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Identification of Swarna x *O. nivara* (RPBio4918) advanced backcross lines performing well under acidic soil conditions

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Abstract

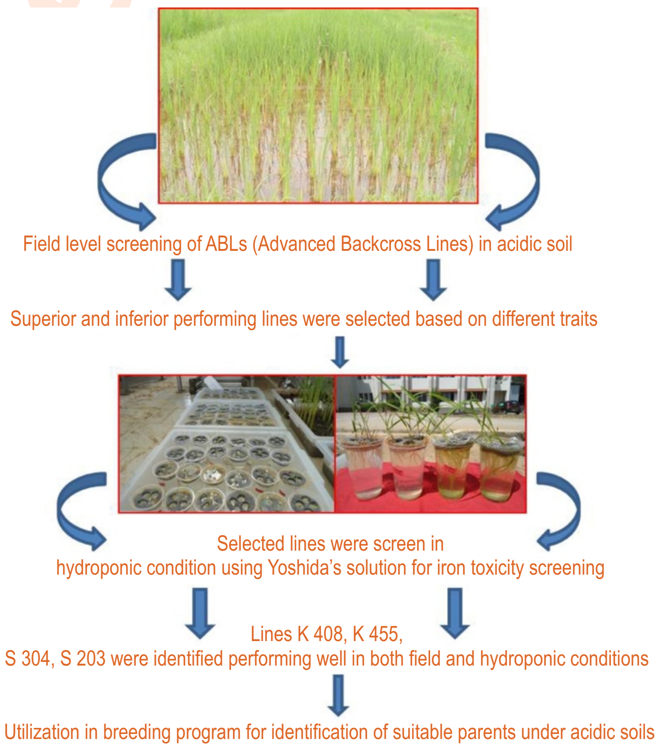
Aim: To evaluate a set of ABLs (Advanced Backcross Lines) to identify lines perfoming well under acidic soil and iron toxicity hydroponics conditions.

Methodology: A total of 194 ABLs were randomly used in lowland field randomly following augmented experimental design. Selected lines were screened in hydroponics condition using Yoshida's solution for iron toxicity tolerance.

Results: Under field conditions, several deficiencies and toxicities often co-exist, and it becomes difficult to partition the effect of different stresses on the genotypes. Therefore, screening genotypes in artificial hydroponics conditions allows us to dissect the response of a genotype to one particular nutrient toxicity or deficiency. Based on different traits superior, average and inferior performing lines were selected from field condition. Hydroponic experiment was conducted using Yoshida's solution for iron toxicity screening, different superior and inferior lines were identified based on the parameters like root growth, root and shoot biomass. Four lines K 408, K 455, S 304, S 203 were identified that performed well in both field and Fe toxic hydroponic conditions.

Interpretation: The information generated would help to identify suitable parents for breeding program under acidic soils, and the phenotypic data on ABLs may serve as a base for future mapping of loci responsible for yield under acidic soils and iron toxic conditions.

Key words: Acidic soil, Advanced backcross lines, Hydroponic condition, Iron toxicity, Seedling stage screening



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Introduction

Rice (*Oryza sativa* L.) constitutes the staple food of 3.5 billion people worldwide and approximately 90% of the world's rice is grown in the Asian continent (El Namaky *et al.,* 2017). In lowland-rice production, iron toxicity is well-recognized as the most widely distributed constraint. Iron toxicity is a complex nutrient disorder in acid soils due to the presence of excessive Fe uptake from flooded soils under reducing conditions. In flooded soil, Fe^{3+} in soil minerals is converted to Fe^{2+}, and this Fe^{2+} is much more soluble in water, leading to an excess of Fe or iron toxicity in the soil solution (Kirk, 2004). Plants suffering with iron toxicity show symptoms like stunted growth, rusty leaf spots, stained leaf edges along with poorly developed root system.

Excessive uptake of Fe^{2+} by roots and its acropetal translocation via xylem flow into the leaves leads to expression of iron-toxicity symptoms. Accumulation of oxidized polyphenols in the leaf occurs due to excess amount of Fe^{2+} which can irreversibly damage cell structural components and lead to typical visual symptom "bronzing" of the rice leaves (Dobermann and Fairhurst, 2000). Majority of visual symptoms of Fe toxicity are commonly observed at tillering and heading stage, but, in severe Fe toxicity conditions, the effects can occur irrespective of any growth stage of rice crop and may lead to complete crop failure (Mahender *et al.,* 2019). Mainly three major morphological and physiological mechanisms or strategies are used by rice plants to survive adverse iron-toxic soil conditions and large amount of iron in plants (Becker *et al.,* 2005). Selection of tolerant or adapted rice genotypes can be done based on these strategies. In case of strategy I (exclusion/avoidance), plants exclude iron at root level and avoid of Fe^{2+} damage to the shoot tissue (root ion selectivity and rhizospheric oxidation).

Iron uptake in the roots of rice is by strategy II (inclusion/avoidance), but tissue damage may be avoided by either compartmentation where immobilization of active iron in old leaves or photosynthetically less active leaf sheath tissue or exclusion from the symplast. Strategy III (inclusion/tolerance) is probably due to enzymatic "detoxification" in the symplast, plants actually tolerate elevated levels of iron within leaf cells. Among the above mentioned three strategies iron exclusion by oxidation in the rhizosphere and detoxification of radicals in leaf cells are well established Fe-tolerance mechanisms of rice (Becker *et al.,* 2005). Tissue tolerance for iron toxicity in rice is considered an important parameter for genetic studies (Gregorio *et al.,* 2002). Genome-wide association study (GWAS) conducted with a population of 329 accessions representing all subgroups of rice to ferrous iron stress has led to better understanding for genetic and physiological mechanisms associated with iron toxicity tolerance (Matthus *et al.,* 2015).

For crop improvement programme against iron toxicity tolerance, two types of selection are distinguished: direct selection which are based on yield and indirect selection using traits such as leaf bronzing score, leaf rolling, plant height, root and shoot biomass, tissue iron concentration, chlorophyll content or agronomic traits other than yield. In case of rice improvement programs against iron toxicity are mainly based on yield as direct selection and leaf bronzing score as a secondary trait for indirect selection (Sikirou *et al.,* 2015). Screening genotypes which are tolerant to iron toxicity stress would be of immense importance in improving rice production in lowland acidic soil conditions. Field screening is essential to estimate the real phenotypic value of genotypes, but it is time and resource consuming.

Under field conditions, several deficiencies and toxicities often co-exist, and it becomes difficult to partition the effect of different nutrient stresses on genotypes. In the present study two sets of ABLs (BC2F8) derived from crosses between a rice mega-variety Swarna and two accessions of *O. nivara* collected from areas affected by problem soils of Bihar (IRGC81832) and Uttar Pradesh (IRGC81848), with Swarna being the recurrent parent (Swamy *et al.,* 2012). Apart from their use as mapping resources, ABLs in the background of popular varieties having chromosomal segments from wild donors that impart stress tolerance may directly be released as improved varieties. Therefore, these ABLs were evaluated under lowland acidic field conditions, and a subset was studied under iron toxic hydroponic condition to identify superior and contrasting ABLs, especially for molecular biological studies, as screening genotypes in artificial hydroponics conditions (Shimizu *et al.,* 2005) allows us to dissect the response of a genotype to one particular nutrient toxicity or deficiency.

Materials and Methods

Plant material: A total of 194 ABLs (Advanced Breeding Lines) were transplanted randomly following augmented experimental design in lowland field of CPGS-AS, CAU(I), Umiam, Meghalaya, situated at 25° 41 latitude and 91° 54 longitude and 950 m above mean sea level.The 194 ABLs are comprised of 123 S lines (Swarna x *O. nivara* (IRGC81848) and 71 K lines Swarna x *O. nivara* (IRGC81832) at BC_2F_9 generation. For screening of ABLs in the field, augmented design was followed where checks were replicated but treatments were used in single entry.

Field experiment: Screening of ABLs in the field, augmented design was followed and the experimental plot was divided into five blocks, each block containing three checks, *i.e.,* Swarna, Shahsarang and Sahbhagi Dhan along with randomly allotted S and K lines. For each line data was recorded on ten plants for different parameters like number. of tillers per plant (30 days old and 60 days old) (TN30; TN60), days to 50 % flowering (DTF), number. of panicles per plant (PPP), number. of grains per panicle, grain yield per plant (GYPP), plant height (PH), biological yield per plant (BY), 100 grain weight, percent spikelet fertility (SF), panicle length (PL), harvest index (HI), bronzing score (BS). Bronzing score was based on a scale of 1-3 (1- less/damage; 2-moderate damage and 3-severe damage) and was usually observed in older leaves (after 60 days of transplanting. Tillers per plant was measured after 30 and 60 days of transplanting. Traits like PPP, GYPP, BY, SF, PL, HI were recorded post-harvest.

Hydroponic experiment: Thirty seeds for each line were germinated on moist filter paper having deionised water and allowed to grow for 5 days. The five-day-old seedlings were transferred to plastic cups (with mesh top) containing Yoshida solution (Yoshida *et al.,* 1976). Each cup contained 4 seedlings and a total of 4 cups (two for control and treatment each) were used per ABL. Control solution was 1X Yoshida solution at pH 5.0 while the treatment solution maintained at pH 4.0 had 200 mg l^{-1} iron sulphate ($FeSO_4$) intially and subsequently 300 mg l^{-1} $FeSO_4$. The solution was changed every 5 days and the seedlings were allowed to grow till 21 days. After 21 days of growth in control and treated solution, data like seedling height, bronzing score and root biomass, shoot biomass (after air drying) was recorded.

Statistical analyses: The data obtained from field experiment was subjected to online augmented design analysis available at Indian Agricultural Statistics Research Institute website (http://www.iasri.res.in/spadweb). The adjusted means were subjected to Analysis of Variance (ANOVA) using Statistical package for agricultural research (SPAR 2.0). The hydroponics data (mean, standard error and graphs) were analysed and plotted using excel.

Results and Discussion

In the present study, 194 ABLs were screened under acidic field conditions with three varieties Swarna, Shahsarang and Sahbhagi Dhan as checks. Variance due to treatments (ABLs) was found to be significant for TN30 (range 2.20-11.00), PPP (range 2.0-15.00), FGPP, SF (range 0.37-87.79) , GY (range 1.33-26.80) and BY (range 28.01-112.36) (Table 1). Coefficient of variation was found to be high for TN60, indicating that the trait was highly affected by microenvironmental conditions. This suggests that sufficient variability was present among the ABLs with respect to the above mentioned key traits. Highly significant (F=165.62, P<0.001) values from one-way ANOVA was also confirmed in a set of Sri Lankan rice varieties and advanced breeding lines where difference in leaf bronzing score were studied at different growth stages(Siriwardana *et al.,* 2019).

Superior and inferior ABLs were identified on the basis of performance with respect to four traits (TN30, PPP, SF and GY) that are mostly influenced by iron toxicity in acidic lowland soil. The performance of K and S lines with respect to these traits are indicated in Fig. 1, 2. Individual ABLs that performed better than the checks, especially the recurrent parent Swarna were identified for each of the four traits and are summarized in Table 2. Simultaneously, ABLs performing poorly with respect to the traits were also identified, which may be useful for subsequent mapping studies.

Table1: Analysis of Variance for Advanced backcross lines (ABLs)

	df	TN30	TN60	PH	DTF	PPP	df	FGPP	SF	BY	GY
Treatments (Adjusted)	1196	22.72**	44.93	2244.24	666.63	55.59**	554	4481.72**	3395.73**	2297.52**	998.40**
Block (Adjusted)	44	00.73**	111.17	3358.87	1148.96	88.15**	44	2211.23**	1183.18**	5544.58**	88.99**
R^2		00.997	00.98	00.99	00.94	11.00		00.99	00.99	00.99	11.00
Coefficient of variance		99.63	221.73	112.00	111.74	55.51		113.00	111.26	66.91	77.92
Root MSE		00.50	22.10	99.11	110.73	00.47		55.23	44.63	44.07	11.13
General mean		55.21	99.66	775.97	991.35	88.56		440.27	441.11	558.94	114.24

Tn30- Tiller number at 30 days; TN60- Tiller number at 60 days; PH- Plant height; DTF- Days to 50% flowering; PPP- Plants per pancilce; FGPP- Filled grains per panicle; SF- Spikelet fertility; BY- Biological yield; GY- Grain yield. df- Degrees of freedom; MSE- Mean square error. (*) = Significant at 5% level of significance; (**) = Significance 1%

Table 2: Contrasting K and S series ABLs identified with respect to tillers per plant (TN30), number of panicles per plant (PPP), percent spikelet fertility (% SF) and grain yield per plant (GYPP)

Traits	Tillers per plant (TN30)		Number of panicles per plant (PPP)		Percent spikelet fertility (SF)		Grain yield per plant (GYPP)	
K and S series	K series	S series	K series	S series	K series	S series	K series	S series
Mean value	4.82	5.61	8.39	9.00	22.46	48.02	9.78	13.45
Variance	2.26	3.23	10.20	4.45	349.93	300.70	28.65	28.09
Range	2.2 – 11	2.2-9.73	0.39-14.89	2.29-14.88	0.37 - 68.72	4.76 -87.79	1.33 - 23.14	4.53 -26.80
Superior performers (lines)	K 417, K 416, K 419, K418, K 415	S 219, S 247, S 203, S 262, S 264	K 410, K 456, K 413, K 419, K 416	S 326, S 328, S 321, S203, S 323	K 450, K 448, K 447, K 455, K 410	S 260. S 319, S 281, S 240, S 203	K 410, K 437, K 447. K 413	S 260, S 319, S 304, S 240, S 328
Inferior performers (Lines)	K 429, K 436, K 440, K 442, K 422	S 313, S 310, S 327, S 299, S 296	K 450, K 449, K 435, K 414, K 436	S 207, S 296, S 208, S 222, S 299	K 422, K 440, K 417, K 409, K 441	S 299, S 258, S 326, S 305, S 328	K 450, K 440, K 422, K 448, K 408	S 299, S 310, S 300, S 222, S 296

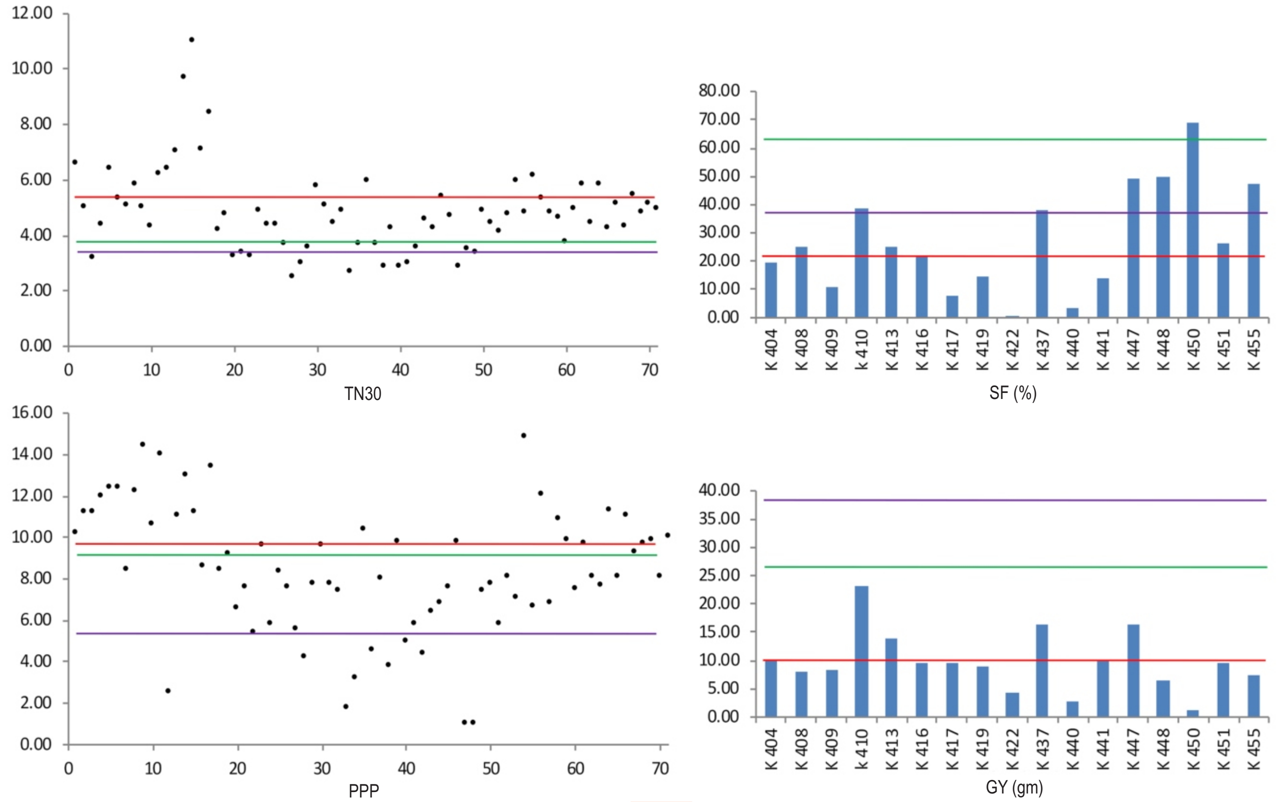

Fig. 1: Phenotypic variation observed under field conditions in 71 K series ABLs as compared with three check varieties with respect to tillers per plant (TN30), number of panicles per plant (PPP), percent spikelet fertility (SF) and grain yield per plant (GY). Post harvest data is only shown for a subset of lines representing the variation observed. Red, green and purple horizontal lines represent mean trait values of checks Swarna, Shahsarang and Sahbhagi dhan, respectively.

A majority of the S series ABLs outperformed the checks for number of tillers at 30 DAT and number of panicles per plant, indicating contribution of favourable alleles for the traits from donor *O. nivara* (IRGC81848). However, transgressive segregants in the case of K series ABLs were much lesser in proportion. Similarly, for grain yield per plant and spikelet fertility, the proportion of transgressive segregants observed were significantly higher in S series ABLs. None of the ABLs were able to outperform the best check (Sahbhagi dhan) in terms of grain yield per plant. ABLs consistently performing well for all the traits were also identified. Differences in root morphology and biomass of K and S lines were also observed between superior and inferior ABLs as depicted in Fig 3. Similar studies in rice revealed that iron toxicity affects rice growth through difference in shoot and root development (Li *et al.,* 2016; Wu *et al.,* 2017). These differences due to Fe toxicity are more prominent in lowland rice field as more iron is available in reduced form. This condition affects the plant nutrition and ultimately decrease the crop yield (Morrissey *et al.,* 2009; Kim *et al.,* 2007). Gbeto-Dansou *et al.* (2017) also reported that plots with iron toxicity gives less yield as compared with plots not affected by toxicity. Depending on the severity of iron toxicity and also form of iron, plant growth is affected (Muller *et al.,* 2015).

Although in some studies, intense iron toxicity symptoms are associated with lowest yield (Diedhiou *et al.,* 2020) but most of the inferior lines identified in our study did not show iron toxicity symptoms. Previously a decrease in rice yield has been reported even in plots where plant did not show any symptoms of iron toxicity (Hua *et al.,* 2001; Sikirou *et al.,* 2015). Also, the leaf bronzing appreance appears to be non-significant in many cases but significant reduction in yield was observed (Sahrawat, 2004; Onaga *et al.,* 2012). Soil heterogeneity or Genotype × Environment interactions (GxE) might be the reason for creating such differences among lines. Even in the same field, variability in the distribution of iron leads to a large environmental errors and express different G x E interactions on rice yield which ultimately affects the breeding efficiency of genotype selection for iron toxicity tolerance (Cherif *et al.,* 2006). Therefore, iron toxicity tolerance should be directed to multi locational trials as well as biophysical environmental analysis before establishing a variety (Piepho, 1996).

Fe toxicity tolerance screening for selected ABLs: Based on the field performance (Fig. 1, 2), a smaller set of contrasting ABLs (7 K lines- K 408, K 437, K 440, K 448, K 450, K 455 and 9 S lines- S203, S 240, S296, S 300, S 304, S 307, S 310, S 312, S 323)

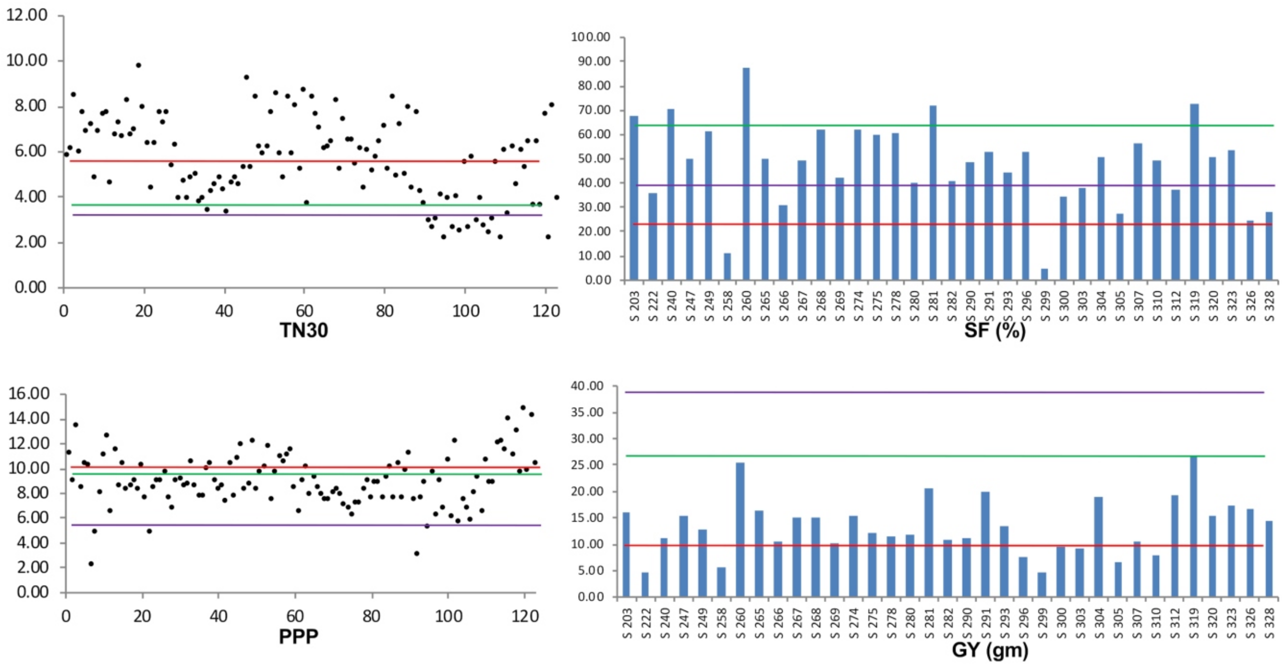

Fig. 2 : Phenotypic variation observed under field conditions in 123 S series ABLs as compared with three check varieties with respect to tillers per plant (TN30), number of panicles per plant (PPP), percent spikelet fertility (SF) and grain yield per plant (GY). Post harvest data is only shown for a subset of lines representing the variation observed. Red, green and purple horizontal lines represent mean trait values of checks Swarna, Shahsarang and Sahbhagi dhan, respectively.

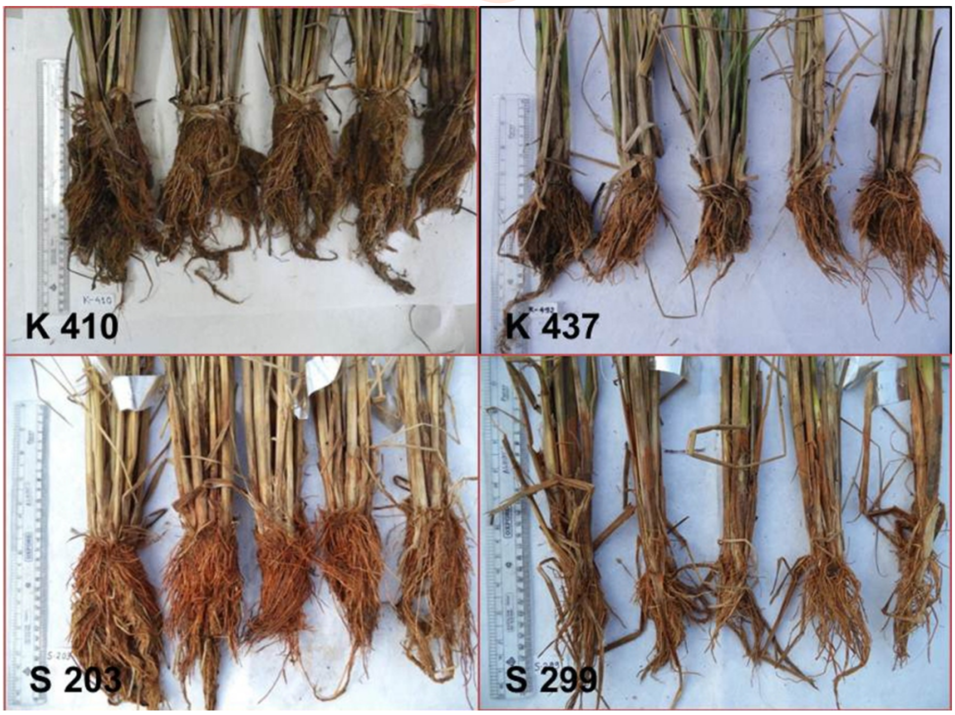

Fig. 3 : Representative picture showing differences in root morphology and biomass of superior and inferior K and S lines under acidic field conditions. Lines K 410, S-203 showed high root biomass and tiller number as compared to K 437, S-299 lines.

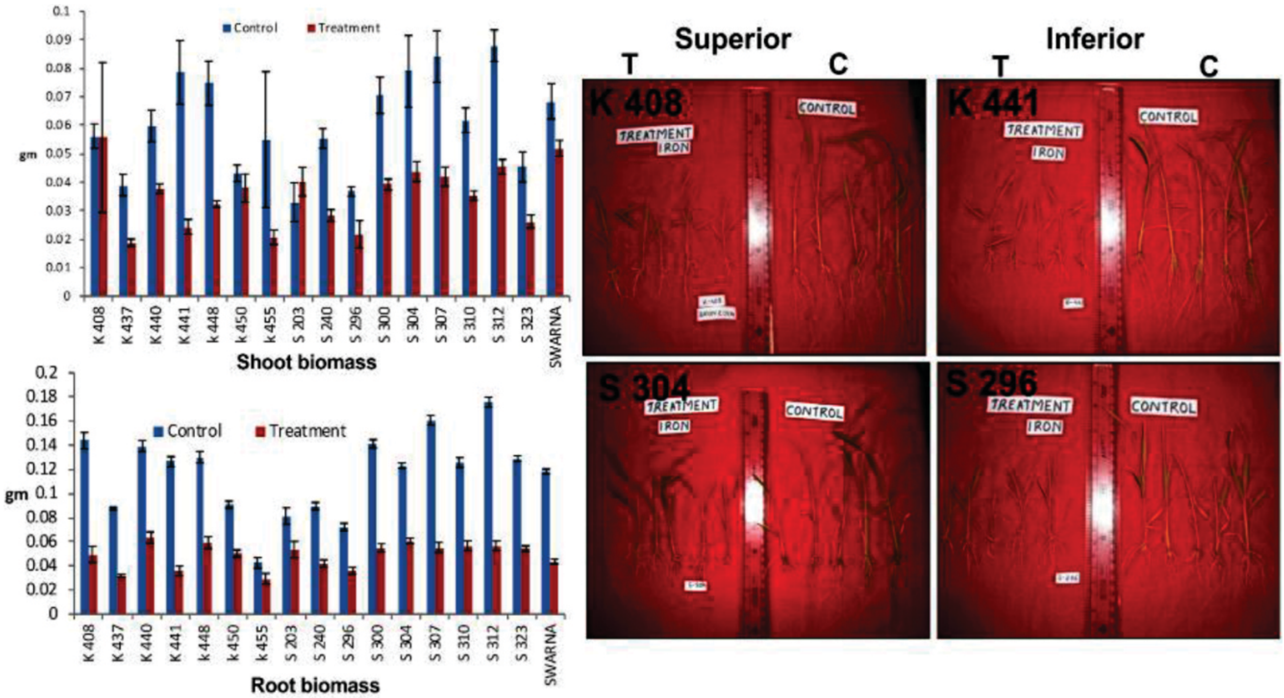

Fig. 4 : Variation in (a) shoot and root biomass among selected K and S lines and (b) representative picture of superior and inferior lines under control (C- 2 mg l^{-1} Fe) and treatment (T- 200 mg l^{-1} Fe) hydroponic conditions.

along with their recurrent parent Swarna were evaluated under hydroponic condition in greenhouse in response to iron toxicity. Elec *et al*. (2013) carried out a similar study by establishing a nutrient-based screening method for rice. Data was recorded after 21 days after stress treatment. Traits like shoot biomass and root biomass were recorded. There was significant decrease in shoot biomass and root biomass in almost all the genotypes in (200 mg l^{-1} Fe) as compared to control (2 mg l^{-1} Fe) (Fig. 4a)). Symptoms like stunted growth, rusty leaf spots, stained leaf edges and a poorly developed root system and bronzing were observed in some lines (K 440, S 240, S 312, and S 222). Superior lines (K 450, K 408, S 203, and S 304) and inferior lines (K 441, S 296) were selected based on shoot biomass and root biomass. Previously, using a set of 220 BC_3DH lines derived from the backcross *O. Sativa* (Caiapo)/ *O. glaberrima* (MG12)// *O. sativa* (Caiapo) screened in the presence or absence of iron (0 or 250 mg l^{-1}) revealed that that iron toxicity mechanism differs in cultivated rice and wild rice can be used as a source of resistance (Dufey *et al.,* 2015).

In the present study, no significant correlation was found between the yield contributing traits under field conditions and the traits studied under hydroponic condition (data not shown). However, four superior ABLs (K 408, K 455, S 304, S 203) that performed consistently in acidic soil and hydropnoics screening were identified based field performance, root and shoot biomass under hydroponics and visual observations. Some of the poor performing lines identified were K 441, K 437, S 296 and S 300 (Fig. 4b)). The superior lines identified in this study can be used as potential donors for toxicity tolerance. As iron toxicity is a complex trait, crosses between superior and inferior lines can be used for mapping study as well. Previously, screening two different bi-parental mapping populations under iron pulse stresses Wu *et al.* (2014), followed by experiments with selected lines were able to shed light on genetic basis of shoot-based Fe toxicity tolerance mechanisms (Wu *et al.,* 2017), Similar strategy using wild rice species as donors has been used to generate important resources for mapping complex trait like yield (Reddy *et al.,* 2005; Tian *et al.,* 2006). In conclusion the phenotypic variations observed in this study suggests variable genome contribution from the donor parent (*Oryza nivara*) and this can be used effectively for QTL mapping. The four superior ABLs identified can be used as donors for mapping novel sources of iron toxicity tolerance in rice after further validation.

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Add-on Information

Authors' contribution: A. Debnath: Performed and designed the experiments, wrote draft and Final Page Proof; **M. Rai:**

conceived the research plan, designed the experiments, analyzed the data and also reviewed and edited the paper, providing helpful comments and discussions; **W. Tyagi:** Conceived the research plan and designed the experiments, reviewed and edited the paper and helped in preparation of Final Page Proof.

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