: C1-C14.
18. Fageria, I.B. and Rabelo, N.A., Tolerance of rice cultivars to iron toxicity. *J. of Plant Nutrition.* 1987; 10: 653-661.
19. Fitter, A.H. and Hay, R.K., *Environmental Physiology of Plants.* An International Series of Monographs. Academic Press, New York, Toronto, Sydney, San Franscisco.1981; PP.200-260.
20. Genon, J.G., Hepee, N., Duffy, J.E.,Delvaux, B. and Hennebert, P.A., Iron and other chemical soil constraints to rice in high land swamps of Burundi. *Plant and Soil.* 1994; 166: 109-161.
21. Halder, B.R. and Mandal, L.N., Effect of calcium carbonate on exchangeable Al content and availability of some nutrient cations in acid soils of Assam under waterlogged condition. *Journal of the Indian Society of Soil Science.* 1987; 35: 35-40.
22. Hartel, C.,Quader, H., Robinson, D.G. and Marme, D., Antimicrotubar herbicides affect Ca^{2+} transport in plant mitochondria. *Planta. 1980;* 149: 336-340.
23. Higinbotham, N., Etherton, B. and Foster, R.J., Mineral ion contents and cell trans-membrane electro potentials of pea and oat seedling tissues. *Plant physiol.* 1987; 42: 37-46.
24. Jackson, M.L. (1973) *Soil Chemical Analysis.* Prntice Hall of India Pvt. Ltd. New Delhi.
25. Jain, R., Chowdhury, I. and Chatterjee, C. (1997) Influence of calcium on growth, composition and yield on black gram (*Vigna mungo* L.) *India J. Plant Physiol.* 1997; 2(3)(NS): 221-224.
26. Jarrett, A.W., Brown, C.J., Black, C.C. and Cormier, M.J., Evidence that calmodulin is in the chloroplast of peas and serves a regulatory role in photosynthesi. *J.Biol. Chem.* 1982; 257: 13795-804.
27. Krishnamurti, G.S.R., Mahavir, A.V. and Sharma, V.A.K., Spectrophotometric determination of Fe with orthophenanthroline. *Micro Chemical Journal.* 1970; 15: 585-589.
28. Legge, R.L. Thompson, j.E., Baker, J.E. and Lieberman, M., The effect of calcium on the fluidity of phase properties of microsomal membranes isolated from post climacteric golden delicious apples. *Plant and Cell Physiol.* 1982; 23: 161-169.
29. Lone, N.A., Khan, N.A., Hayat, S., Azam, Z.M. and Samiullaht, K.N.A., Evaluation of effect of some B-vitamins on root development of mustard. *Ann. Appl. Bio.* 1999*;* 134(Supplement): 30-37.
30. Mahapatra, I.C., Singh, K.N., Pillai, K.G. and Bapat, S.R., Rice soil and their management. *Indian J. Agron.* 1985; 3(1).
31. Mecklon, A.E.S. and Sim, A., Cortical cell fluxes and transport to the stele in excised root segments of *Allium cepa* L. *Planta.* 1981; 152: 381-387.
32. Medhi et, B.K., Borah, D.K. and Borkakati, K., Effects of levels of iron and time of application of fresh plant meterials on nutrient uptake and enzymes of rice. *JASS.* 1975; 8(2): 228-231.
33. Mengel, K. and Kirby, E.A.(1978) *Principles of Plant Nutrition.* International Potash Inst. Berne, Switzerland.
34. Mitsui, S. (1955) *Inorganic Nutrition. Fertilization and soil Amelioration for Lowland Rice*, Yokendo Ltd., Tokyo.
35. Mutto, S., Kinetic nature of calmodulin-dependent NAD kinase from pea seedlings. *Zeitsctrift fur pflanzenphysiologie.* 1983; 109: 385-393.
36. Nath, B.C., Gogoi, N. And Baruah, K.K., Iron toxicity induced physiological changes in rice. Proceedings of National Seminar on ‘Water and Land Management including CAD For Socio economic Upliftment of NE Region’ held on 22-23rd Nov. 2001.
37. Nayak, B., Biswas, A.K. and Jr. Choudhury, M.A., Effects of calcium on water stress induced biochemical changes and yield of field grown rice. *Plantarum,* 1983; 25 (2): 117-123.
38. Nobel, P.S., *Introduction to Biophysical Plant Physiology*, 1975; Pp.35 Freeman W.H. and Company, San Francisco.
39. Ohishi, S.L. and Ito, T., Calcium induced phase separations in phosphatidylserine-phosphtidy lcholine membranes. *Biochem.* 1974; 13: 881-887.
40. Panse V.G. and Sukhatme, P.V. (1978) *Statistical methods for Agricultural Workers*, ICAR, New Delhi.
41. Perur, N.G., Subramanium, C.K., Muhr, G.R. and Roy, H.E. (1973) *Soil fertility evaluation to serve Indian farmers.* Mysore Dept. of Agril. USAID, Bangalore.
42. Price, A.H, and Hendry, G.A.F., Iron catalysed oxygen radical formation and its possible contribution to drought damage in nine native

grasses and three cereals. *Plant Cell and Environment.* 1991; 14: 477-848.

43. Rasmussen, H., Pathways of amplitude and sensitivity modulation in the calcium messenger system. In: '*Calcium and Cell Functions'*. Cheng, W.Y. Ed. Vol.4. New York Academic. 1983; PP 1-61.
44. Samiullah Khan, N.A., Ansari, S.A. and Afridi, M.M.R.K., B-vtamin in relation to crop productivity. *Indian Rev. Life Sci.* 1988; 8: 51-74.
45. Sarma, P. and Bharali, B., Effects of Calcium on Physiology of Rice (*Oryza sativa* L.) under iron toxic condition. *Indian Journal of Plant and Soil.* 1995; 2(2): 47-54.
46. Shear, C.B., Symptoms of calcium deficiency on leaves and fruit of 'York Imperial' apples. *J. Amer. Soc. Hort.Sci.* 1971; 96: 415-417.
47. Singh, B.P. and Singh, B.N., Responses of K application on rice in iron rich valley soils. *IRRN.* 1987; 12(5): 31-32.
48. Singh, B.P., Das, M., Ram, M., Dwivedi, B.S. and Prasad, R.N., Characterisation of Fe-toxic soils and affected plants in Meghalaya. *J. Indian Soc.Sci.* 1992; 40: 329-428.
49. Singh, K.P., Micronutrients. In: *Acid Soil Management,* Edt. Mohsin, M.K., Sarkar, A.K. and Mathur, B.S. Kalyani Publishers (India). 1995; Pp. 291-231.
50. Takagai, S., Studies on the inhibiting factors of the rice plant grown in the much paddy soil under pot experiment condition (in Japanese, English Summary) *Bull. Inst. Agric. Res.* Tokohu Univ. 1958; 10: 13-28.
51. Tanaka, A. and Yoshida, S. (1970). Nutritional disorders of the rice plant in Asia, *IRRI, Tech. Bull.* No. 10.
52. Venkateswar, B. and Visperas, S., Changes in source-sink relationship in rice through different canopy profile under field conditions. *J. Plant Physiol*. 1981; 19(3): 195-210.
53. Venkateswrulu, B., Vergara, B.S. , Parao, F.T. and Visperas, R.M., Enhancing grain yield potential in rice by increasing the number of high density grains. *Philippine J. Crop Sci.* 1986; 11: 145-152.
54. Verma, T.S., Bronzing disease of rice in relation to critical levels of iron and iron-manganese ratio in North-West Himalayan Region. *J. Indian Soil Sci.* 1991; 39: 201-203.
55. White, J.P. and Broadly, R.M., Calcium in plants. *Annals of Botany. 2003;* 92: 477-485.