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Selection of F_2 plants through hydroponics screening at higher iron toxic levels for bulk segregant analysis (BSA) in rice

M Amaranatha Reddy, Rose Mary Francies, Jiji Joseph, CR Elsy and PS Abida

M Amaranatha Reddy
Department of Plant Breeding and Genetics, College of Horticulture, Kerala Agricultural University, Thrissur, Kerala, India

Rose Mary Francies
Department of Plant Breeding and Genetics, College of Horticulture, Kerala Agricultural University, Thrissur, Kerala, India

Jiji Joseph
Department of Plant Breeding and Genetics, College of Horticulture, Kerala Agricultural University, Thrissur, Kerala, India

CR Elsy
Department of Plant Breeding and Genetics, College of Horticulture, Kerala Agricultural University, Thrissur, Kerala, India

PS Abida
Department of Plant Breeding and Genetics, College of Horticulture, Kerala Agricultural University, Thrissur, Kerala, India

Correspondence
M Amaranatha Reddy
Department of Plant Breeding and Genetics, College of Horticulture, Kerala Agricultural University, Thrissur, Kerala, India

Abstract
Phenotypic screening of 300 F_2 plants at higher toxic iron levels of hydroponics revealed that wide variability is observed for shoot length, root length, total number of roots, number of fresh roots, shoot weight, root weight and visual scoring for iron-toxicity symptoms. A total of 15 F_2 plants and 87 F_2 plants out of 300 plants had exhibited leaf bronzing score 1 and 9 respectively at six weeks after exposure to iron stress at 800ppm of Fe. Ten most tolerant and ten most susceptible plants selected from 15 and 87 F_2 plants respectively based on ranking of total score which is obtained by adding individual ranks of all characters of particular plant. Plant number 248, 320, 309, 111, 156, 20, 287, 268, 52 and 300 respectively from 15 F_2 plants with highest overall score were selected as ten most tolerant plants. The selected susceptible plants with the least overall score were plant number 12, 202, 66, 18, 109, 113, 122, 334, 231 and 213 respectively. Resistant bulk DNA sample and Susceptible DNA sample was prepared by mixing equal amounts of DNA at same concentration from ten most tolerant F_2 plants and ten most susceptible F_2 plants respectively.

Keywords: Hydroponics, leaf bronzing, resistant bulk and susceptible bulk

Introduction
Globally, rice is the most important food crop, serving as staple food for more than half of the world's population (Khush 2005) [1]. It occupies almost one-fifth of the total land area cropped with cereals. During 2015, the total global rice production reached 740.2 million tonnes from an area of 161.1 Mha (FAO, 2016) [2]. Rice and wheat are the major food crops grown in India. In 2015, the total rice production in the country reached 104.8 million tonnes with a production of 44.16 Mha and productivity of 2373 kg/ha (Indiastat, 2015) [3].

In acidic soils of Kerala, iron content of the root to the order of 50,000 ppm under submerged conditions was found to inhibit morphological and physiological development leading to low yield (Bridgit, 1999) [4]. During recent years, the problem of iron toxicity has become even more severe due to the introduction of modern high-input rice varieties susceptible to excess iron. Several management and cultural practices have been proposed for the control of iron toxicity in the field. Great inter-varietal differences in iron toxicity tolerance in rice have been reported (Mohanty and Panda, 1991) [5]. Therefore, exploiting the varietal tolerance to iron toxicity is accepted as the most cost-effective and practical means for increasing rice production under iron toxic soils (Shimizu, 2009) [6].

Rice varieties are different in their tolerance for iron toxicity and this selection of rice variety with better iron tolerance is important to avoid yield reduction. Genetic differences in adaptation and tolerance for iron toxic soil conditions have been exploited for rice variety with tolerance for iron toxicity (Gunawardena *et al.*, 1982; Fageria *et al.*, 1990) [7, 8]. The existence of genetic variability for various desirable maturity and yield related traits in segregating generations is of utmost importance in crop breeding programs to develop desirable recombinant inbred lines and cultivars. Breeders have developed a wide array of cultivars with various degrees of adaptation, using both traditional breeding methods (Akbar *et al*., 1987; Gunawardena *et al.*, 1982; Luo *et al*., 1997; Mahadevappa *et al*., 1991) [9,7,10,11] and quantitative trait loci (QTL) analysis combined with marker-assisted breeding (Bennett, 2001; Wan *et al.*, 2003a and 2003b; Wissuwa, 2005) [12,13,14,15].

According to De Datta *et al.* (1994) [16] delineating the genetic differences in tolerance and adaptation to iron stress, requires screening the genotypes in iron toxic soil conditions.

Since, obtaining uniform field experimental conditions to evaluate iron toxicity tolerant genotypes is difficult to come by, the use of molecular markers to assist selection of tolerant genotypes offer a better alternative. Mackill *et al.* (1999) [17] advocated that characterization of QTL mapping populations combined with marker-assisted selection would be a promising approach for improving the resistance of cultivars to iron toxicity. Bulk Segregant Analysis (BSA) helps in quick identification the loci governing for screened character based on molecular polymorphism between resistant bulk and susceptible bulk by making genetic background of tolerant plants and susceptible plants except screened character as homogenous. Hence, selection of most tolerant plants and most susceptible plants for preparation of resistant bulk and susceptible bulk is discussed in this investigation.

Materials and methods

The experimental material for the study comprised of thirty rice genotypes selected from the KAU rice germplasm maintained at Regional Agricultural Research Station (RARS), KAU, Pattambi. The 30 rice genotypes were subjected to further screening to confirm their tolerance or susceptibility to iron toxicity. One most tolerant genotype (Tulasi) and most susceptible genotype (CUL-8709) selected and used for development of F_2 population. 300 F_2 plants and their parents were screened at 800 ppm of Fe through hydroponics. In the present study, an attempt has been made to understand the influence of iron at toxic level (800ppm) on growth parameters *viz.*, shoot length, root length, total number of roots, number of fresh roots, shoot weight, root weight and visual scoring for iron-toxicity symptoms of F_2 plants. The amount of iron reversibly adsorbed on root surface, iron content in root and leaf were also assessed.

DNA bulks were constituted for each trait by pooling the DNA of phenotypic extremes. Two bulks (one for higher extremes and the other for lower extremes) were made for each of the traits considered for marker analysis. Ten F_2 plants found most tolerant to iron toxicity and ten most susceptible F_2 individuals were identified based on outcome of Experiment 4(i): Phenotyping of F_2 plants for iron toxicity tolerance. An equimolar amount (10 µl) of genomic DNA from the selected ten susceptible F_2 individuals was bulked to constitute the susceptible bulk (SB). Similarly, an equimolar amount (10 µl) of genomic DNA from the selected ten resistant F_2 individuals was bulked to constitute the resistant bulk (RB).

Results and Discussion

Results indicated presence of wide variability for these traits among the F_2 plant population studied. Wu *et al.* (1997) [18] had also observed wide variability among double haploid (DH) populations for leaf bronzing index and shoot weight in confirmation with the results of the present study.

Mean visual scoring for iron-toxicity symptoms of 300 F_2 plants after 4 weeks of 800ppm of Fe treatment was 5. Mean visual scoring for iron-toxicity symptoms of 300 F_2 plants after 6 weeks of 800ppm of Fe treatment was 8. Visual scoring for iron-toxicity symptoms ranged from 1 to 9 after both after 4 weeks and 6weeks of 800ppm of Fe treatment.

A total of 15 F_2 plants (Table 1) had exhibited leaf bronzing score 1 at six weeks after exposure to iron stress at 800ppm of Fe. These 15 F_2 plants are plant number 20, 52, 110, 111, 156, 246, 248, 268, 287, 300, 308, 309, 319, 320 and 354. Since under iron stress, tolerance found to be positively correlated with traits root length, shoot length, total number of roots, number of fresh roots, root weight, shoot weight, iron reversibly adsorbed on root surface and iron content in the root, the 15 plants were ranked serially (1, 2…) in ascending order of magnitude for individual traits. However, plants were scored in descending order of magnitude with respect to traits iron reversibly adsorbed on root surface and iron content in leaf as these traits were found to be negatively correlated with iron stress tolerance. The total score for each plant was then ascertained by summation of the ranks obtained by the plant for the different traits studied. Finally, the top ten plants with the highest total score were selected as the most promising tolerant plants to constitute the resistant bulk (RB). The ten most tolerant F_2 plants selected from these 15 F_2 plant were plant number 248 (Score: 84), 320 (Score: 79), 309 (Score: 77), 111 (Score: 75), 156 (Score: 74), 20 (Score: 60), 287 (Score: 59), 268 (Score: 55), 52 (Score: 50) and 300 (Score: 49) respectively (Table 2).

Through a similar exercise, out of the 87 F_2 plants (Table 3) that recorded a leaf bronzing score of 9 on four weeks of exposure to iron stress (800ppm of Fe), ten most susceptible F_2 plants were selected. In this instance unlike in the above case, the ten plants with the least total score were selected to constitute the susceptible bulk (SB). The selected susceptible plants with the least overall score were plant number 12(Score: 48), 202 (Score: 57), 66 (Score: 73), 18 (Score: 80), 109 (Score: 81), 113 (Score: 84), 122 (Score: 90), 334 (Score: 93), 231 (Score: 95) and 213 (Score: 107) respectively. These top ten susceptible are selected for development of susceptible bulk used for bulk segregant analysis (Table 4).

Based on the LBI from among the 300 F_2 phenotype plants, fifteen F_2 individuals with an LBI score of 1 at Fe stress (800ppm) were identified to be tolerant iron stress while 87 F_2 individuals with an LBI score of 9 were scored as susceptible. Since significant negative association was evident between LBI and traits *viz.*, root length, shoot length, total number of roots, number of fresh roots, root weight, shoot weight, iron reversibly adsorbed on root surface and iron content in the root characters, the tolerant and susceptible individuals were further evaluated on the basis of a collective score obtained by the individuals based on the *per se* performance for various traits (Table 1 and 3). Considering both the LBI score and the collective performance of each of the F_2 individual for various traits, ten plants (Plant number 248, 320, 309, 111, 156, 20, 287, 268, 52 and 300) were identified as the most tolerant F_2 plants while plant number 12, 202, 66, 18, 109, 113, 122, 334, 231 and 213 were identified to be the ten genotypes most susceptible to Fe stress. Resistant bulk DNA sample was prepared by mixing equal amounts of DNA at same concentration from ten most tolerant F_2 plants and DNA of susceptible bulk was prepared by mixing equal amounts of DNA at same concentration from ten most susceptible F_2 plants as listed above. Each pool or bulk contains individuals selected to have identical genotypes for a particular genomic region (target locus or region). Therefore, the two resultant bulked DNA samples differ genetically only in the selected region and are seemingly heterozygous and monomorphic for all other regions (Michelmore *et al.*, 1991) [19].

The iron toxicity symptoms were more pronounced in the sensitive bulk than in resistant bulk. Extreme leaf bronzing score values were observed between susceptible bulk and resistant bulk. The values of susceptible bulk and resistant bulk were found to be on par with that of susceptible parent and resistant parent respectively. Similarly, traits like shoot length, root length, total number of roots, number of fresh roots, shoot weight, root weight, iron reversibly adsorbed on

Table 1: Phenotypic data of individual F_2 plants resistant to iron toxicity at 800ppm of iron

F_2 plant no / Parent	Leaf bronzing score (4th week)	Leaf bronzing score (6th week)	No of roots	Root length (cm)	Shoot length (cm)	Root weight (g)	Shoot weight (g)	No of fresh roots	Fe adsorbed on root (mg l^{-1})	Root Fe content (mg kg^{-1})	Leaf Fe content (mg kg^{-1})
248	1	1	33	25.9	62.8	7.55	11.60	32	12.81	13932.69	688.75
320	1	1	33	23.6	62.3	7.35	12.60	28	14.87	13685.57	656.25
309	1	1	34	24.3	62.8	7.80	13.45	29	12.08	12979.04	658.75
111	1	1	34	25.6	62.3	8.20	12.25	31	13.38	11514.12	692.50
156	1	1	32	24.9	62.6	8.20	11.50	27	15.13	13723.96	726.25
20	1	1	34	24.4	61.8	7.85	12.05	31	12.60	11763.89	736.84
287	1	1	32	23.6	62.1	7.25	12.30	28	13.46	13360.82	748.75
268	1	1	32	25.1	61.8	7.45	11.25	27	12.57	12189.29	678.75
52	1	1	33	23.5	62.5	7.20	11.95	28	14.35	11318.52	732.50
300	1	1	31	23.5	61.6	7.20	11.35	26	12.62	13762.20	691.25
246	1	1	32	25.3	61.6	7.40	11.20	30	12.16	11965.36	716.25
319	1	1	31	23.1	61.4	7.10	11.85	26	13.04	12468.93	665.00
308	1	1	32	23.6	61.6	7.30	12.95	26	12.41	11719.95	760.00
110	1	1	32	23.9	61.1	7.15	11.55	28	11.92	12691.29	763.75
354	1	1	31	23.3	60.9	7.15	10.90	26	12.85	11687.85	706.25
*PGC14	1	1	31	23.4	61.2	7.80	11.30	27	12.19	11918.52	731.25

* PGC 14 – Tulasi (Resistant parent)

Table 2: Ranking of F_2 genotypes that exhibited high tolerance to iron stress

S. No.	Plant no	(1) Leaf bronzing score –4 weeks	(2) Leaf bronzing score - 6 weeks	(3) No of roots	(4) Root Length (cm)	(5) Shoot length (cm)	(6) Root weight (g)	(7) Shoot weight (g)	(8) No of fresh roots	(9) Fe reversibly adsorbed on root (mg l^{-1})	(10) Root Fe content (mg/kg)	(11) Leaf Fe content (mg/kg)	Total score (Σ 1 to 11)	Final ranking
1	248	1	1	3	12	10	9	7	7	8	15	11	84	14
2	320	1	1	3	4	7	6	13	3	14	12	15	79	13
3	309	1	1	4	6	10	10	15	4	2	10	14	77	12
4	111	1	1	4	11	7	12	11	6	11	2	9	75	11
5	156	1	1	2	8	9	12	5	2	15	13	6	74	10
6	20	1	1	4	7	5	11	10	6	6	5	4	60	9
7	287	1	1	2	4	6	4	12	3	12	11	3	59	8
8	268	1	1	2	9	5	8	3	2	5	7	12	55	7
9	52	1	1	3	3	8	3	9	3	13	1	5	50	6
10	300	1	1	1	3	4	3	4	1	7	14	10	49	5
11	246	1	1	2	10	4	7	2	5	3	6	7	48	4
12	319	1	1	1	1	3	1	8	1	10	8	13	48	4
13	308	1	1	2	4	4	5	14	1	4	4	2	42	3
14	110	1	1	2	5	2	2	6	3	1	9	1	33	2
15	354	1	1	1	2	1	2	1	1	9	3	8	30	1

Table 3: Phenotypic data of individual F_2 plants susceptible to iron toxicity at 800ppm of iron

F_2 plant no	Leaf bronzing score (4th week)	Leaf bronzing score (6th week)	No of roots	Root Length (cm)	Shoot Length (cm)	Root Weight (g)	Shoot Weight (g)	No of fresh roots	Fe adsorbed on root (mg l^{-1})	Root Fe content (mg kg^{-1})	Leaf Fe content (mg kg^{-1})
12	9	9	22	15.8	50.1	2.85	4.00	0	2.80	6391.67	2560.00
202	9	9	22	15.8	49.5	2.90	3.80	0	2.86	6666.67	2646.25
66	9	9	23	16.1	50.6	3.00	4.20	0	2.71	6746.53	2533.75
18	9	9	21	15.6	49.4	2.85	3.90	0	2.81	6547.15	2255.00
109	9	9	22	15.7	49.0	3.00	3.95	0	3.07	6583.33	2483.75
113	9	9	22	16.0	51.2	3.05	4.35	0	2.82	6558.14	2468.75
122	9	9	22	16.2	50.3	3.20	4.70	0	2.84	6160.38	2382.50
334	9	9	23	15.9	51.1	3.05	4.15	0	3.17	6546.51	2541.25
231	9	9	23	15.9	51.3	3.45	4.85	0	2.95	6443.07	2585.00
213	9	9	23	16.4	52.1	3.05	4.30	0	3.03	6724.45	2548.75
194	9	9	21	15.9	49.9	3.25	4.75	0	2.85	6775.59	2417.50
303	9	9	23	16.2	52.4	3.30	4.40	0	2.74	7041.26	2653.75
131	9	9	22	16.6	51.6	3.20	4.50	0	3.19	6722.22	2448.75
168	9	9	22	16.9	52.3	3.25	4.30	0	2.75	7911.11	2511.25
84	9	9	22	16.8	52.6	3.15	4.25	0	2.99	7361.84	2477.50
242	9	9	22	16.3	51.8	3.15	4.20	0	2.82	7573.01	2313.75
65	9	9	24	17.9	52.9	3.35	4.70	0	3.11	6982.95	2816.58
96	9	9	22	18.9	53.5	3.50	4.30	0	3.16	6753.68	2468.75
24	9	9	22	16.7	52.9	3.20	4.80	0	3.52	6357.14	2307.50
77	9	9	22	16.8	52.7	3.15	4.40	0	2.90	7461.65	2417.50
1	9	9	24	19.3	55.1	3.05	4.25	0	2.90	6567.86	2265.00
270	9	9	23	16.3	52.3	3.10	4.35	0	3.21	6335.99	1890.00
183	9	9	23	16.6	51.2	3.25	4.30	0	3.24	6688.30	1961.25
15	9	9	22	16.6	54.4	3.35	4.55	0	3.28	6504.08	2232.50
174	9	9	25	17.6	52.3	3.35	4.45	0	2.91	8365.74	2585.00
78	9	9	22	16.9	51.8	3.20	4.35	0	2.77	8685.90	2310.00
224	9	9	23	17.7	54.2	3.20	4.50	0	2.94	6998.03	2375.00
173	9	9	20	17.0	53.9	3.35	4.70	0	2.88	8976.42	3177.78
64	9	9	24	18.7	53.5	3.20	4.85	0	2.89	6891.79	2247.50
85	9	9	25	18.6	53.7	3.25	4.60	0	2.95	6781.91	2200.00
115	9	9	21	16.6	55.3	3.15	5.00	0	2.95	6943.63	2246.25
75	9	9	26	16.9	56.2	3.35	4.90	0	2.68	7954.27	2653.75
87	9	9	23	19.4	52.7	3.50	4.30	0	2.88	8889.53	2682.50
108	9	9	22	18.0	53.6	3.25	4.95	0	2.65	7424.42	2276.25

Table 3: Continued………

F_2 plant no	Leaf bronzing score (4th week)	Leaf bronzing score (6th week)	No of roots	Root length (cm)	Shoot length (cm)	Root Weight (g)	Shoot weight (g)	No of fresh roots	Fe adsorbed on root (mg l^{-1})	Root Fe content (mg kg^{-1})	Leaf Fe content (mg kg^{-1})
215	9	9	23	16.2	51.3	3.25	4.25	0	2.98	7454.08	2108.75
39	9	9	22	17.9	53.0	3.85	5.10	0	3.00	8254.90	3324.22
35	9	9	25	20.1	54.0	3.70	4.50	0	3.13	6383.93	2238.75
124	9	9	21	16.7	51.5	3.15	4.45	0	3.37	7495.28	2438.75
164	9	9	24	18.1	54.6	3.35	4.30	0	3.09	8089.55	3153.74
291	9	9	25	20.6	55.8	3.50	4.30	0	2.96	7354.35	2952.50
50	9	9	23	19.5	52.9	3.15	4.65	0	2.95	8951.92	2691.25
203	9	9	23	19.4	53.4	3.15	4.50	0	2.83	6906.25	2046.25
205	9	9	22	16.5	52.0	3.20	4.35	0	2.80	7465.75	1922.50
217	9	9	22	16.5	53.2	3.20	4.30	0	3.15	6921.88	2012.50
182	9	9	26	19.0	55.5	3.55	5.20	0	2.77	6625.00	2168.75
48	9	9	22	16.9	51.8	4.25	4.15	0	3.17	7185.42	2230.00
264	9	9	25	19.3	54.2	3.55	4.30	0	3.16	6841.73	2283.75
269	9	9	26	19.4	55.3	3.25	4.35	0	3.67	6323.25	2283.75
148	9	9	24	18.5	53.0	3.55	5.20	0	3.20	7668.18	2821.25
71	9	9	26	18.1	54.6	3.30	4.05	0	2.85	7366.94	2198.75
249	9	9	24	19.8	55.1	2.85	3.90	0	3.03	7398.76	2333.75
166	9	9	24	18.1	54.6	3.20	4.85	0	3.01	8768.94	2730.00
340	9	9	24	16.3	52.1	3.30	4.45	0	3.11	7055.28	1991.25
46	9	9	25	22.3	54.7	3.65	4.35	0	3.13	6966.39	2506.25
67	9	9	22	18.7	52.7	3.85	5.00	0	2.85	7584.91	2308.75
11	9	9	23	18.8	53.9	3.35	6.65	0	3.17	6990.00	2477.50
99	9	9	26	21.2	56.5	3.45	4.60	0	2.89	6819.03	2287.50
19	9	9	21	16.8	53.6	4.35	4.65	0	3.19	6935.55	2230.00
195	9	9	24	17.6	53.1	4.25	4.30	0	3.34	7750.00	2510.00
63	9	9	23	19.1	54.2	3.65	5.20	0	3.31	6932.04	2471.25
192	9	9	23	17.9	53.9	3.50	4.25	0	3.23	6954.55	2148.75
40	9	9	23	22.6	54.5	3.65	4.90	0	3.31	6785.42	2457.50
17	9	9	23	21.6	53.5	4.75	4.95	0	3.13	6953.13	2350.00
134	9	9	23	19.1	53.1	3.55	5.10	0	3.19	7063.73	2292.50
216	9	9	23	17.0	54.5	3.30	4.40	0	2.96	7489.58	2097.50
282	9	9	24	19.3	55.0	3.70	4.50	0	3.48	6412.83	2110.00
36	9	9	24	19.4	52.3	3.15	4.45	0	3.35	7823.68	2355.00
73	9	9	25	19.9	53.9	3.95	5.95	0	2.78	6861.70	2016.25
275	9	9	26	21.1	56.3	5.10	5.35	0	3.27	6691.06	2521.25
86	9	9	22	16.5	52.9	3.15	4.40	0	3.49	8100.00	2256.25

Table 3. Continued………….

F_2 plant no / parent	Leaf bronzing score (4th week)	Leaf bronzing score (6th week)	No of roots	Root length (cm)	Shoot length (cm)	Root weight (g)	Shoot weight (g)	No of fresh roots	Fe adsorbed on root (mg l^{-1})	Root Fe content (mg kg^{-1})	Leaf Fe content (mg kg^{-1})
93	9	9	26	18.9	53.8	3.20	4.80	0	2.83	7533.02	2092.50
312	9	9	26	19.2	53.5	3.35	4.30	0	3.41	6902.88	2061.25
366	9	9	27	20.8	55.9	6.50	6.75	0	3.07	6972.92	2621.25
68	9	9	27	18.2	53.1	3.70	5.35	0	3.10	8259.76	2390.00
274	9	9	24	19.1	55.3	3.25	4.80	0	3.18	6766.39	1933.75
53	9	9	26	22.6	55.9	5.90	7.30	0	3.06	6924.07	2515.00
165	9	9	25	19.4	55.0	3.50	4.60	0	2.99	7268.18	2127.50
185	9	9	28	18.8	54.9	3.20	4.10	0	3.11	7346.59	2006.25
256	9	9	26	21.2	55.2	5.20	6.35	0	3.07	6980.00	2436.25
47	9	9	23	16.6	55.5	3.35	5.00	0	3.16	8500.00	2175.00
8	9	9	24	16.1	55.7	4.80	8.05	0	3.00	8068.18	2145.00
152	9	9	27	21.6	58.2	3.25	5.35	0	2.85	7097.50	2157.50
297	9	9	26	18.9	54.1	4.80	5.30	0	3.25	6901.32	2012.50
301	9	9	26	20.1	56.3	5.10	7.20	0	3.26	6386.65	2023.75
26	9	9	24	19.4	55.0	3.15	5.65	0	3.15	8857.14	2170.00
94	9	9	24	20.8	55.1	3.90	5.15	0	3.14	7856.56	1975.00
281	9	9	26	22.2	56.5	4.10	5.10	0	3.58	6845.07	1873.75
**PGC31	9	9	22	16.3	49.7	2.95	3.95	0	3.36	6889.42	2258.75

** PGC 31 – Cul-8709 (Susceptible parent)

Table 4: Ranking of F_2 genotypes that exhibited high susceptibility to iron stress

S. No.	Plant no	(1) Leaf bronzing score - 4 weeks	(2) Leaf bronzing score - 6 weeks	(3) No of roots	(4) Root length (cm)	(5) Shoot length (cm)	(6) Root weight (g)	(7) Shoot weight (g)	(8) No of fresh roots	(9) Fe reversibly adsorbed on root (mg l^{-1})	(10) Root Fe content (mg/kg)	(11) Leaf Fe content (mg/kg)	Total score (Σ 1 to 11)	Final ranking
1	1	1	1	5	1	30	39	4	9	17	14	46	167	20
2	8	1	1	5	1	6	43	23	37	4	75	61	257	65
3	11	1	1	4	1	25	29	10	33	36	47	24	211	45
4	12	1	1	3	1	3	5	1	4	8	7	14	48	1
5	15	1	1	3	1	1	33	10	15	46	10	52	173	23
6	17	1	1	4	1	40	25	22	23	32	41	36	226	50
7	18	1	1	2	1	1	2	1	2	9	12	48	80	4
8	19	1	1	2	1	13	26	21	17	38	39	53	212	46
9	24	1	1	3	1	12	20	7	20	54	4	41	164	18
10	26	1	1	5	1	31	38	6	30	34	84	57	288	69
11	35	1	1	6	1	35	30	15	14	32	5	51	191	32
12	36	1	1	5	1	31	16	6	13	49	71	35	229	53
13	39	1	1	3	1	18	21	16	25	24	78	1	189	31
14	40	1	1	4	1	43	34	14	22	47	27	27	221	49
15	46	1	1	6	1	42	36	14	11	32	43	22	209	43
16	47	1	1	4	1	1	42	10	24	35	81	56	256	64
17	48	1	1	3	1	14	13	20	7	36	53	53	202	37
18	50	1	1	4	1	32	20	6	17	20	86	8	196	34
19	53	1	1	7	1	43	45	26	36	27	37	19	243	61
20	63	1	1	4	1	28	32	14	27	47	38	25	218	48
21	64	1	1	5	1	24	25	7	21	16	32	49	182	27
22	65	1	1	5	1	18	20	10	18	31	46	6	157	16
23	66	1	1	4	1	6	7	3	8	3	22	17	73	3
24	67	1	1	3	1	24	19	16	24	13	68	40	210	44

Table 4: Continued……

S. No.	Plant no	(1) Leaf bronzing score - 4 weeks	(2) Leaf bronzing score - 6 weeks	(3) No of roots	(4) Root length (cm)	(5) Shoot length (cm)	(6) Root weight (g)	(7) Shoot weight (g)	(8) No of fresh roots	(9) Fe reversibly adsorbed on root (mg l^{-1})	(10) Root Fe content (mg/kg)	(11) Leaf Fe content (mg/kg)	Total score (Σ 1 to 11)	Final ranking
25	68	1	1	8	1	21	22	15	29	30	79	32	239	59
26	71	1	1	7	1	20	35	9	5	13	58	55	205	40
27	73	1	1	6	1	34	29	18	31	7	31	70	229	53
28	75	1	1	7	1	14	46	10	22	2	74	10	188	30
29	77	1	1	3	1	13	19	6	12	17	62	31	166	19
30	78	1	1	3	1	14	13	7	11	6	82	39	178	25
31	84	1	1	3	1	13	18	6	9	23	57	24	156	15
32	85	1	1	6	1	23	27	8	16	20	26	54	183	28
33	86	1	1	3	1	10	20	6	12	53	77	47	231	55
34	87	1	1	4	1	31	19	12	10	15	85	9	188	30
35	93	1	1	7	1	26	28	7	20	11	66	66	234	56
36	94	1	1	5	1	37	39	17	26	33	72	74	306	70
37	96	1	1	3	1	26	25	12	10	35	23	26	163	17
38	99	1	1	7	1	39	48	11	16	16	28	43	211	45
39	108	1	1	3	1	19	26	8	23	1	60	45	188	30
40	109	1	1	3	1	2	1	3	3	28	15	23	81	5
41	113	1	1	3	1	5	9	4	11	10	13	26	84	6
42	115	1	1	2	1	1	41	6	24	20	40	50	187	29
43	122	1	1	3	1	7	6	7	18	12	1	33	90	7
44	124	1	1	2	1	12	11	6	13	50	65	29	191	32
45	131	1	1	3	1	1	12	7	14	38	20	28	126	13
46	134	1	1	4	1	28	22	13	25	38	51	42	226	50

Table 4: Continued……

S. No.	Plant no	(1) Leaf bronzing score - 4 weeks	(2) Leaf bronzing score - 6 weeks	(3) No of roots	(4) Root length (cm)	(5) Shoot length (cm)	(6) Root weight (g)	(7) Shoot weight (g)	(8) No of fresh roots	(9) Fe reversibly adsorbed on root (mg l^{-1})	(10) Root Fe content (mg/kg)	(11) Leaf Fe content (mg/kg)	Total score (Σ 1 to 11)	Final ranking
47	148	1	1	5	1	22	21	13	27	39	69	5	204	39
48	152	1	1	8	1	40	49	8	29	13	52	59	261	66
49	164	1	1	5	1	20	35	10	10	29	76	3	191	32
50	165	1	1	6	1	31	38	12	16	23	54	62	245	62
51	166	1	1	5	1	20	35	7	21	25	83	7	206	41
52	168	1	1	3	1	14	16	8	10	5	73	20	152	14
53	173	1	1	1	1	15	29	10	18	15	87	2	180	26
54	174	1	1	6	1	16	16	10	13	18	80	13	175	24
55	182	1	1	7	1	27	42	13	27	6	16	58	199	36
56	183	1	1	4	1	1	9	8	10	42	18	75	170	22

57	185	1	1	9	1	25	37	7	6	31	55	72	245	62
58	192	1	1	4	1	18	29	12	9	41	42	60	218	48
59	194	1	1	2	1	4	4	8	19	13	25	31	109	11
60	195	1	1	5	1	16	22	20	10	48	70	21	215	47
61	202	1	1	3	1	3	3	2	1	14	17	11	57	2
62	203	1	1	4	1	31	24	6	14	11	35	68	196	34
63	205	1	1	3	1	10	14	7	11	8	63	77	196	34
64	213	1	1	4	1	9	15	4	10	26	21	15	107	10
65	215	1	1	4	1	7	10	8	9	22	61	64	188	30
66	216	1	1	4	1	15	34	9	12	21	64	65	227	51
67	217	1	1	3	1	10	23	7	10	34	36	71	197	35
68	224	1	1	4	1	17	32	7	14	19	48	34	178	25

Table 4: Continued……

S. No.	Plant no	(1) Leaf bronzing score - 4 weeks	(2) Leaf bronzing score - 6 weeks	(3) No of roots	(4) Root length (cm)	(5) Shoot length (cm)	(6) Root weight (g)	(7) Shoot weight (g)	(8) No of fresh roots	(9) Fe reversibly adsorbed on root (mg l^{-1})	(10) Root Fe content (mg/kg)	(11) Leaf Fe content (mg/kg)	Total score (Σ 1 to 11)	Final ranking
69	231	1	1	4	1	4	10	11	21	20	9	13	95	9
70	242	1	1	3	1	8	13	6	8	10	67	38	156	15
71	249	1	1	5	1	33	39	1	2	26	59	37	205	40
72	256	1	1	7	1	39	40	25	32	28	45	30	249	63
73	264	1	1	6	1	30	32	13	10	35	29	44	202	37
74	269	1	1	7	1	31	41	8	11	56	2	44	203	38
75	270	1	1	4	1	8	16	5	11	40	3	78	168	21
76	274	1	1	5	1	28	41	8	20	37	24	76	242	60
77	275	1	1	7	1	38	47	24	29	45	19	18	230	54
78	281	1	1	7	1	41	48	19	25	55	30	79	307	71
79	282	1	1	5	1	30	38	15	14	52	8	63	228	52
80	291	1	1	6	1	36	44	12	10	21	56	4	192	33
81	297	1	1	7	1	26	31	23	28	43	33	71	265	67
82	301	1	1	7	1	35	47	24	35	44	6	69	270	68
83	303	1	1	4	1	7	17	9	12	4	49	10	115	12
84	312	1	1	7	1	29	25	10	10	51	34	67	236	57
85	334	1	1	4	1	4	8	4	7	36	11	16	93	8
86	340	1	1	5	1	8	15	9	13	31	50	73	207	42
87	366	1	1	8	1	37	45	27	34	28	44	12	238	58
88	PGC31	1	1	3	1	8	3	2	3	49	31	46	148	14

Table 5: Performance of Resistant parent (PGC 14 - Tulasi), Resistant Bulk (RB), Susceptible parent (PGC 31 - Cul 8709) and Susceptible Bulk (SB)

S. No	Character	Resistant parent (Tulasi)	Resistant Bulk (RB)	Susceptible parent (Cul-8709)	Susceptible Bulk (SB)
1	Leaf Bronzing score after 4 weeks	1	1	9	9
2	Leaf Bronzing score after 6 Weeks	1	1	9	9
3	Total number of roots	31	33	22	22
4	Number of fresh roots	27	29	0	0
5	Root length (cm)	23.4	24.4	16.3	15.9
6	Shoot length (cm)	61.2	62.3	49.7	50.5
7	Root weight (g)	7.80	7.60	3.00	3.00
8	Shoot weight (g)	11.30	12.00	4.00	4.20
9	Iron reversibly adsorbed on root (mg l^{-1})	12.20	13.40	3.40	2.90
10	Iron content in root (mg kg^{-1})	11,918.52	12,823.01	6,889.42	6,536.79
11	Leaf iron content (mg kg^{-1})	731.25	701.06	2,258.75	2,500.50

root surface, iron content in root and leaf of sensitive bulk and resistant bulk differed from each other (Table 5).

Higher values of shoot length, root length, total number of roots, number of fresh roots, shoot weight, root weight, iron reversibly adsorbed on root surface, iron content in root and lower values of leaf bronzing score and iron content in leaf were observed in resistant parent Tulasi and individuals of resistant bulk (Table 2), while the inverse relationship among the above traits was observed in susceptible parent Cul-8709 and individuals of susceptible bulk (Table 4).

These results confirmed that the resistant parent (Tulasi) and individuals constituting the resistant bulk differed from susceptible parent (Cul-8709) and individuals of susceptible bulk in their response to excessive iron. Thus, the variability in the response of the different genotypes to the stress suggests that resistance mechanisms to iron toxicity are genetically determined and can be manipulated through breeding.

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