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Identification of Potential Genotype Influencing Stress Tolerance to Fe Toxicity and P Deficiency under Low Land Acidic Soils Condition of North Eastern Rice, "Shasarang"

Sudip Das, Wricha Tyagi*, Mayank Rai and Ashim Debnath

School of Crop Improvement, College of Post-Graduate Studies, CAU (Imphal), Umiam, Meghalaya (793 103), India

Corresponding Author

Wricha Tyagi
e-mail: wtyagi.cau@gmail.com

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Abstract

P is one of the macronutrients required for plant growth and development, whereas Fe is a micronutrient required for various metabolic functions of plant. However, under acidic soil conditions, due to low pH Fe^{2+} toxicity and P deficiency occurs leading to decrease in crop productivity. The present investigation was undertaken to understand molecular physiology of acidity tolerance with special reference to P-deficiency and Fe-toxicity tolerance in Shasarang (LR11). Under lowland acid soils (pH=5.2), KMR3 showed a higher bronzing score of 3 at 110 DAT and LR11 showed very little or no bronzing. The field performance of LR11 was better as compared to KMR3 with respect to yield related traits. Furthermore, our data confirmed that LR11 is infact Fe toxicity tolerant and "Shasarang" means "tolerant to Fe" in Khasi. The susceptible genotype, KMR3, showed significant decrease in shoot and root dry weight under low P and Fe toxicity conditions in hydroponic. However, in LR11, the difference in root-shoot dry weight in control and treatment were not significant. Excess amounts of iron in hydroponics could lead to distinct variation in the bronzing score, root-shoot biomass. Based on this criteria *Nipponbare* has been considered tolerant and Kasalath as susceptible. In our case, screening using excess of Fe and low P simultaneously, Kasalath performed *at par* with LR11. This study, therefore, suggests that identified tolerant LR11 can be a potential target for enhancing rice production introducing tolerance to acidity under lowland rainfed field conditions.

Keywords: Fe toxicity, P deficiency, tolerant genotype, bronzing score

1. Introduction

In response to soil acidity, rice is the most tolerant of all cereals crops. Therefore, understanding acidity tolerance in rice definitely can help in better understanding of the abiotic stress tolerance in other cereal crops. As far as soil acidity is concerned, it is mainly contributing by ferrous iron (Fe^{2+}) overload or toxicity and phosphorous (P) limiting or deficiency condition.

Iron is an essential trace element for living organisms. It has very important role in DNA synthesis, respiration, and photosynthesis and in various physiological and biochemical pathways in plants too are activated by iron, and it is a prosthetic group, constituent of many enzymes and maintains chloroplast structure and function (Rout and Sahoo, 2015). About 80% of iron is present in photosynthetic cells essential for the biosynthesis of cytochromes and other heme molecules- chlorophyll, the electron transport system, and the of Fe-S clusters formation (Briat *et al*., 2007; Hansch and Mendel, 2009). Two or three iron atoms are found in molecules directly related to photosystem II (PS-II), 12 atoms in photosystem I (PS-I), five in the cytochrome complex, and two in the ferredoxin molecule (Varotto et al., 2002). Such distributions show that iron is directly involved in the photosynthetic activity of plants and, consequently, their productivity (Briat et al., 2007).

But the imbalance in Fe solubility, uptake from soil, distribution from root rhizosphere to plant tissue and all other parts leads to deficiency (chlorosis), toxicity and disorder or diseases. The solubility of ferric (Fe^{3+}) form of iron is increased in waterlogged condition and due to this the Fe^{3+} is converted to Ferrous (Fe^{2+}) form which is readily absorbed by plants and hence causes the Fe^{2+} toxicity. This is potentially toxic and favors ROS (reactive oxygen Species) formation. ROS like free radicals, superoxide can damage the cells by lipid peroxidation.

The most severe and prominent symptom of iron toxicity is bronzing which is nothing but coalesced tissue necrosis. The leaves and roots become bronze or brown and even blackening in color and in severe toxicity cases twigs, stem and whole plants mortality could takes place. As a result of severe Fe^{2+} toxicity, stunting in plants growth takes place. Iron toxicity on other hand leads to yellowing, bronzing in rice and also an enhanced requirement for nutrients like P and K. Plant cannot obtain the essential nutrients like Zn, Mg, Ca, Mn, P

etc. due to this toxicity as it interfere in their absorption, forming complex of iron plaque and other interaction with macro/micro elements (Figure 1).

Phosphorus (P) is one of the essential macro nutrients required for the plant growth and development. It is the constituent of

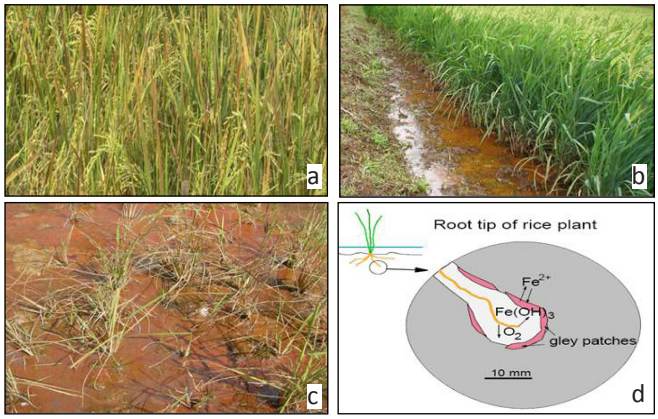


Figure 1: Leaf bronzing score on older leaves (a), Fe precipitation (b, c), and plaque formation (d) when ferrous iron is oxidized and precipitates as iron oxide on root surface

key cell molecules such as ATP, nucleic acids and phospholipids and pivotal regulator involved in many biological processes including energy transfer, protein activation, carbon and amino acid metabolism. Phosphorus deficiency symptoms in rice seedling may include moderate to severe stunting; small, very erect, dark green leaves; small stem diameter; reduced or no tillering and delayed plant development.Plants have developed several mechanisms to adapt to low P availability, which can broadly be classified into three main categories: root interception of Pi, P acquisition efficiency, and internal P-use efficiency. The size of the root system is consequently considered to be an important characteristic of plants that tolerate P deficiency (Wissuwa and Ae, 2001a).

Majority soil of North East India is acidic and this leads to P being limiting for plant growth because soil P-fixation capacity is generally high at low pH and in lowland conditions this problem is aggravated due to Fe^{2+} toxicity. Insufficient plant-available soil phosphorus is a major constraint for rice production. This is particularly apparent under upland conditions, which are commonly characterized by infertile, highly acidic, P-fixing soils, normally in areas where little or no fertilizer is applied. Even under lowland conditions, P deficiency is a main factor limiting performance of modern rice varieties and is likely to become increasingly important as P is removed from soils under intensive rice production (De Datta et al., 1998). The lack of locally available P sources and the high cost of importing and transporting fertilizers prevent many resource-poor rice farmers from applying P. Some rice soils can quickly fix up to 90% of the added P fertilizer into less soluble forms (Dobermann et al., 1998). An attractive, cost-effective and sustainable strategy is to develop rice cultivars capable of extracting higher proportion of fixed P. Genetic variability among lowland and upland rice cultivars in their ability to exploit soil and fertilizer P has been reported (Fageria et al., 1988). Hence, genetic variation in tolerance to P deficiency could effectively be exploited for rice improvement. Mechanisms of P solubilization in aerobic soils are probably different and mainly involve the secretion of low molecular weight organic acids, such as citrate, that increase P availability through the formation of soluble metal-citrate chelates. Chelating agents such as organic acids may help solubilize P in the soil by dissolving Al and Fe solid phases on which P is held. High rates of release of P-solubilizing organic acid anions from roots in response to Pdeficiency have been reported (Kirk et al., 1999). Although genotypic differences in P deficiency and iron toxicity tolerance in rice are reported, efforts have been few to develop new genotypes especially adapted to low pH problem soils. The fact that many traditional varieties were more superior to modern varieties (Wissuwa and Ae, 2001b) indicates the need for such breeding and molecular biology programs to understand and incorporate deficiency and Fe^{2+} toxicity tolerance into modern cultivars. Genotype 'Shasarang' is one of the popular, medium yielding lowland varieties in the North-Eastern part of India and tolerant to iron toxicity and growing well in P-deficient condition. Therefore, novel source of P deficiency and Fe^{2+} toxicity tolerance can be a base in Shasarang as a potential acidity donor in molecular breeding programme and directly for betterment of stress management.

2. Materials and Methods

The detailed materials and methods adopted in the present investigations involving the screening of upland rice genotypes for phosphorus deficiency and iron toxicity tolerance as well as identifying potentialtolerantdonor for iron toxicity tolerance and P deficient, which may be used for further molecular study are as follows:

2.1. Study area

The study was conducted in greenhouse as well as field of College of Post Graduate Studies (CPGS), Central Agricultural University, Barapani, Umiam, Meghalaya (Figure 2). Greenhouse day temperature of 32°C and night temperature of 22 °C was maintained for initial pot raising and crossing. The study area is situated at latitude of 25˚ 41' and longitude of 91˚ 54' and an elevation of 950 m above mean sea level. The climatic condition of the region as a whole is sub-tropical humid having hot summer and cold winter. The average temperature of Umiam, in particular, varies from 4 ˚C to 32˚C in winter and summer months, respectively.The parent genotypes Shasarang (LR11), LR15, LR60 and KMR3 were planted in pots inside the green house of College of Post Graduate Studies (CPGS), Central Agricultural University, Barapani, Umiam, Meghalaya (under protected environmental condition). The sowing was done in the month of the June, 2014 and transplanted on July 17, 2014 in the field of CPGS (lowland transplanting). During the time of transplanting, the soil acidity (pH) was recorded to be acidic and ranged from 5.7 to 5.8.

Figure 2: Field experimental view of CPGS, CAU (Low land acidic field condition)

2.2. Experiment

Four Parents genotypes seeds viz. LR11, KMR3, LR60 and LR15 were procured from CPGS, Barapani, Meghalaya. Shasarang (LR11), a drought tolerant rice variety performing well in acidic soils of Meghalaya, North Eastern India (Tyagi et al., 2012) and KMR3; an acidic tolerant line of Kerala. As the plants were grown under rainfed conditions, no additional water was provided to the plant during the entire rice growing season. Visual leaf bronzing symptoms on leaves were observed in range of 0-5 were assigned based on percentage of leaf area affected. From an individual plant, total number of tillers was recorded after 30 DAT. The second tiller reading was taken at 60 DAT along with the three checks. The scoring for bronzing symptom (0-5 scale) in the leaves was very important trait for this study as it is an indication of Fe^{2+} toxicity and the data for bronzing score was taken at 60 DAT and 90 DAT and it was seen that after 60 DAT, the bronzing score started developing in leaves, gradually in stem and after 90 DAT the severity become higher and distinctly visible in the individual progeny (Figure 1; Figure 2). Total weight (plant dry weight) of the plant was taken after the drying of plants completely. No. of panicles $plant^{-1}$, no. of filled grains and filled, grains $panicle^{-1}$, grain yield $plant^{-1}$ was also taken into account. The phosphorous (P) and iron estimation was done from flag leaf, which harbors maximum sunlight results in higher photosynthetic matters accumulation. Then the nutrients get remobilized to the developing grain in P starvation response (Jeong et al., 2017).

P estimation was done from the flag leaf of individual plants using spectrophotometric method (Gupta, 2007). The fresh weight of the each leaf from each plant was measured and allowed to dry. Dry weight was taken and P content was estimated through spectrophotometer. The standard curve for P was prepared in a concentration range of 0, 0.1, 0.2, 0.3., 0.4, 0 .5, 0.6, 0.7, 0.8, 0.9 and 1 ppm. P Uptake was calculated in unit of flag leaf dry weight (g) x P content (mg) and P Use Efficiency was calculated in unit of flag leaf dry weight (g)/ P content (mg).The flag leaf for iron (Fe^{2+}) estimation was also collected similarly from each individual parent plant and fresh weight was measured and the leaves were allowed to dry for taking the dry weight. Iron content was analyzed in acidic (HNO_3: $HCLO_4$, 3:1) leaf tissue digest prepared in MilliQ water. Samples were incubated for 24 hrs at 37^0C in the presence of 5 mM 2, 2' bipyridine and 100 mM ascorbic acid (pH 7.0) prepared in MilliQ water. For each sample; both a reactive blank, and a sample blank without adding 2, 2'bipyridine were prepared and absorbance was measured at 520 nm. A standard curve having a concentration range of 0, 25, 50, 100, 500, 1000 and 2000 uL iron ($FeSO_4$/$FeCl_3$) was used. The correlations among the thirteen traits (i.e., TN 30, TN 60, BS 60, BS 110, PDW, PN, FGPP, GW, P Content, PUP, PUE, Fe Content, Fe uptake) were conducted using Excel (Microsoft, Redmond, WA, USA) CORREL function, and the statistical levels of significance were examined by t-test. Correlation test and t-test were carried out at both 1% and 5% level of significant.

2.3. Observations to be recorded and field data generation

The following data were taken under field conditions. The number of tillers was recorded for each individual progeny at 30 and 60 DAT. The scoring for the bronzing was very important job for this study and as it is the indication of Fe^{2+} toxicity or deficiency was done in 0-5 scale. So, accordingly the data for bronzing score was taken at 60 DAT and 90 DAT and it was seen that after 60 DAT, the bronzing score started developing in leaves, gradually in stem and after 90 DAT the severity The sampling for P estimation was done by collecting the flag leaf for each individual at the time of their physiological maturity because the Phosphorous (P) deficiency (yellowing of leaf) and maximum chlorophyll syntheses in flag leaf at the time of the maturity. The fresh weight of the each leaf from each plant was measured and allowed to dry. The leaf sample for iron (Fe^{2+}) was also collected similarly from each individual parent progeny plant and fresh weight was measured and the leaves were allowed to dry for taking the dry weight. The remaining data like number of panicles per plant, dry biomass in grams (g), number of filled grains per plant and filled grains per panicle, etc. were taken after the harvesting of each plant.

2.4. Formulae for calculation

P %={concentration/ wt. of sample (g)} x {100/ aliquote (ml) taken } x {Volume of digest (ml)/ 10,000}
P Uptake: Plant dry weight (g) x P content (mg)
P use Efficiency: Plant dry weight (g)/ P content (mg)

2.5. Screening methodology for iron (Fe^{2+}) toxicity and phosphorus (P) deficiency

Screening methodology was carried out in order to find the tolerant or susceptible genotypes in hydroponics. Two different types of treatments were given (modified Yoshida medium) to 5-6 days old seedlings: (A) 290 mg/L Fe^{2+}, pH 4, 1:2 molar ratio of Fe: EDTA and 0.5 mg/L P and (B) 290 mg L^{-1} Fe^{2+}, pH 4, 1:2 molar ratio of Fe: EDTA. The following parameters

were studied after 20-30 days of treatment: root and shoot length, fresh root and shoot biomass, dry root and shoot biomass, leaf bronzing score, root architecture (Root Nav software), root staining (perl blue), P content was measured by spectrophotometer, Fe content by atomic spectra. Screening was done in hydroponics nutrient media called modified Yoshida nutrient media. This system is suitable for seedling studies. Hydroponics requires adequate aeration and greater expertise.The pH of the medium should be kept 5.

Rice may be evaluated for Fe-toxicity tolerance in between seedling or transplanting and booting stages on 0-5 scale as outlined by IRRI (1988). Lines scoring 0-2 can be classified as tolerant and those scoring 3-5 as susceptible (Table 1).

2.6. Parameters studied

Leaf bronzing score, dry matter content, Fe-content in leaf tissue chemically, shoot biomass, root biomass, plant height, root length, etc.

3. Results and Discussion

3.1. Screening for iron toxicity and phosphorous deficiency tolerance under hydroponics conditions

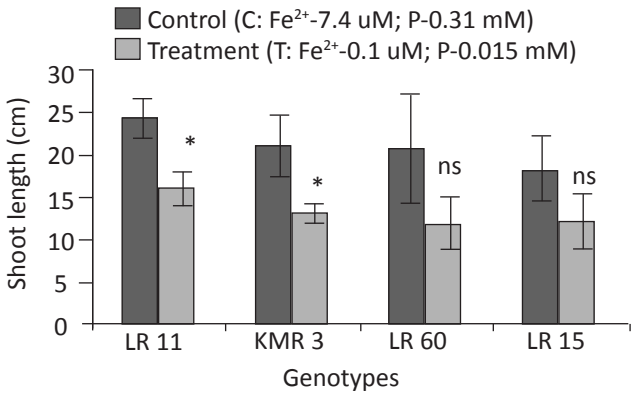
Table 1: Standard scale of bronzing score in respect to Fe toxicity tolerance by IRRI (1988)

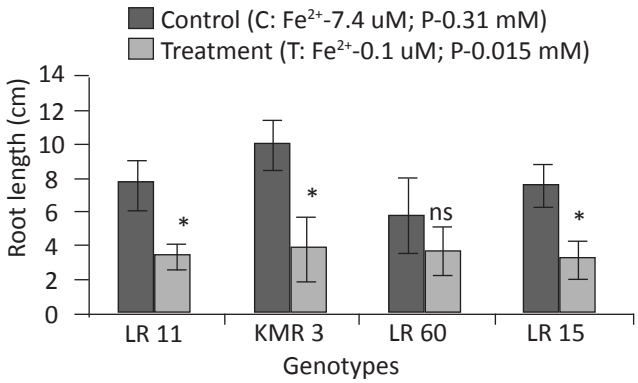
Scale	Expression	Category
0	Growth and tillering nearly normal	Tolerant
1	Growth and tillering nearly normal, reddish brown spots or orange discoloration on older leaves	Tolerant
1-2	Growth and tillering nearly normal, older leaves reddish brown, purple or orange yellow	Tolerant
2-3	Growth and tillering retarded, most leaves discarded or dead	Susceptible
3-4	Growth and tillering ceases, most leaves discarded or dead	Susceptible
4-5	Almost all plants dead or dying	Susceptible

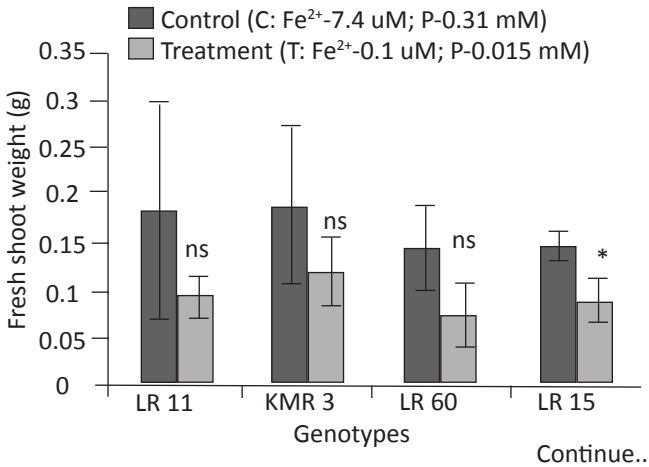
Four genotypes LR11, KMR3, LR60 and LR15 were taken for hydroponic screening. Screening was done for iron toxicity tolerance which was already described in materials and methods.

The shoot length in mean± SD value for LR11, KMR3, LR60 and LR15 was found to be 24.52±2.26, 21.3±3.49, 21±6.28, and 18.6±3.64 cm in control condition and 16.28±1.96, 13.24±1.08, 12.18±2.96 and 12.4±3.18 cm, respectively in treatment condition (Figure 3.1a). A decrease in shoot length under treatment conditions was observed for all the four genotypes. However, the decrease in length was significant for LR11 and KMR3 only. The average root length were for LR11, KMR3, LR60 and LR15 were found to be 7.57±1.41, 9.86±1.58, 5.76±2.11 and 7.5±1.29 cm, respectively in control and 3.4±0.64, 3.8 ± 1.8, 3.7±1.39 and 3.18 ± 1.03 cm, respectively under treatment conditions (Figure 3.1a). The decrease in root length was significant for LR11, KMR3 and LR15.

The mean fresh shoot weight for LR11, KMR3, LR60 and LR15 were found to be 0.184±0.11, 0.191±0.082, 0.146±0.043, 0.147±0.014 g, respectively under control conditions. Under Fe toxicity and P deficiency condition, the mean fresh weight for LR11, KMR3, LR60 and LR15 was 0.093±0.021, 0.12±0.035, 0.075±0.032, 0.09±0.022 g, respectively (Figure 3.1a). The genotype LR15 showed significant reduction in fresh shoot weight under treatment conditions.The mean fresh root weight for LR11, KMR3, LR60 and LR15 were recorded to be 0.1131±0.025, 0.1136 ± 0.484, 0.0363±0.027 and 0.0421±0.02 g, respectively in control. The treatment conditions, mean fresh root weight were found to be 0.0994±0.024 g,







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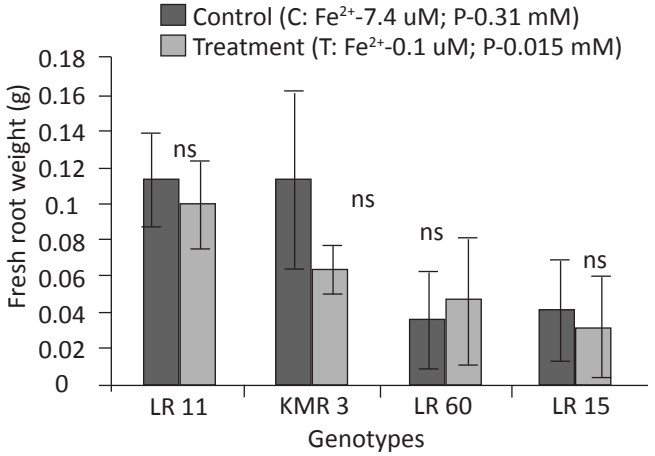

Figure 3.1a: Variation for root length, shoot length fresh shoot and root weight un four rice genotypes (LR 11, KMR 3, LR 60 and KMR 3) in response to 10 days Fe toxicity and P deficiency treatment. The histoginous represent average phenotype value under control (C: Fe2+-7.4 uM; P-0.31 mM) and treatment conditions (T: Fe2+-0.1 uM; P-0.015 mM). Error her represent the standard errors of responsive means (n=10), asterisk (*) indicates that the average gene expression values of the treatment and control are significantly different ($p<0.05$); whereas, ns indicates non-significant difference

0.0638±0.012 g, 0.046±0.035 g and 0.032±0.028 g for LR11, KMR3, LR60 and LR15, respectively. There was no significant difference for fresh root weight for all the four genotypes under control and treatment conditions.

The average shoot dry weight for LR11, KMR3, LR60 and LR15 in control conditions were 0.017±0.008 g, 0.023±0.004 g, 0.0154±0.0013 g and 0.0178±0.0023 g, respectively. Mean values of 0.007±0.003 g, 0.0098±0.004 g, 0.0136±0.0099 g and 0.0106±0.004 g, respectively were obtained for LR11, KMR3, LR60 and LR15 under treatment conditions (Figure 3.1b). Significant decrease in shoot dry weight was observed for LR11, KMR3 as well as LR 15 under P deficiency and Fe toxicity conditions.The mean dry root weight values of 0.00916±0.009 g, 0.00978±0.0075 g, 0.0056±0.001 g, 0.0044±0.001 g were obtained for LR11, KMR3, LR60 and LR15, respectively under control conditions. Under low P and Fe toxicity conditions, LR11, KMR3, LR60 and LR15 showed mean values of 0.0059±0.002 g, 0.0044±0.002 g, 0.0046±0.0034 g and 0.0031±0.001 g, respectively (Figure 3.1b). Only KMR3 showed significant reduction in root weight under treatment conditions.

3.1.1. Data for key traits under Hydroponics Screening

Fe content of LR11, KMR3, LR60 and LR 15 was found to be 37.186±19.352, 44.108±28.12, 49.62±33.378 and 57.531±45.795 ppm, respectively. The P uptake (PUP) was found to be 0.322±0.749, 0.079 ± 0.52, 0.128 ± 0.064 and 0.363±0.934 mg/flag leaf dry weight for LR11, KMR3, LR60 and LR15, respectively. Similarly, PUE was recorded to be 12.458±6.711, 17.413±7.29, 17.764±30.37 and 16.152±9.403 mgPi/g, respectively (Figure 3.1.1).

3.2. Field Screening Data

The following data were taken under field condition in the low land: flowering date, tiller number and bronzing score at 30 DAT and 60 DAT respectively, dry biomass (g), no. of panicles plant^{-1}, no. of filled grains, filled grains panicle^{-1}, grain weight (g). Bronzing score denotes that LR 11 was more tolerant as compare to KMR3. The bronzing score at 110 DAT and 60 DAT are found to be significantly correlated to each other. Bronzing score denotes that LR11 was more tolerant as compare to KMR3. Correlation between bronzing score at 110 DAT and Filled grain panicle^{-1} were found to be significant.

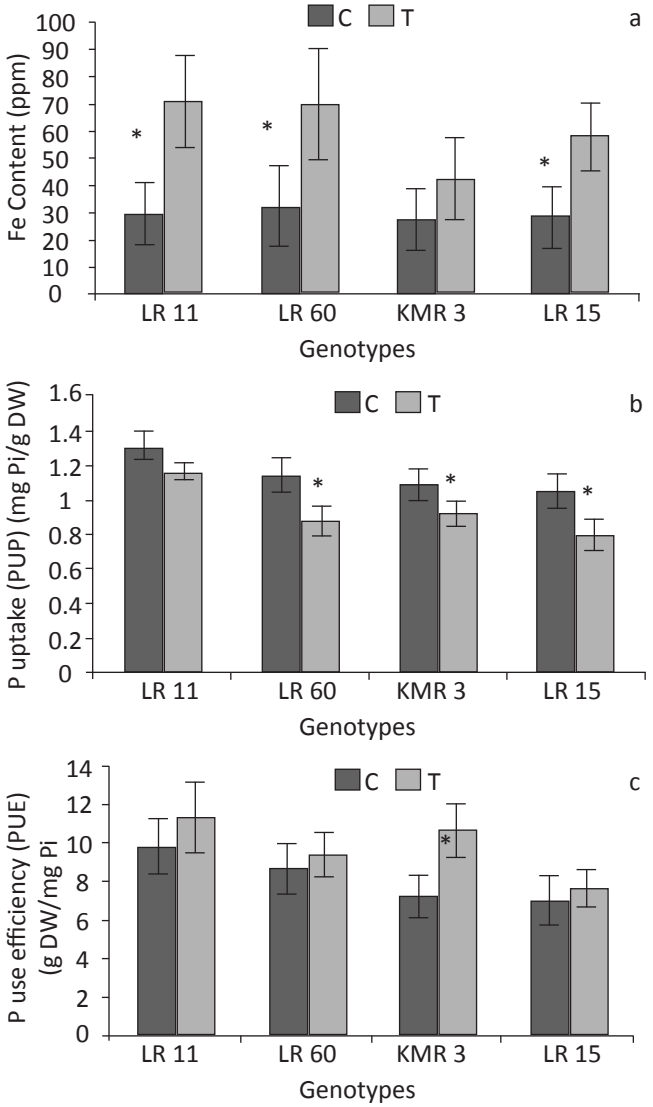

Figure 3.1.1: a) Fe content (ppm) of 4 genotype viz. LR 11, LR 60, KMR 3 and LR 15 grown under hydroponics in control as well as treatment; b) P uptake (PUP) (mg Pi/ g DW) of 4 genotypes in control as well as treatment under hydroponics; c) P use efficiency (PUE) of all the 4 genotypes in g DW/mg Pi. Significant values are indicated by symbal 'star'. Data allotted are average value on n-10

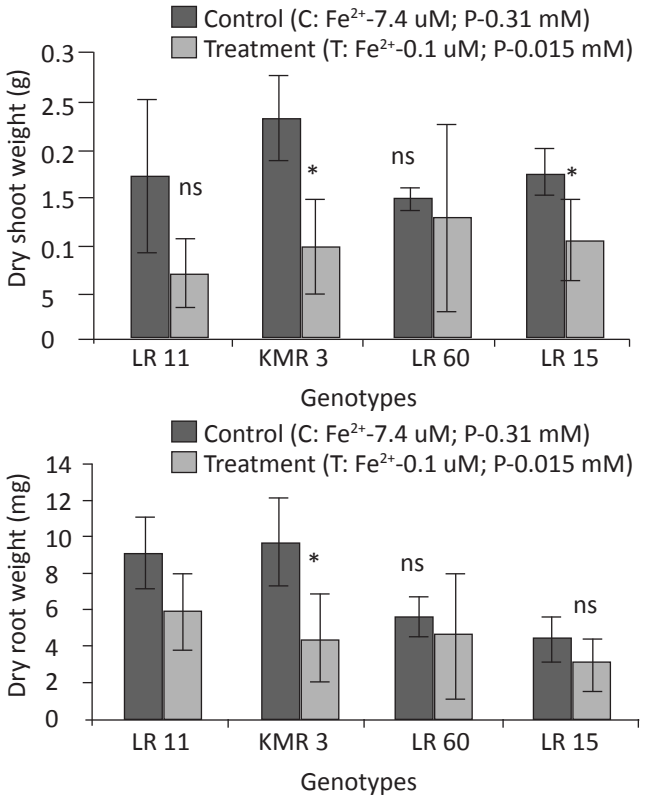

Figure 3.1b: variation for shoot and dry weight in four rice genotypes (LR11, KMR 3, LR 60 and KMR3) in response to 10 days Fe toxicity and P deficiency treatmetn. The hostrograms represent average phenotypoc value inder control (Fe if sepective means (n-10), Asterisk (*) indicates that the average fene expression values of the treatment and conteol are signicatly different (p<0.005); whereas, ns indicates nosignificant diffrence

Tiller number at 60 DAT was found to be positively correlated to tiller number at 30 DAT. Filled grain was significantly correlated with plant dry weight and panicle number. Again the grain weight was positively correlated to plant dry weight, panicle number and filled grain panicle^{-1}. The tiller number and Panicle number were found to be positively correlated to each other. The graph shows that bronzing score, tiller no., yield are highly controlled by environmental condition and these are polygenic traits so shows normal distribution and continuous variation in case parents as well.

The average panicle number for LR11 is 12 and KMR3 is 9. The mean bronzing score was found to be 1.83 and 4.66 at 60 DAT and 110 DAT in LR11 and 2.66 and 6.22 in KMR3 at 60DAT and 110 DAT respectively. The tiller number on an average was 5.5 and 8.5; whereas 4.3 and 10.8 for LR11 and KMR3 respectively. The grain weight was 14.85 g and 9.52 g for LR11 and KMR3, respectively. Fe content was 1.134 and 0.92, respectively.

Bronzing score at 60 days after transplanting (DAT) ranged from 0 to 3 (Table 2). Parents, LR11, LR60, LR15 and KMR3 had bronzing score of 0, 1, 2 and 2, respectively. A set of plant having bronzing score of '0' were also identified.Bronzing score at 110 DAT ranged from 1 to 4 with bronzing score of 3 having maximum frequency (Table 2). The parents, LR 11 and KMR3 had a score of 1 and 3, respectively. LR60 and LR15 had bronzing score of 2 and 4.

In parents, the average tiller number was found to be 6 ± 2 in case of LR 11 and 4±2 in KMR3 (Table 2). In case of tiller number at 60 DAT, the range observed was from 1 to 17. The average tiller number was found to be 9 ± 4 in LR11, 10 ± 2 in LR60, 7±3 in LR15 and 11±3 in KMR3.

The panicle number ranged from 2 to 23 and the average panicle number for LR11 was 12±5, LR60 was 10±4, 7±3 was in LR15 and KMR3 was 9 ± 5, respectively (Table 2). Filled grains per panicle.The average filled grains per panicle were found to be 58±25, 55±27, 49±26 and 53.27±29 in LR11, LR60, LR15 and KMR3, respectively. Continuous variation was observed for this trait (Table 2).

The average P content value for LR11 was 132.72 ± 11.54 ppm and KMR 3 was 132.48±17.32 ppm (Table 2). The average P content value for LR60 was 130.82±9.72 ppm and LR15 was 118.92±15.4 ppm. The PUP value for the parents LR11, LR60, LR15 and KMR3 was found to be 0.015 ± 002, 0.03±0.003,

Table 2: Field screening data on four rice genotypes

	Genotypes			
	LR11	LR60	LR15	KMR3
Bronzing score (60 DAT)	0	1	2	2
Bronzing score (110 DAT)	1	2	4	3
Tiller no. (30 DAT)	6±2	5±2	3±2	4± 2
Tiller no. (60 DAT)	9±4	10±4	7±3	11± 3
Panicle Nubmer	12±5	10± 4	7±3	9± 5
Filled grains panicle^{-1} (FGPP)	58±25	55±27	49±26	53.27± 29
Plant dry Weight (g)	29.83 ± 14.4	40.48 ± 16.42	25.44± 10.24	58.88 ± 111.01
P content (ppm)	132.72 ± 11.54	130.82 ± 9.72	118.92± 15.41	132.48± 17.32
P uptake (PUP) (mg flag^{-1} leaf dry weight)	0.015 ± 0.002	0.03 ± 0.003	0.016± 0.008	0.011± 0.005
P use efficiency (PUE) mg Pi g^{-1}	58.28 ± 9.44	67.25± 14.28	72.32± 18.28	80.01± 23.54
Fe content (ppm)	113.45 ± 37.32	117.69 ± 32.8	74.68± 10.45	92.85± 25.8
Fe Uptake (PUP) (mg flag^{-1} leaf dry weight)	13.42 ± 4.15	8.2±2.7	9.4± 3.21	8.2± 2.7

0.016±0.008 and 0.011±0.005, respectively. The values for ranged from 0-200 mg Pi g^{-1} dry weight of flag leaf. The average value for P use efficiency for LR11 was 58.28±9.44 mg g^{-1} DW and KMR3 had a value of 80.01±23.54 mg g^{-1} whereas the PUE values were found to be 72.32±18.28 for LR15 and 67.25±14.28 for LR60.

The average dry weight for parent LR11 was 29.83±14.4 g and for KMR3 was 58.88±111.01 g. In case of Fe content, KMR3 had a value of 92.85±25.8 ppm and LR 11 had a value of 113.45±37.32 ppm.The LR60 had 117.69±32.8 ppm and LR15 had 74.68±10.45 ppm. For amount of Fe taken up in the flag leaf, LR11 showed a value of 13.42±4.15 mg while KMR3 had a value of 8.2±2.7 mg. LR60 and LR15 had values of 8.2±2.7 mg and 9.4±3.21 mg, respectively.

3.3. Correlation among various phenotypic parameters in the four genotypes

Upon evaluation parents' genotypes, to study the association between different physiological and agronomic traits, correlation coefficients were obtained for each pair of traits. Positive correlation was found between tiller numbers at 60 days with plant dry weight (PDW) (0.642) and panicle number (PN) (0.984). PDW was positively correlated with panicle number (PN) (0.667) and filled grains per panicle (FGPP) (0.295). Bronzing score at 60 DAT (BS 60) was positively correlated with bronzing score at 110 DAT (BS110) (0.454). Interestingly, BS60 was negatively correlated with Fe content (0.21) at 5% level of significance. P content was positively correlated with P uptake (0.572) and negatively correlated with P use efficiency (PUE) and Fe content at both the levels of significance, respectively. P uptake was positively correlated with PUE (0.398) and Fe uptake (0.557) but negatively correlated with Fe content (-0.41). PUE was positively correlated with Fe uptake (0.623), Fe content with Fe uptake (0.619) at both 5% and 1% level of significance. Understandably, number of tillers at 60 days, plant dry weight, number of panicles per plant and 100 grain weight showed significant positive correlation with grain yield per plant (Table 3).

3.4. Physiological difference between parents

In response to growth under lowland acid soils (pH 5.2), it was observed that the genotype KMR3 showed a higher bronzing score of 3 at 110 DAT. On the other hand, LR11 showed very little or no bronzing. The occurrence of bronzing symptoms' in LR11 when observed, were delayed (50-55 days) as compared with KMR3 (30 days). The field performance of LR11 was better as compared to KMR3 with respect to yield related traits like 100 grain weight and filled grains $panicle^{-1}$. It has been suggested that higher bronzing score is strongly correlated with yield loss (Audebert and Fofana, 2009). Based on bronzing score, KMR3 was denoted as a susceptible genotype to Fe toxicity. Furthermore, our data confirmed that LR11 is in fact Fe toxicity tolerant. The name "Shasarang" means "tolerant to Fe" in Khasi language.

Table 3: Correlation among various phenotypic parameters in all four genotypes under acidic field conditions

	TN30	TN60	PDW	PN	FGPP	100 GW	BS60	BS110	P content	P uptake	PUE	Fe content	Fe uptake
TN30	1.00	0.02	0.09	0.03	0.00	-0.09	0.05	0.04	-0.02	0.11	0.14	0.09	0.19
TN60		1.00	0.642**	0.984**	0.14	-0.01	-0.04	0.03	0.03	0.05	0.01	0.02	0.05
PDW			1.00	0.668**	0.295*	-0.04	-0.04	0.04	0.06	0.12	0.05	-0.05	0.03
PN				1.00	0.17	-0.12	-0.04	0.05	0.05	0.06	0.01	0.00	0.05
PGPP					1.00	-0.27	-0.18	0.01	0.12	0.09	-0.09	-0.10	-0.03
100 GW						1.00	0.07	0.01	-0.07	-0.03	0.08	-0.13	-0.01
BS60							1.00	0.454**	0.03	0.08	0.09	-0.21**	0.01
BS110								1.00	0.12	0.08	0.03	-0.17	-0.02
P content									1.00	0.572**	-0.87**	-0.42**	-0.02
P uptake										1.00	0.398**	-0.21**	0.557**
PUE											1.00	0.04	0.623**
Fe content												1.00	0.619**
Fe uptake													1.00

Star (*) symbol represents significant coorelation among traits at 1% (P=.01) level of significance, whereas ** denotes significance at 5% (p=0.05) level of significance. TN=Tiller Number, FGPP=Filled Grains Per Panicle, PUP=Phosphorus Uptake, PUE=Phosphorus Use Efficiency, BS=Bronzing Score

The susceptible genotype, KMR3, showed significant decrease in shoot and root dry weight under low P and Fe toxicity conditions in hydroponic. However, in LR11, the difference

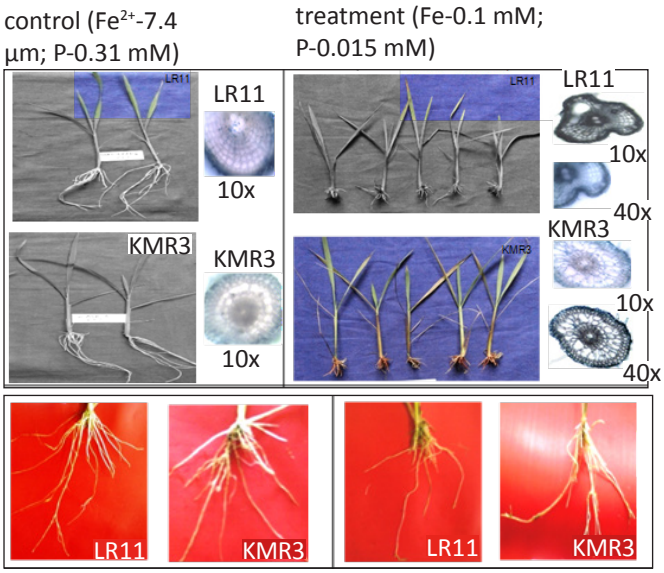

Figure 3.2: Variation observed in 21 days old roots and shoots of LR 11 and KMR 3 in hydroponics under control (Fe-7.4 µm; P-0.31 mM) and treatment (Fe-0.1 mM; P-0.015 mM) conditions. Seedling morphology (top panel) and root sections after pearl's blue staining are shown

in root and shoot dry weight in control and treatment were not significant.

Previously, Wu et al., 2014 had suggested that excess amounts of iron in hydroponics could lead to distinct variation in the bronzing score and root or shoot biomass. In our case, screening using excess of Fe and low P simultaneously, Kasalath (LR60) performed at par with LR11. The phenotypic variation in parent's treatment and control under hydroponics screenings are shown in Figure 3.2 along with their cross section of roots in 10X and 40X after pearl blue staining.

3.5. Probable mechanism of tolerance in Shasarang (LR11)

The Fe content and Fe uptake in flag leaves for LR11 was higher as compared to the KMR3 under acidic lowland field condition. The P uptake values in the flag leaf of the two genotypes were comparable. However, significant differences in values for the amount of Pi in total dry shoot were found (Yumnam, 2015) with LR11 and KMR3 having values of 4.17±1.03 mg $plant^{-1}$ and 3.07± 1.02 mg $plant^{-1}$, respectively.

4. Conclusion

The tolerance of Shasarang (LR11) was proven in all the quality traits attributed for acidity tolerance and hence, LR11 can be used as a tolerant donor under acidic lowland conditions. This study has led to a better understanding of a rice genotype, LR11 and will help to generate and evaluate novel molecular breeding resources to understand the tolerance mechanism suitable to soils of North Eastern Region of India.

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