# IDENTIFICATION OF MOLECULAR MARKERS LINKED TO IRON TOXICITY TOLERANCE THROUGH BULK SEGREGANT ANALYSIS (BSA) IN RICE (*Oryza sativa* L.)

By

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(2012-21-130)

THESIS

Submitted in partial fulfillment of the requirement
for the degree of

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# DECLARATION

I hereby declare that the thesis entitled **'Identification of molecular markers linked to iron toxicity tolerance through bulk segregant analysis (BSA) in rice (*Oryza sativa* L.)'** is a bonafide record of research work done by me during the course of research and the thesis has not been previously formed the basis for the award to me any degree, diploma, fellowship or other similar title, of any other University or Society.

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# CERTIFICATE

Certified that thesis entitled **'Identification of molecular markers linked to iron toxicity tolerance through bulk segregant analysis (BSA) in rice (*Oryza sativa* L.)'** is a bonafide record of research work done independently by **Mr. M. Amaranatha Reddy (2012-21-130)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to him.

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Amaranatha Reddy 16/11/2016

*M. Amaranatha Reddy*

Dedicated

to

Rice Science

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## ABBREVIATIONS

| | |
|---|---|
| °C | Degree celsius |
| % | Per cent |
| µl | Micro litre |
| bp | Base pairs |
| BSA | Bulk Segregant Analysis |
| cm | Centi meter |
| CRD | Completely Randomized Design |
| DAS | Days after sowing |
| DNA | Deoxy-ribo Nucleic Acid |
| *et al* | And others |
| FAO | Food and Agriculture Organization |
| Fe | Iron |
| g | Gram |
| ha | Hectare |
| IRRI | International Rice Research Institute |
| ITS | Iron Toxicity Score |
| KAU | Kerala Agricultural University |
| Kg | Kilo gram |
| LAI | Leaf area index |
| LBI | Leaf Bronzing Index |
| LOD | Logarithm of the Odds |
| mM | milli Molar |
| ηg | Nanogram |
| NILs | Near Isogenic Lines |
| NS | Non significant |
| PCR | Polymerase Chain Reaction |
| PGC | Pattambi Germplasm Collections |
| ppm | Parts Per Million |
| QTL | Quantitative Trait Loci |
| r | Correlation coefficient |
| RARS | Regional Agricultural Research Station |
| RB | Resistant Bulk |
| RILs | Recombinant Inbred Lines |
| RM | Rice Microsatellites |
| ROS | Reactive Oxygen Species |
| SB | Susceptible Bulk |
| SSR | Simple Sequence Repeats |

# Introduction

# I. INTRODUCTION

Globally, rice is the most important food crop, serving as staple food for more than half of the world's population (Khush, 2005). 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). 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).

As in other parts of the country, in Kerala too, rice is the major food crop. However, there has been a steep decline in the area under rice in the state. Rice occupied an area of 7.90 lakh ha during 1960 with a production and productivity of 10.68 lakh tones and 1371 kg/ha respectively, while during 2015 the area under rice is estimated to have shrunk to 1.53 lakh ha with a production and productivity of 3.92 lakh tonnes and 2565 kg/ha respectively (Indiastat, 2015). The total annual production of rice is however insufficient to meet the total demand in the state. Urbanisation, conversion of land for non-agricultural purposes, labour deficit and other socio-economic reasons, leaves little scope to increase production through increasing land under rice cultivation. Under such circumstances, the only option to increase rice production is by increasing productivity.

Kerala occupying the extreme southern end of west coast enjoys a tropical humid climate with an average annual rainfall of 3000 mm (Krishnakumar *et al.,* 2009). The high rainfall is mainly responsible for leaching of nitrates from soils, accumulation of iron and aluminium oxides in surface soils and rendering it acidic in reaction (Becker and Asch, 2005). Iron (Fe) though an essential element in plants that is involved in many physiological processes can also be toxic when provided in excess. Iron toxic soils are characterized by floating brackish red scums on soil surface. Excessive absorption and translocation of Fe in the rice plants occur under such conditions leading to toxicity. Excessive iron has been identified to be one of the major yield-limiting abiotic factors affecting rice productivity in lowland acid soils, inland valley swamps, coastal swamps and irrigated lowlands in ultisols and oxisols (Tanaka and Yamaguchi, 1973; Brigit *et al.,* 1993; Maschner, 1995).

Occurrence of high soil acidity (Benkiser *et al.*, 1982) coupled with increasing occurrences of iron at toxic levels in Kerala (320 to 2000 ppm of Fe) make it a serious long term threat to rice production (Dobermann and Fairhurst, 2000; Thampatti *et al.*, 2005). According to Majerus *et al.* (2007), yield losses associated with iron toxicity commonly ranges from 30 to 60 per cent. Prevalence of severe toxicity at younger stage may result in complete crop failure (Audebert and Sahrawat, 2000).

In well-aerated soils, Fe is present as ferric form ($Fe^{3+}$) with low plant availability (Conte and Walker 2011). However, in anaerobic soils and at low redox potential (Eh), Fe is reduced to its soluble ferrous form ($Fe^{2+}$) and can be taken up excessively by plants. In plant tissues, $Fe^{2+}$ participates in Fenton reactions, catalyzing the generation of hydroxyl radicals (-OH) and other reactive oxygen species (ROS) (Thongbai and Goodman, 2000). These radicals cause irreversible damage to membrane lipids, proteins and nucleic acids (Becana *et al.*, 1998). Eventually they oxidize chlorophyll and subsequently reduce leaf photosynthesis (Pereira *et al.*, 2013), thereby leading to yield reductions.

The typical symptoms associated with iron toxicity are leaf discoloration (bronzing) and reddish spots (Ponnamperuma *et al.*, 1955; De Datta *et al.*, 1994, Dobermann and Fairhurst, 2000; Becker and Asch, 2005). Typically, iron toxicity symptoms are manifested as tiny brown spots starting from the upper tips and spreading towards the bases of the lower leaves. At increased levels of toxicity, the brown spots coalesce on the interveins of the leaves to the extent that the entire affected leaves look purplish brown, followed by drying of the leaves, which gives the rice plant a scorched appearance. Growth and tillering become depressed. Equally important, the roots of rice plants affected by iron toxicity become scanty, coarse, short, blunted and dark brown in color. The roots may slowly recover to the usual white color with the alleviation of the stress (Sahrawat *et al.*, 1996; Audebert and Sahrawat, 2000; Sahrawat, 2004). Toxicity symptoms on rice leaves and changes in root color and morphology are proven diagnostic indicators of iron stress.

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). 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). 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).

Heritability of tolerance to iron toxicity has been confirmed by Wu *et al.* (1997). Two different genes were reported to govern iron tolerance in rice. Variety Suakokes is reported to harbour a dominant gene while variety Gossi 27 possesses a recessive gene for imparting tolerance to iron toxicity (Abifarin, 1986). However, research reports also indicate that resistance to iron toxicity is a complex trait controlled by several genes and largely depend on the environment.

According to De Datta *et al.* (1994) 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) 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.

The effectiveness of marker assisted breeding programme heavily relies on the use of reliable molecular markers. According to Dufey *et al.* (2015), use of reliable tightly linked molecular markers can hasten the identification of tolerant genotypes at an early stage of crop growth, avoid yield losses and increase productivity. Hence, the present study was formulated with the following objective:

To detect linkage between markers and genomic regions influencing iron toxicity tolerance in native rice genotype through bulk segregant analysis (BSA).

# Review of Literature

# II. REVIEW OF LITERATURE

Iron toxicity is a nutrient disorder, usually occurring in the plants with excess uptake of $Fe^{2+}$ (ferrous iron) concentrations in the soil exceeds 300 mg $L^{-1}$ (Yamauchi and Peng, 1995). Excessive uptake of $Fe^{2+}$ by plants under such conditions leads to toxicity. In rice, the toxicity leads to disruption or over expression of several metabolic processes (Bode *et al.*, 1995). Toxicity usually manifests itself as rusty leaf spots (bronzing), stained leaf edges and a dark brown and poorly developed root system (Dobermann and Fairhurst, 2000). Although several approaches to ameliorate iron toxic soil conditions have been attempted, the use of tolerant varieties was found to be the most economic and viable approach. Rampant iron toxicity especially among the native germplasm in rice fields of Kerala has resulted in evolution of inherent mechanisms in the plant system, to cope with and survive adverse iron-toxic soil conditions and large amounts of iron. The plants are found to have developed morphological and physiological avoidance and/or tolerance mechanisms. Breeders have been successful in developing an array of cultivars with various degrees of adaptation, relying mainly on traditional breeding methods. Marker-assisted breeding methods have proved to hasten identification and isolation of preferred genotypes. However, the approach requires information on molecular markers associated with genomic region governing iron toxicity tolerance. Considering the above, an attempt has been made to identify such molecular markers linked to iron toxicity tolerance in the native rice germplasm. The literature on the above aspects in rice is reviewed under the following headings.

2.1. Iron toxicity in the soil

2.2. Iron toxicity in the rice plant

2.3. Mechanisms of tolerance to iron toxicity in rice

2.4. Variability in response to iron toxicity in rice genotypes

2.5. Correlation between growth responses under iron toxic conditions in rice

2.6. Bulk segregant analysis and markers linked to QTLs conferring iron toxicity tolerance

## 2.1. Iron toxicity in the soil

Iron (Fe) toxicity is a major nutrient disorder of rice grown on acid sulfate soils, ultisols and sandy soils with low CEC, moderate to high acidity and active Fe

(Sahrawat, 2004). Toxicity of iron occurs with presence of excess $Fe^{2+}$ (Ferrous ions) ions in the soil solution. High amounts of soluble $Fe^{2+}$ (100 to 1000 mg $L^{-1}$) may be found in acid soils (Ponnamperuma, 1972). In acid sulfate soils, concentrations of up to 5000 mg $kg^{-1}$ have been reported (Harmsen and Breemen, 1975). A soil solution concentration of 300 mg water soluble Fe $l^{-1}$ is generally considered the critical limit for the cultivation of lowland rice (Lantin and Neue, 1989).

The relation between $Fe^{2+}$ (Ferrous ions - soluble form) and $Fe^{3+}$ (Ferric ions - less soluble form) depends on soil texture, clay content, bulk density, gaseous porosity of soils and other environmental conditions (Kirk and Solivas, 1990). In most cases, iron will interact with other nutrients in the soil through antagonistic or synergistic interaction. One of the antagonists is manganese. Application of manganese can suppress iron desorption and subsequently total iron uptake from the soils. Similarly, high amounts of iron suppress the uptake of manganese. On the other hand, iron oxides are known to have a strong zinc binding capacity. The reduction of iron oxides and the resulting increase in Fe availability to plants is generally associated with an increased availability of Zn. Silicon as a beneficial (not essential) element in the soil can reduce concentration and uptake of iron in the plant (Verma and Minhas, 1989). In general, the uptake of cationic nutrients by plant is reduced with increasing iron concentration $Fe^{2+}$ in the growth medium. The inhibition of plant nutrient uptake by iron can be put in the order: P > K > N > Mg > Ca and for micronutrients: Mn > Zn > Cu. Consequently, with increasing iron concentrations in lowlands, phosphorous, potassium and zinc deficiencies are likely to be the first to appear (Fageria, 1988). Wang and Liu (1992) concluded that $Fe^{3+}$ deposition on plant roots and in rhizoshpere might block the uptake of other nutrients.

Depending on the site and the cultivars used, reported critical concentrations of iron can range from 20 to 2500 mg $kg^{-1}$, indicating that factors other than pH and Fe concentration influence the occurrence of Fe toxicity symptoms. Given the large diversity of soil and environmental conditions that do affect the rate of reduction and the amount of $Fe^{2+}$ in the soil, the time of occurrence and severity of Fe stress, a systematic categorization of iron-toxic environments is required to improve the targeting of intervention strategies (Becker and Asch, 2005).

## 2.2. Iron toxicity in the rice plant

Adequate Fe concentration in the plant tissue is reported to be in the range of 70 to 300 mg $kg^{-1}$ (Welch *et al.*, 1993). Iron deficiency or toxicity occurs at concentrations below or above this sufficiency range (Tanaka and Yoshida, 1972). According to Fageria *et al.* (1981), the toxic concentration also depends on rice cultivars.

In green leaves, about 80 per cent of the iron is accumulated in the chloroplast (Bienfait, 1985; Marschner, 1993). Stroma of plastids is a hollow shell which can store up to 5000 atoms of iron as $Fe^{3+}$, often as well-defined crystalls (Seckbach, 1982). In the stroma, iron is stored as phytoferritin. Physiologically active iron present within plant tissues may catalyse the generation of reactive oxygen species such as superoxide, hydroxyl-radical, and $H_2O_2$ resulting in oxidative stress which can eventually damage plant cells. It is now established that excess amounts of $Fe^{2+}$ in the plant tissue causes elevated production of toxic oxygen radicals.

Membrane lipids (Thompson and Legge, 1987), membrane charge proteins and nucleic acids (Elstner, 1982) are irreversibly damaged by oxygen radicals. Free radical formation will eventually lead to stimulation of chlorophyll oxidation and subsequently to a decrease of chlorophyll content upon accumulation of high concentrations of iron (Monteiro and Winterbourn, 1988). The typical visual symptom in rice plant associated with processes of iron toxicity and particularly with the accumulation of oxidized polyphenols, is the 'bronzing' of the leaves. The bronzing symptoms start in fully developed older source leaves with the occurrence of tiny brown spots that spread from the leaf tip to the base. In the further development of the symptom, the leaf tips become orange-yellow and dry up in some rice varieties. These symptoms are particularly developed in older leaves having higher transpiration rates (Yamanouchi and Yoshida, 1981). Eventually, the entire transpired leaf becomes orange to rusty brown or purple brown when toxicity is extremely severe (Fairhurst and Witt, 2002).

These symptoms can occur at different growth stages and may affect rice at the seedling stage, during the vegetative growth and at the reproductive stages. Depending on the growth stage at which leaf bronzing occurs, the effect on growth and productivity may vary. In the case of toxicity occurring during seedling stage, the rice plants remain

stunted with extremely limited tillering (Abraham and Pandey, 1989). Toxicity at seedling or early vegetative stages can strongly affect plant growth and result in a complete yield loss (Abifarin, 1988).

Toxicity during the vegetative stage is associated with reduced plant height and dry-matter accumulation (Abu *et al.*, 1989), with the shoot being more affected than the root biomass (Fageria, 1988). Both the tiller formation and the share of productive tillers can be severely reduced (Cheema *et al.*, 1990). When iron toxicity occurs during the late vegetative or early reproductive growth phases, it is associated with fewer panicles per hill (Singh *et al.*, 1992), and increase in spikelet sterility (Virmani, 1977), and delayed flowering and maturity by up to 20 to 25 days. In highly susceptible cultivars, flowering may not occur (Ayotade, 1979). Root growth stops after booting and the aerenchyma starts to senesce and decay. As a result, the oxidation power of the root breaks down, and the root surface is coated with dark brown to black precipitates of $Fe(OH)_3$, and many roots die (Morel and Machado, 1981).

Correlation between the severity of iron-toxicity symptom expression and yield has been proved. This relationship can vary within as well as between cropping season. Seasonal and inter-seasonal variation in the relationship between symptom expression and yield loss are mainly related to transpiration and differences in acropetal Fe translocation. Hence, bronzing symptoms and Fe uptake (Kpongor *et al.*, 2003), and iron-induced yield losses and leaf bronzing were more pronounced in dry season as compared to a wet season crop (Sahrawat and Diatta, 1995).

### 2.3. Mechanisms of tolerance to iron toxicity in rice

Evidently, rice plants have developed morphological and physiological avoidance and/or tolerance mechanisms to cope with and survive adverse iron-toxic soil conditions and large amounts iron in the plant. These mechanisms are important in the selection of tolerant or adapted rice genotypes (Tanaka *et al.*, 1966; Marschner, 1993).

The formation of iron plaque on rice roots not only reduced the $Fe^{2+}$ concentrations in the soil solution, but is also thought to form a physical barrier for further influx of reduced iron (Tanaka *et al.*, 1966).

Tadano (1975) observed three important functions of rice roots to counter iron toxicity and these include (i) oxidation of iron in the rhizosphere to keep iron concentration low in the growth media, (ii) rice roots exclude iron at the root surface and thus prevent iron entering the root and (iii) rice roots are able to retain iron in the root tissue and thus decreases the translocation of iron from the root to the shoot.

Tolerance mechanism based on tissue iron concentrations is prevalent rather than avoidance or exclusion mechanisms of majority rice cultivars (Yamanouchi and Yoshida, 1981).

Fe stress avoidance occurs due to oxidation of available ferrous ions ($Fe^{2+}$) to unavailable $Fe^{3+}$ in the rhizosphere (Ando, 1983; Narteh and Sahrawat, 1999; Silveira *et al.,* 2007; Nyamangyoku and Bertin, 2013; Onaga *et al.,* 2013a). The precipitation of Ferric hydroxide in the rhizosphere by healthy roots (indicated by reddish brown coatings on the roots) prevents excessive $Fe^{2+}$ uptake (Kirk *et al.,* 1990; Shamshuddin *et al.,* 2013; Harahap *et al.,* 2014).

Rice roots diffuse molecular oxygen into the root medium through air chambers and aerenchyma in the rice plant leaves, stem nodes and roots, which makes the rhizosphere more oxidative than the bulk growing soil. This leads to the oxidation of ferrous iron in soil solution to ferric iron, which can be seen as deposits on the surface of the rice roots. The oxidizing power of the rice roots is greater at the growing points and at the elongating parts of the roots than at the basal parts of the roots (Yoshida, 1981).

Avoidance of toxic Fe levels in plant tissues through regulation of Fe uptake is achieved through an oxidation barrier in the rhizosphere established by channeling molecular oxygen from the atmosphere through the stems into the roots *via* a gas-conducting tissue or aerenchyma (Ando, 1983).

Root oxidation power includes the excretion of oxygen (transportation of $O_2$ from the shoot to the root through aerenchyma) from roots and oxidation mediated by enzymes such as peroxidase or catalase. An inadequate supply of nutrients (K, Si, P, Ca and Mg) and excessive amounts of toxic substances ($H_2S$) reduce root oxidation power (Ponnamperuma, 1972; Benckiser *et al.*, 1984). Rice varieties differ in their ability to

release $O_2$ from roots to oxidize $Fe^{2+}$ in the rhizosphere and protect the plant from iron toxicity. Plants actually tolerate elevated levels of $Fe^{2+}$ within leaf cells, probably via enzymatic detoxification in the symplast. In some cases, $Fe^{2+}$ is taken up into the rice root, but tissue damage may be avoided by either compartmentation (immobilization of active iron in 'dumping sites', e.g., old leaves or photosynthetically less active leaf sheath tissue) or exclusion from the symplast (immobilization in the leaf apoplast) (Kosegarten *et al.,* 1999; Lucena, 2000; Asch *et al.,* 2005; Engel *et al.,* 2012).

Plants may exclude $Fe^{2+}$ at the root level and hence avoid of $Fe^{2+}$ damage to the shoot tissue (rhizospheric oxidation and root iron selectivity) (Majerus *et al.,* 2007; Engel, 2009; Engel *et al.,* 2012; Onaga *et al.,* 2013a). Strongly reduced soils contain very large amounts of $Fe^{2+}$. Due to insufficient oxygen at the root surface to oxidize $Fe^{2+}$ to less available $Fe^{3+}$ ions, the iron uptake by plants becomes excessive and roots appear black due to presence of ferrous sulfide (Morel and Machado, 1981).

The iron concentration in the shoots of a rice plant is determined by the iron accumulation rate relative to dry matter production. Increase in the dry matter production results in the decrease of the iron concentration if the accumulation rate is constant, which is called a dilution effect (Yamauchi, 1989).

Luo *et al.* (1997) found significant correlations between the genotypic variation and the decrease in N, P, K, and Mg uptake and in their tolerance to $Fe^{2+}$ toxicity, suggesting that the ability to maintain higher nutrient element uptake under a Fe-toxic condition contributes the tolerance to $Fe^{2+}$ toxicity.

Wu *et al.* (1998) observed that bronzing may not occur in tolerant lines at a Fe concentration that causes severe bronzing in sensitive lines, suggesting that tolerant lines have higher tissue tolerance to iron in plants.

Sahrawat and Singh (1998) observed that high temperatures affected crop physiology, especially during grain maturity mainly due to enhanced transpiration rates of rice plants, could cause a higher uptake of iron through a passive uptake mechanism.

The aerenchyma starts to disintegrate with root senescence, thus losing its capacity for gas transport, and little Fe oxidation occurs in the root zone after flowering

stage of rice. Consequently, late season Fe-toxicity symptoms in flag leaf of rice grown in acid sulfate soil were primarily associated with the breakdown of the root oxidation power (Tinh, 1999).

Audebert and Sahrawat (2000) observed that iron-tolerant rice cultivar (CK4) absorbed less iron or transported less from roots to leaves, indicating the presence of a physiological avoidance mechanism. The iron-tolerant cultivar (CK4) owed its superior performance under iron-toxic conditions partly to avoidance (less iron accumulation in leaves) and tolerance (superior photosynthetic potential in the presence of absorbed iron in the leaves).

Asch *et al.* (2005) revealed that higher leaf-bronzing score and tissue Fe concentrations were observed in older seedlings of 14 rice genotypes. This allowed distinguishing between sensitive includers (IR31785 and MR123) and resistant excluders (WITA7 and CK4) with the exclusion mechanisms being either oxidation power of the root or symplastic discrimination. While ITA320, WITA7, and CK4 apparently efficiently excluded $Fe^{2+}$, Suakoko8 showed a similar bronzing score but tissue Fe concentration is three times higher than other excluders, therefore obviously tolerating elevated levels of Fe in the tissue. Tox4004 had intermediate tissue Fe concentration with only about half the bronzing score of the genotypes described above, suggesting a combination of exclusion and tolerance mechanisms.

High iron concentration in the above ground plant biomass without the expression of the typical damage symptom (bronzing) does not necessarily indicate symplastic tolerance. It cannot be excluded that such cultivars may have exhibited an efficient mechanism of symplastic exclusion or stem/leaf sheath retention (Becker and Asch, 2005).

Dorlodot *et al.* (2005) revealed that iron concentration in the shoot tissue of inter-specific rice hybrid (*Oryza sativa* × *Oryza glaberrima*) *i.e.*, 3356 mg $kg^{-1}$, largely exceeded this critical toxicity level, although the plants were near an optimum in terms of vegetative growth and survival rate, and showed no bronzing symptoms. Considering such a high internal iron content, the resistance mechanism here should be attributed to

the storage of iron in the leaf tissue and/or the activity of antioxidant enzymatic systems, rather than to the excluding power of rice roots.

Another mechanism involves the retention of Fe in root tissue *i.e.*, oxidation of $Fe^{2+}$ and precipitation as $Fe^{3+}$ (Jayawardena *et al.*, 1977; Engel, 2009). Although $Fe^{2+}$ exclusion by oxidation in the rhizosphere and the detoxification of leaf cells well are established Fe-tolerance mechanisms of rice, the other mechanisms are not well understood and therefore usually not considered in rice breeding or screening for iron tolerance (Becker and Asch, 2005).

Majerus *et al.* (2007) screened seedlings of two cultivars differing in their level of resistance to iron stress (TOG7105: resistant and IRGC104047: sensitive) in hydroponic culture. It was found that iron concentration in roots was higher in TOG7105 than IRGC104047, while an opposite trend was recorded in laminae, thus suggesting that iron exclusion could be an efficient mechanism of iron resistance.

Silveira *et al.* (2007) carried out experiments culture solutions using with rice cultivars BR-IRGA 409 (I409, susceptible) and EPAGRI 108 (E108, resistant) grown with Fe excess (500 mg $L^{-1}$ Fe), control (6.5 mg $L^{-1}$ Fe) concentration and deficiency (zero mg $L^{-1}$ Fe). Analysis of shoot dry weight confirmed the E108 plants resistant to excess Fe had lower Fe concentrations than I409 plants when exposed to excess Fe. E108 plants seem to make use of the avoidance mechanism in the resistance to Fe overload. Both cultivars responded to Fe deficiency with allocation of P from roots to shoots.

The longer period for maturity in the elite breeding lines than that in IR64 and its NILs could be one of the factors that were responsible for the tolerance because it could keep the root activity for a longer time and alleviate the Fe-toxicity. NILs might have shown a high survival rate to the Fe-toxicity because of vigorous growth during the early stage of cultivation. The finding suggested that one of the factors that suppressed the growth of IR64 and its NILs during the late stage was early root senescence. Therefore, the growth operating factor such as root senescence might have attributed to the Fe-toxicity during the late stage (Nozoe *et al.*, 2008).

Engel (2009) evaluated ten contrasting rice genotypes in hydroponic culture regarding their iron tolerance and the involved mechanisms. $Fe^{2+}$ stress was applied at the seedling, vegetative and early reproductive growth stages with varying concentrations and durations and under conditions of high and low vapor pressure deficit. Both the mechanisms (exclusion and tissue tolerance) and their effectiveness to counteract elevated $Fe^{2+}$ levels differed between cultivars.

Effects of excessive ferrous ion on growth and iron content between two varieties of rice; susceptible variety Bw 272-6b and resistant variety Bw 267-3 to $Fe^{2+}$ stress were studied by Samaranayake *et al.* (2012). They suggested two possible mechanisms regarding this study. Firstly, the shoot system of the resistant variety may have a mechanism of partitioning iron in their tissues without causing cell damage, whereas the susceptible variety does not possess such a mechanism. Secondly, the leaf symptoms may be linked to a chemical signal transmitted by the root system. Having significantly greater iron content in the root system, the signal transmitted by the roots of the susceptible variety may be stronger than the signal transmitted by the low iron containing root system of the resistant variety.

Nyamangyoku and Bertin (2013) identified the resistance mechanisms and strategies of rice in the presence of an excess of ferrous iron by submitting a wide range of cultivars of both cultivated rice species and their inter specific hybrids under two levels of $Fe^{2+}$ (0 and 250 mg $L^{-1}$ supplied as $FeSO_4$). They considered that the iron coating must be as a symptom of sensitivity to ferrous iron toxicity rather than as a mechanism of resistance. Obvious differences were found between cultivars, especially discriminating the *glaberrima's* from the remaining ones. The *glaberrima's* produced high biomass, both under control and treated conditions. They showed low levels of bronzing. This suggests that one of their main resistance mechanisms could be related to a dilution effect. Hence, they considered as ferrous-iron resistance mainly because of avoidance mechanism.

Harahap *et al.* (2014) evaluated morphological and physiological responses as well as the level of tolerance of paddy genotypes to iron toxicity. Based on several parameters such as the differences observed in ethylene content, aerenchyma size, Fe content in root tissue, Fe content in shoot tissue and the percentage of leaf bronzing,

Indragiri was considered as highly tolerant genotype to iron toxicity. This genotype is not only had excluder tolerance (avoidance) but also had includer tolerance mechanism.

Stein *et al.* (2014) elucidated mechanisms involved in tolerance to iron toxicity in plants from one cultivar susceptible to iron toxicity (BR-IRGA 409) and two tolerant cultivars (EPAGRI 108 and EPAGRI 109). Only plants from the susceptible cultivar showed symptoms of iron toxicity when grown at the iron-toxic site, accumulating high levels of iron in leaves. EPAGRI 108 plants had the lowest iron concentration in leaves and reached the highest iron concentration in the root symplast, suggesting that the capacity to safely store iron in root cells and to limit iron translocation to shoots could be a tolerance mechanism in this cultivar.

Wu *et al.* (2014) observed that the shoot Fe concentration was significantly lower in Pokkali and FL510 compared to IR29, while FL483 did not differ significantly from any other genotypes. Lower Fe concentrations in Pokkali despite higher absolute Fe uptake may have partly occurred due to higher biomass leading to a 'dilution effect'. However, FL510 had even lower Fe concentration than Pokkali, despite a significantly lower biomass than Pokkali, suggesting that dilution was not the dominant factor leading to low Fe concentrations in FL510. FL483 did not differ significantly from IR29 in shoot Fe concentration, dry weight, or shoot Fe uptake, suggesting that it was tolerant due to a shoot-based mechanism.

Dufey *et al.* (2015) observed that the sensitive lines have lower SDW (shoot dry weight) than the resistant ones under iron toxicity screening. This could imply that having higher biomass can be considered as a tolerance mechanism, through the mechanism referred to as dilution effect, i.e. at a given Fe uptake the tissue concentration would be lower in lines with high biomass than in lines with low biomass production.

Matthus *et al.* (2015) conducted a genome wide association study (GWAS) by exposing a population of 329 accessions representing all subgroups of rice to ferrous iron stress (1000 ppm, 5 days). Both iron including and excluding tolerant genotypes were observed, and shoot iron concentrations explained around 15.5 per cent of the variation in foliar symptom formation.

The photosynthetic parameters decreased the most, when the plants were treated with high levels of iron source. Despite the toxicity to cultivars, the levels of accumulation in roots and the translocation of iron were different among the sources of iron evaluated. Under cultivation with ferrous sulfate, the symplastic iron was mainly accumulated in the roots of rice plants. This can be considered an exclusion mechanism. However, the iron citrate was highly translocated to the shoots in the upland cultivar but still showed a lower toxicity compared with ferrous sulfate, which indicates an internal tolerance mechanism to iron excess in the shoots (Muller *et al.,* 2015).

Shrestha and Becker (2015) evaluated root iron (Fe) exclusion capacity of four lowland rice genotypes in increasing rate of $Fe^{2+}$ stresses (0, 500, 1000 and 1500 mg $L^{-1}$) in growing medium under the conditions of low and high vapor pressure deficit. Rice root excluded significantly higher amount of iron under dry atmospheric condition (655 mg Fe/g) than moist atmospheric condition (118 mg Fe/g). But their iron exclusion capacity reduced when they were gradually exposed to the higher levels of Fe stress. Tolerant genotype such as TOX3107 excluded more iron when they were exposed to dry atmospheric condition.

**2.4 Variability in response to iron toxicity in rice genotypes**

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). 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) and quantitative trait loci (QTL) analysis combined with marker-assisted breeding (Bennett, 2001; Wan *et al.,* 2003a and 2003b; Wissuwa, 2005).

Breemen and Moorman (1978) observed that seedlings of variety IR31785 sensitive to iron toxicity when subjected to high concentrations of external $Fe^{2+}$ at 28 days after sowing (DAS) developed leaf-bronzing symptoms faster and possessed higher leaf-bronzing scores than seedlings of less sensitive variety Suakoko 8.

The reactions of tolerant and sensitive genotypes to P deficiency, Zn deficiency and Fe toxicity in wetlands and iron deficiency in drylands was studied by Mahadevappa *et al.* (1979). They found that tolerance varied widely with the stress level and the genotype. Sensitive genotypes suffered severe yield losses even under mild stress, whereas tolerant ones resisted the yield decline until the stress became moderate. Under severe stress, both tolerant and sensitive genotypes perished.

Solivas and Ponnamperuma (1980) screened 536 rice genotypes for tolerance to iron toxicity. They found marked varietal differences in tolerance. Tolerance for iron toxicity in rice conferred a yield advantage of about 2 t/ha over susceptible genotypes. There were marked seasonal differences in the performance of rices on the acid sulfate soil. Costly amendments such as liming may be avoided by using tolerant varieties.

Winslow *et al.* (1989) evaluated two genotypes ITA 212 (susceptible) and ITA 247 (resistant) under iron toxic conditions. Variety ITA 212 yielded 10 to 250 per cent more than ITA 212 as toxicity increased from moderate to severe levels.

Elsy (1994) conducted the varietal performance trial with 40 genotypes in lateritic rice soil of Kerala and revealed that the high yielding genotypes exhibited characteristics visual symptoms of Fe toxicity. The average yield recorded by the high yielding genotypes in the Fe toxic and non-toxic fields was 2.6 t $ha^{-1}$ and 4 to 4.5 t $ha^{-1}$ respectively.

A pot trial implemented to assess Fe toxicity to rice using flooded highland swamp soils rich in organic carbon contents revealed that leaf iron content of more than 250 mg $g^{-1}$ of dry matter induced total grain weight reduction by 50 per cent (Genon *et al.*, 1994).

Results of field experiments to evaluate the iron toxicity tolerance of promising rice cultivars showed that genetic tolerance to iron toxicity can significantly improve rice production in iron-toxic soils (Sahrawat *et al.*, 1996). Iron toxicity scores ranged from 2 to 9. The application of N, P, K and Zn in the field decreased the uptake of iron in rice crops, and this can be a significant factor in the iron-toxicity tolerance of the cultivars.

A study conducted with twenty eight rice genotypes, two *O. glaberrima* land races and two checks (Suakoko 8 and Bouak 189 were tolerant and susceptible checks) revealed that iron toxicity caused significant reductions in agronomic parameters (yield, plant height and tiller number) as compared with the control plot and the scores were significantly correlated with reductions in yield and plant height (Nipah, 1997).

Wu *et al.* (1998) observed segregation for leaf bronzing and growth reduction due to $Fe^{2+}$ toxicity in a doubled haploid (DH) population with 135 lines derived from a $Fe^{2+}$ tolerant japonica variety, Azucena and a sensitive indica variety, IR64 in a solution culture with $Fe^{2+}$ stress condition. A non-normal distribution of Leaf Bronzing Index (LBI) was found. The total iron concentration in the 38 tolerant lines ranged from 1.76 mg Fe $g^{-1}$ to 4.12 mg Fe $g^{-1}$ and was in a similar range as in the non-tolerant genotype (2.04 to 4.55 mg Fe $g^{-1}$).

Mendoza *et al.* (2000) screened 161 genotypes at seedling stage under iron toxic conditions. They observed the seven genotypes of *O. sativa* and three accessions from *O. rufipogon* showed tolerance in 400ppm concentration, whereas none of the accessions from *O. glaberrima* species was tolerant. Varieties BW2673, Suakoko 8, IR9884, IR685442921312, and Azucena showed good levels of tolerance at 400ppm iron concentration. Three *O. rufipogon* accessions, 105909, 106412 and 106423 were found to be highly tolerant and these could be good donors for iron toxicity tolerance.

A field experiment at a Fe toxic site in Korhogo, Ivory Coast revealed that in both Fe-tolerant and susceptible varieties, there was no differences in elemental composition except for Fe. At harvest, the concentration of Fe in grain and straw was lower in CK 4 than Bouake 189 (Sahrawat, 2000).

Olaleye *et al.* (2001) conducted a pot experiment involving two rice cultivars (ITA 212 and Suakoko 8), two soil types with four $Fe^{2+}$ levels (control, 1000, 3000 and 4000mg $Fe^{2+}$ $L^{-1}$). The results clearly showed that tissue phosphorus (P), potassium (K) and manganese (Mn) contents decreased with age and increasing $Fe^{2+}$ levels while there was an increase in calcium (Ca), magnesium (Mg) and Fe contents. Increasing $Fe^{2+}$ levels was also observed to reduce dry matter yields, tiller numbers and plant height significantly.

Sahrawat and Sika (2002) observed that varieties CK4 and Bouake189 showed iron toxicity symptoms in varying degrees. The intensity of iron toxicity based on the extent of bronzing symptoms was higher without nutrient application. Applying nutrients reduced iron toxicity as indicated by a lower ITS (Iron Toxicity Score). They recommended that CG14 has a high tolerance for iron toxicity and remains an obvious choice as a donor for iron tolerance in a breeding program.

The two genotypes (Suakoko8 and Nipponbare) and five control cultivars were evaluated by Wan *et al.* (2003a) for ferrous iron toxicity tolerance. The leaf bronzing index of Nipponbare is equal to that of control cultivar Suakoko8, and the differences of leaf bronzing index between Kasalath and IR26, IR64 (susceptible to ferrous iron toxicity) are not significant also.

Fourteen rice genotypes were screened under Fe-toxicity conditions. Seedlings subjected to high concentrations of external $Fe^{2+}$ at 28 DAS developed leaf-bronzing symptoms faster and recorded higher leaf-bronzing scores than seedlings subjected to the same conditions at 14 DAS (Asch *et al.,* 2005).

Hydroponics screening experiment applying different ferrous iron concentrations (0, 125, 250, and 500 ppm $Fe^{2+}$) by Dorlodot *et al.* (2005) revealed that inter-specific rice hybrid (*Oryza sativa* × *Oryza glaberrima*) did not show iron toxicity symptoms at 125 mg litre$^{-1}$ $Fe^{2+}$, despite an iron concentration in its leaves (3356 mg kg$^{-1}$) well above the usual critical toxicity concentration (700 mg kg$^{-1}$).

Iron toxicity tolerance levels of local varieties varied assessed based on iron toxicity symptoms, concentration of Fe-leaves and roots, plant growth, and decreased relative plant growth. Local rice variety Siam Unus Putih was relatively more tolerant than Lemo Kwatik and Lakatan Hirang. The grain yield of local varieties ranged from 2.0 to 3.0 t ha$^{-1}$ (Khairullah *et al.*, 2005).

Roy and Mandal (2005) screened *in vitro,* seed derived calluses of rice cultivars, IR72 (susceptible) and C148 (tolerant) under increasing levels (50, 100, 200 or 400 ppm) of Fe-toxicity and found that higher concentration of iron was detrimental to plantlet regeneration. C148 showed higher degree of tolerance than IR72.

Many promising accessions (Nerica-L19, CK 4, Suakoko 8, TOX 3069-66-2-1-6 and WAT 1282-B-3-3) with performance higher than the best *O. sativa* and *O. glaberrima* checks were identified as new sources of tolerance to Fe toxicity (Sarla and Swamy, 2005).

Screening to iron toxicity of 130 local varieties from tidal swamplands in South Kalimantan and South Sumatera showed variations in Fe toxicity tolerant. In soils with 156 ppm Fe concentration and 0.44 mg $L^{-1}$ Fe soluble in water, seedlings (1 week old) of 35 local rice varieties were found tolerant to iron toxicity, whereas at 2 weeks there were 29 tolerant varieties. However, after 3 weeks of exposure to toxic levels of iron, there were only 20 varieties that exhibited tolerance to iron toxicity (Khairullah *et al.*, 2006).

Screening three rice varieties at three levels of iron (Fe) in nutrient solutions *viz.*, 0.045 (control) 5.34 and 7.12 mM Fe revealed that shoot length, root and shoot dry weights were reduced significantly by higher levels of Fe in the medium. Results of leaf bronzing have revealed higher bronzing score in the seedlings grown at 7.12 mM Fe in the growth medium (Baruah *et al.*, 2007).

Nozoe *et al.* (2008) screened IR64 (check variety) and four lines of rice (*Oryza sativa* L.) developed at IRRI in an iron (Fe) toxicity field and also under normal soil conditions. They found that the yield reduction of elite breeding lines was smaller than that of IR64 indicating that the tolerance of elite breeding lines to iron toxicity.

Various screening methodologies (field, pot, hydroponics) have been used to identify promising lines/varieties among 172 entries. From these, 80 entries were found to be tolerant based on their yields under stress, iron-toxicity score and other agronomic characters like total biomass, pant height, grain weight, harvest index and number of panicles (Drame *et al.*, 2010).

Kang *et al.* (2011) tested twenty-six upland lines of New Rice for Africa (NERICA) along with four *Oryza sativa* varieties in relation to Fe toxicity tolerance under hydroponic culture containing 1.44 mM Fe (+Fe) and 0 mM Fe (as a control). Three NERICAs, among the 30 lines/varieties tested were found to exhibit tolerance to iron at toxic levels judging from reduction of root length and dry weight and shoot dry

weight. These recorded significantly lower Fe content in the shoots than BL2-DV2, suggesting that the tolerant NERICAs could have some mechanism to inhibit the absorption of Fe.

Engel *et al.* (2012) assessed the response of 21 rice genotypes (symptom score, biomass, Fe concentrations and uptake) to 1500 $mgl^{-1}$ $Fe^{2+}$. Eight selected genotypes were further compared at different stress intensities (0, 500, 1000, and 1500 $mgl^{-1}$ $Fe^{2+}$) and different developmental stages. Resistant and sensitive genotypes were identified based on Fe-induced biomass reduction and leaf-bronzing score.

Panda (2012) compared two indica rice cultivars *viz.*, Swarna and Kalinga 3 for their response to iron (Fe) stress in hydroponic culture. Plant growth, soluble protein, chlorophyll content and phytoferritin were more adversely affected in Swarna than Kalinga3 at 10 mg $L^{-1}$ of Fe indicated that higher Fe tolerance is observed in Kalinga3 than Swarna.

Three rice varieties: Mahsuri, Ranjit and SiyalSali were screened in four different levels of $Fe^{2+}$ iron viz., control, 100ppm, 200ppm and 300ppm. Iron 300 ppm in the medium was found to induce severe bronzing disorder in the variety Ranjit and SiyalSali. Variety Mahsuri maintained higher total soluble protein, higher superoxide dismutase and catalase activity. Significant reductions in superoxide dismutase and catalase activities were observed in the varieties Ranjit and SiyalSali (Saikia and Baruah, 2012).

Samaranayake *et al.* (2012) used two varieties of rice; Bw 272-6b (susceptible) and Bw 267-3 (resistant) and imposed Fe stress by adding 250 mg $Fe^{2+}$ $L^{-1}$. The relative decrease of shoot dry weight was 10 times greater in Bw 272-6b than Bw 267-3. Root dry weights of both varieties remained unaffected by the ferrous stress. The iron content of the shoots of the two varieties under stress condition was not significantly different from each other. Although the iron content of the shoots of susceptible and resistant varieties was not significantly different, the leaf symptoms were severe in the susceptible variety.

Onaga *et al.* (2013a) found that iron toxicity reduced grain yield by 34.2 per cent under field conditions and 28.3 per cent under greenhouse conditions. Tolerance to iron

toxicity was associated with high biomass production and phosphorus content in the leaves. Resistant cultivars retained more iron in the root tissue, confirming earlier findings that root retention is more efficient as avoidance/exclusion mechanism.

Cultivars of *O. glaberrima* were confirmed to be more resistant to the iron toxicity than those of *O. sativa* and inter-specific hybrids. *O. glaberrima* cultivars showed fewer bronzing, weak iron contents in the leaves and few iron coating on the roots than those of the other two groups (Nyamangyoku and Bertin, 2013).

Wang *et al.* (2013) conducted an experiment to find out the effect of excess iron between iron sensitive and iron resistant rice cultivars. It was found that excessive iron concentration significantly inhibited the growth of both Fe-sensitive cultivar IIyou838 and Fe-resistant cultivar Xieyou9308, including the shoot and root lengths, root and shoot fresh weights and dry weight.

Morphological and physiological responses as well as the level of tolerance of paddy genotypes to iron toxicity were evaluated by Harahap *et al.* (2014). The results showed that there were significant differences among each genotype of the ethylene content, aerenchyma size, plaque content, Fe content in the root, leaf bronzing and Fe content in the shoot. Based on observations of several parameters, it was concluded that the genotype Indragiri was very tolerant to iron toxicity, whereas IR64 was very sensitive to iron toxicity.

Matthus *et al.* (2015) conducted a genome wide association study (GWAS), identifying iron tolerance loci in a panel of 329 varieties, representing all subgroups of *O. sativa* from 79 countries. All phenotypic traits yielded genomic loci significantly associated with tolerance to excess iron. Temperate japonica and aromatic sub-populations proved more tolerant than tropical japonica, indica and aus ($p < 0.001$).

Fifty one varieties of upland and lowland rice were tested for their tolerance to different levels of iron (0, 50 mM, 100 mM and 200 mM) in nutrient solution at pH 6.8. The tolerant, medium tolerant and susceptible to iron were classified on the basis of relative root and shoot growth and biochemical analysis. Based on observations, it is concludes that out of 51 varieties, 16 varieties were tolerant (> 200mM Fe), 11 varieties

were medium tolerant (<200 mM Fe) and 24 varieties were susceptible (<100 mM) to selected iron concentration (Rout *et al.*, 2014).

Genotypes IR29 and Pokkali were exposed to a pulse stress of 1,000 mg $L^{-1}$ $Fe^{2+}$ in hydroponics. The genotypes IR29 and Pokkali showed a significant difference in leaf bronzing score after 2 and 5 days of treatments. The relative root and shoot dry weights of Pokkali were significantly higher than those of IR29. The bronzing scores and the root biomass of Nipponbare were significantly lower than of those of Kasalath, but no significant difference was found in shoot biomass. Pokkali showed markedly higher tolerance than IR29 in terms of symptom score and relative shoot and root growth. Nipponbare was more tolerant than Kasalath in terms of symptom score and root growth (Wu *et al.*, 2014).

**2.5 Correlation between growth responses under iron toxic conditions in rice**

Abifarin (1988) observed correlation between the severity of iron-toxicity symptom and yield. However, toxicity at seedling and early vegetative stages can strongly affect plant growth and result in a complete yield loss.

Snowden and Wheeler (1995) observed negative correlation between the shoot iron concentrations and iron concentration in the leaves.

Significant reductions in agronomic parameters (yield, plant height and tiller number) were observed under iron toxicity as compared with the control plot, but the scores were significantly correlated with reductions in yield and plant height (IRRI, 1996).

Hu *et al.* (1997) conducted a hydroponic culture experiment with 5 iron-tolerant and 5 iron-sensitive rice lines derived from Azucena × IR64. Results revealed that the peroxidase (POD) activity in the rice shoot was closely correlated with tolerance to iron stress, it being higher in tolerant lines than in sensitive lines. Iron stress significantly increased POD activity in all lines, but this increase was positively correlated with iron concentration in tolerant lines and negatively correlated with iron concentration in sensitive lines.

Wu *et al.* (1998) observed that the LBI (Leaf bronzing index) values and the relative decrease in shoot dry weight were positively correlated ($r = 0.56^{**}$). The results indicate that leaf bronzing was associated with growth reduction due to $Fe^{2+}$ toxicity in the DH (Double Haploid) population.

Luo *et al.* (1997) observed that significant correlations were found between N, P, K, and Mg uptake and in their tolerance to $Fe^{2+}$ toxicity, which suggests that the ability to maintain higher nutrient element uptake under a Fe-toxic condition contributes the tolerance to $Fe^{2+}$ toxicity.

Audebert and Sahrawat (2000) observed strong correlations between grain yield and scored leaf iron toxicity symptoms across seasons and cultivars. Higher evapo-transpiration in the dry season in an iron toxic soil causes greater uptake of iron to the plant. The greater uptake of iron may cause more severe toxicity. In the iron-toxicity susceptible cultivar Bouake 189, grain yield steadily decreased with the increase in total iron content of the leaves. Under the same growing conditions, CK 4 leaves absorbed considerably less iron than those of Bouake 189. Since Bouake 189 is susceptible to iron toxicity, absorption of more iron in its leaves led to a decrease in yield.

Negative correlation of visual bronzing symptom (VBS) with dry matter yield and plant height was observed by Olaleye *et al.* (2001).

Wan *et al.* (2003a) identified significant negative correlation of leaf bronzing index with stem weight, tiller number and root weight.

Asch *et al.* (2005) observed that leaf-bronzing scores were highly correlated with tissue Fe concentration (visual differentiation in includer and excluder types). The combination of these two parameters also identified genotypes tolerating high levels of Fe in the tissue while showing only few leaf symptoms (tolerant includers).

Audebert (2006) observed that the large rice genetic variability in response to iron toxicity. The correlation between leaf-symptom score and grain yield across genotype could be a breeding advantage for producing improved rice cultivars rapidly under iron-toxic conditions.

Majerus *et al.* (2007) observed negative correlation of root iron concentration with sheath iron concentration and laminae iron concentration.

IR64 (check variety) and four lines of rice (*Oryza sativa* L.) developed at IRRI were screened in an iron (Fe) toxicity field and also under normal soil conditions by Nozoe *et al.* (2008). They suggested that the absorption of Fe in the root was responsible for the changes in Fe concentration of leaf especially during the late stage of cultivation.

Audebert and Fofana (2009) identified that leaf bronzing was strongly correlated with yield loss under Fe-toxic conditions. It was estimated that increment of each visual symptom score is associated with a yield loss of approximately 400 kg $ha^{-1}$.

Field and greenhouse experiments were conducted by Onaga *et al.* (2013a) to determine variation in iron toxicity tolerance and uptake of macronutrients in 19 rice cultivars. Growth and nutrient uptake showed negative correlation with iron content in the leaves, suggesting that both traits were impacted by iron toxicity. Results showed a significant negative correlation between iron in leaves with root weight, shoot weight and tiller number under iron toxic conditions.

Samaranayake *et al.* (2012) observed positive correlation between shoot iron content and root iron content of two rice cultivars (Bw 272-6b and Bw 267-3) under stressed conditions.

Nyamangyoku and Bertin (2013) stated that leaf iron concentration and the level of bronzing correlated positively and highly significantly. Both parameters correlated negatively and highly significantly with leaf dry weight, thus showing that efficient regulation of leaf iron concentration play a primordial role in resistance to iron toxicity.

Wang *et al.* (2013) studied the effects of excess iron on rice plant stature, production, acid metabolism and content by hydroponic experiments. Excessive $Fe^{2+}$ significantly inhibited the shoot length, root length, root fresh weight, shoot fresh weight and dry weight of both Fe-sensitive cultivar Ilyou838 and Fe-resistant cultivar Xieyou9308.

A set of 220 $BC_3DH$ lines derived from the backcross *O. sativa* (Caiapo)/*O. glaberrima* (MG12)//*O. sativa* (Caiapo) was tested by Dufey *et al.* (2015) in hydroponics in the presence or absence of $Fe^{2+}$ (0 or 250 mg $L^{-1}$). A high and positive correlation was found between the LBI (Leaf bronzing index) and the Fe concentration in the blade, sheath and root-plaque system. The highest positive correlation coefficient was found between SDW (Shoot dry weight) and RDW (Root dry weight).

## 2.6. Bulk segregant analysis and markers linked QTLs conferring iron toxicity tolerance

Bulked segregant analysis involves screening for differences between two pooled DNA samples derived from a segregating population that originated from a single cross. Each pool, or bulk, contains individuals selected to have identical genotypes for a particular genomic region ('target locus or region') but random genotypes at loci unlinked to the selected 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. The two bulks are screened for differences the same way as NILs, with several RFLP probes simultaneously or individual RAPD primers or SSR primers (Michelmore *et al.,* 1991). Of these markers, SSR (simple sequence repeats) primers provide the most efficient way of identifying new loci.

Bulked segregant analysis does not reveal novel types of variation but rather allows the rapid screening of many loci and therefore the identification of segregating markers in the target region (Michelmore *et al.,* 1991). Molecular markers linked to iron toxicity tolerance in rice have been reported by several workers. A brief review on this aspect is presented below.

Double haploid (DH) population consisting of 123 lines derived from a japonica variety (Azucena) and an indica variety (IR64) and 100 $BC_1F_1$ lines were screened by Wu *et al.* (1997). Two gene loci were identified to be flanked by RG345 and RG381and linked to RG810 on chromosome 1 for both index values and shoot weight (SW), respectively. The variation in SW was also explained by a locus linked to RG978 on chromosome 8 by about 10 per cent. Comparison of the two marker genotypic class

means indicated that the tolerant alleles were from Azucena at the first locus on chromosome 1 and the locus on chromosome 8, and that at the second locus on chromosome 1 from IR64.

Segregation for leaf bronzing and growth reduction due to $Fe^{2+}$ toxicity in a doubled haploid (DH) population with 135 lines derived from a $Fe^{2+}$ tolerant japonica variety (Azucena) and a sensitive indica variety (IR64) observed by Wu *et al.* (1998). A non-normal distribution of LBI was found. Single locus analysis and interval mapping analysis based on 175 molecular markers revealed that the interval flanked by RG345 and RZ19 on chromosome one was an important location of gene(s) for $Fe^{2+}$ tolerance. A gene locus with relative small effect on root ability to exclude $Fe^{2+}$ was also detected.

Two parents (Nipponbare and Kasalath) and 96 BILs were phenotyped by growing them in $Fe^{2+}$ toxicity nutrient solution. A total of four QTLs were detected on chromosome 1 and 3, with LOD of QTLs ranging from 3.17 to 7.03. One QTL controlling LBI (Leaf Bronzing Index), SDW (Shoot Dry Weight), TN (Tiller Number) and RDW (Root Dry Weight) was located at the region of C955-C885 on chromosome 1. The QTL located at the region of C955-C885 on chromosome 1 may be important to ferrous iron toxicity tolerance in rice. Another QTL for SDW and RDW was located at the region of C25-C515 on chromosome 3. Further, two QTLs on chromosome 1 were located for RDW at the region of R2329-R210 and for TN at the region of R1928-C178 (Wan *et al.*, 2003b).

The genetic factors for excess iron accumulation under K or P deficiency, in a set of seedlings in $F_3$ and $F_8$ generations from an *Oryza sativa* cross between Gimbozu and Kasalath analyzed by Shimizu *et al.* (2005). QTLs for the Fe, P and Mg content in shoots were compared in the maps of $F_3$ and $F_8$. The QTLs for the Fe content in shoots varied in three types of nutritional conditions, but consistently indicated two overlapping regions on chromosome 3 and 4.

Wan *et al.* (2005) used $F_2$ and $F_3$ populations derived from a Longza8503/IR64 cross under iron-enriched solution cultures to map QTLs controlling ferrous iron toxicity tolerance. A total of 20 QTLs for LBI (Leaf Bronzing Index), PH (Plant Height) and RL (Root Length) under the $Fe^{2+}$ stress were detected over 10 of the 12 rice

chromosomes, reflecting multigenic control of these traits. QTLs controlling LBI were located at the region of RM315-RM212 on chromosome 1, RM6-RM240 on chromosome 2 and RM252-RM451 on chromosome 4.

Identification of many QTLs with a small effect suggests that tolerance to Fe toxicity may involve additive effects of several genes. This implies that several QTLs/genes must be manipulated at the same time in order to have a significant impact on the phenotype. Alternatively, the search should be targeted to large-effect QTL associated with grain yield under Fe toxic conditions among germplasm adapted to Fe toxicity in West Africa (Sikirou *et al.*, 2015).

Morphological traits were measured on all 164 RILs derived from a cross between Azucena and IR64 by Dufey *et al.* (2009). Physiological traits were measured on the two parents and extreme individuals only, selected on the basis of their leaf bronzing index and shoot dry weight. A total of 24 putative QTLs were identified on chromosomes 1, 2, 3, 4, 7 and 11 for leaf bronzing index, shoot water content, shoot and root dry weight, relative variation of shoot and root dry weight, shoot iron concentration, stomatal resistance and chlorophyll content index. Several QTLs were detected in overlapping regions for different parameters.

Shimizu (2009) conducted a QTL analysis for iron-toxicity tolerance in rice. On the basis of quantified score, QTL analysis for bronzing tolerance was conducted using $F_3$ lines from a cross between tolerant cultivar (Gimbozu) and susceptible cultivar Kasalath. A single QTL near RM221 marker on chromosome 2 was detected by composite interval mapping and additional five QTL were detected by multiple interval mapping.

Dufey *et al.* (2012) checked the consistency of quantitative trait loci (QTLs) for traits related to resistance mechanisms using 164 recombinant inbred lines derived from Azucena and IR64. A total of 44 putative QTLs were identified for morphological, physiological and agronomic traits. From these 44 QTLs, 20 were found in overlapping regions for the same or related traits in different environments, identifying six regions of great interest for the determinism of resistance to iron toxicity.

A quantitative trait locus (QTL) analysis for susceptibility to ferrous iron using chromosomal segments substitution lines (CSSLs) was performed by Fukuda *et al.* (2012). The shoot iron concentration was examined in 39 CSSLs carrying Kasalath chromosomal segments in a background of Koshihikari, a *japonica* cultivar. Of the CSSLs, SL208, which carried the Kasalath chromosomal segment on chromosome 3, had a significantly higher shoot iron concentration than Koshihikari, and none of the CSSLs had a shoot iron concentration significantly lower than Koshihikari. This finding suggests that the putative QTL affecting the shoot iron concentration is between the markers R663 and S1571 on chromosome 3.

Two genotypes, IR61612-313-16-2-2-1 and Suakoko8 showed significantly high resistance with an average score of $\leq 3.5$ on 1 to 9 scale. The SSR markers were highly informative and showed mean polymorphism information content (PIC) of 0.68. The PIC values revealed that RM10793, RM3412, RM333, RM562, RM13628, RM310, RM5749 and RM154 could be the best markers for genetic diversity estimation of these rice cultivars (Onaga *et al.*, 2013b).

Wu *et al.* (2014) detected 7 QTLs for leaf bronzing score on chromosome 1, 2, 4, 7 and 12 in an IR29/Pokkali $F_8$ recombinant inbred population. Two tolerant recombinant inbred lines carrying putative QTLs were selected for further experiments. In a Nipponbare/ Kasalath/Nipponbare backcross inbred population, 3 QTLs were mapped on chromosomes 1, 3 and 8 respectively. The effect of QTLs on chromosome 1 and 3 were confirmed by using chromosome segment substitution lines (SL), carrying Kasalath introgressions in the genetic background on Nipponbare. The Fe uptake in shoots of substitution lines suggests that the effect of the QTL on chromosome 1 was associated with shoot tolerance while the QTL on chromosome 3 was associated with iron exclusion.

Dufey *et al.* (2015) tested a set of 220 $BC_3DH$ lines derived from the backcross *O. sativa* (Caiapo) / *O. glaberrima* (MG12) // *O. sativa* (Caiapo) in hydroponics in the presence or absence of $Fe^{2+}$ (0 or 250 mg $L^{-1}$). A total of 28 QTLs were detected in 18 distinct chromosomal regions for 11 morphological and physiological traits. The single and joint composite interval mappings confirmed the interest of region RM5-RM246 on chromosome 1. Several QTLs were detected in new regions, including five QTLs and

one joint QTL on chromosome 5 and one QTL on chromosome 10. The favorable allele for all these seven new QTLs were provided by the *O. glaberrima* cultivar MG12, *i.e.* the lesser investigated species. These QTLs corresponded to leaf bronzing index, dry weight and Fe concentration in the root-plaque system and stomatal conductance.

Chrisnawati *et al.* (2016) performed molecular analysis using STS markers associated with iron tolerance trait in double haploid rice population. The results of the association between the genetic and phenotypic analysis showed that there were three markers, i.e. OsIRT1, OsIRT2, and OsFRO2 presented on chromosome 3, 7 and 4 respectively, associated with iron tolerance trait in rice. The markers have potential as selection markers for iron tolerant lines.

High-density SNP bin markers were used by Liu *et al.* (2016) in two reciprocal introgression line (IL) populations to identify QTL tolerant to iron and zinc toxicities. The results indicated that the japonica variety 02-428 had stronger tolerance to iron and zinc toxicities than the indica variety Minghui 63. Nine and ten QTL contributing to iron and zinc toxicity tolerances, respectively, were identified in the two IL populations. The favorable alleles of most QTL came from 02-428. Among them, qFRRDW2, qZRRDW3, and qFRSDW11 appeared to be independent of genetic background. The region C11S49–C11S60 on chromosome 11 harbored QTL affecting multiple iron and zinc toxicity tolerance-related traits, indicating partial genetic overlap between the two toxicity tolerances.

# Materials and methods

# III. MATERIALS AND METHODS

The present investigation on 'Identification of molecular markers linked to iron toxicity tolerance through bulk segregant analysis (BSA) in rice (*Oryza sativa* L.)', was conducted in the Department of Plant Breeding and Genetics, College of Horticulture, Kerala Agricultural University (KAU), Vellanikkara, Thrissur during 2013 to 2015. The study comprised of four major experiments. 1) Hybridization programme [1a) Parental selection and 1b) Hybridisation], 2) Parental polymorphism study using molecular markers, 3) Raising of $F_1$'s and 4) Bulk Segregant Analysis (BSA) [4a) Phenotyping of $F_2$ plants for iron toxicity tolerance, 4b) Genotyping parents, susceptible and resistant bulks and 4c) Confirmation of putative markers]. The details of the material used and methods employed in the present investigation are presented below.

## 3.1. Experimental location

The experimental site was located at the College of Horticulture (COH), Kerala Agricultural University (KAU), Vellanikkara P.O., Thrissur 680 656, 40 m above MSL between $10^0$31' N latitude and $76^0$13' E longitude and experiencing humid tropical climate.

## 3.2. Experimental material

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 selection of the genotypes was based on their tolerance reaction to iron toxicity as assessed under KSCSTE project: 'Donor identification for tolerance to iron toxicity in rice (*Oryza sativa* L.)'. List of thirty rice genotypes is given in table 1. The genotypes thus selected included individuals that were either tolerant or susceptible to iron toxicity.

## 3.3. Experimental method

### 3.3.1. Experiment 1: Hybridization programme

#### 3.3.1.1. Parental selection

The 30 genotypes were subjected to further screening (Confirmation test 1 and test 2) to confirm their tolerance or susceptibility to iron toxicity. The laboratory screening of the thirty genotypes was undertaken via hydroponics following the method

**Table 1. List of 30 rice genotypes**

| Sl.No. | PGC No. | Details | S.No. | PGC No. | Details |
|---|---|---|---|---|---|
| 1 | 33 | Cul-18714 | 16 | 50 | PTB-10 |
| 2 | 60 | PM-709 | 17 | 43 | ASD-18 |
| 3 | 48 | ASD-16 | 18 | 100 | Cul-90-03 |
| 4 | 115 | IVT-33 | 19 | 31 | Cul-8709 |
| 5 | 34 | Cul-18716 | 20 | 28 | T(N)-1 |
| 6 | 46 | Abhaya | 21 | 20 | IR-1552 |
| 7 | 12 | Kanchana | 22 | 84 | ASD(Peringotukurussi) |
| 8 | 29 | Cul-8759 | 23 | 59 | PM-706 |
| 9 | 192 | CSR 13 | 24 | 64 | PM-717 |
| 10 | 104 | Cul-210-29 | 25 | 27 | Cul-8755 |
| 11 | 157 | Moncompu-519 | 26 | 16 | Supriya |
| 12 | 39 | Cul-3 | 27 | 73 | Karangi |
| 13 | 133 | AM-10-7 | 28 | 36 | Cul-8723 |
| 14 | 14 | Thulasi | 29 | 125 | JM-10-31 |
| 15 | 17 | IR-36 | 30 | 71 | Kargi |

advocated by Shimizu *et al.* (2005). Confirmation test 1 and 2 were laid out as completely randomized design with thirty genotypes and two replications. Observations were recorded on ten seedlings in each replication. The genotypes were screened at three iron concentrations [0ppm Fe (control), 600ppm Fe and 800ppm Fe] to elucidate their response to iron stress. The laboratory procedure followed for the Confirmation test 1 and 2 is enumerated under 3.3.1.1.1.

### 3.3.1.1.1. Laboratory screening for iron toxicity tolerance (Confirmation test 1 and 2)

Rice seedlings were screened through hydroponics using Yoshida nutrient medium (Yoshida *et al.,* 1976). The experimental setup consisted of rectangular plastic trays of 10 litre capacity. A float was fabricated with a rectangular polysterene sheet of size 28 x 32 x 1.25 cm with 100 holes and fitted with nylon net at the bottom. The float was then placed in the tray containing deionized water (10 litre).

Four day old seedlings were transferred to the hydroponics system containing deionized water to enable pre-conditioning. After five days, when seedlings were well established, the deionized water was replaced with Yoshida solution with the graded concentrations of iron (0 ppm, 600ppm and 800pp Fe). Yoshida culture solution (Yoshida *et al.,* 1976) was prepared by adding 12.5 ml each from each of the six stock solutions (Table 2) prepared and volume made up to ten liters with de-ionized water. The culture was maintained at pH 5.0 and pH adjusted to 5.0 daily using either with 1N sodium hydroxide (NaOH) or 1N hydrochloric acid (HCl). The culture solution was renewed weekly. The culture [0ppm Fe (control), 600ppm Fe and 800ppm Fe] was maintained for 30 days and visually scored for iron-toxicity symptoms, using a scale of 1 to 9 based on the International Rice Research Institute standard evaluation system (IRRI, 1996) and the biomass recorded.

### 3.3.1.2. Hybridization

A non-replicated crossing block was laid out during January to June, 2014. Staggered sowing of each genotype was done at weekly intervals from 20/01/2014 to 10/02/2014 to ensure synchronized flowering between males and females and ensure pollen availability for hybridization. Usual agronomic practices were adopted. Hybrid

**Table 2. Nutrient composition of Yoshida's stock solution**

| Sl.No. of Stock solution | Element | Source | Quantity (g /500ml) |
|---|---|---|---|
| | **Macronutrients Stock solution** | | |
| 1 | N | Ammonium nitrate ( $NH_4NO_3$) | 45.700 |
| 2 | P | Sodium dihydrogen phosphate ($NaH_2PO_4$) | 17.800 |
| 3 | K | Pottassium sulphate ($K_2SO_4$) | 35.700 |
| 4 | Ca | Calcium chloride ($CaCl_2.2H_2O$) | 58.675 |
| 5 | Mg | Magnesium sulphate ($MgSO_4.7H_2O$) | 162.000 |
| 6 | | | **Micronutrients Stock solution** |
| | Mn | Manganese chloride ($MnCl_3.4H_2O$) | 0.7500 |
| | Mo | Ammonium molybdate tetrahydrate ($NH_4\ Mo_7O_{24}.\ 4H_2O$) | 0.0375 |
| | Zn | Zinc sulphate hepta-hydrate ($ZnSO_4.7H_2O$) | 0.0175 |
| | B | (Boric acid $H_3BO_3$) | 0.4670 |
| | Cu | Cupric sulphate penta-hydrate ($CuSO_4.5H_2O$) | 0.0155 |
| | Fe | Ferric chloride anhydrous ($FeCl_3$) | 2.3100 |
| | | Citric acid | 5.9500 |

seeds between the lines and testers were produced by emasculation through clipping method followed by hand pollination.

#### 3.3.1.2.1 Emasculation

Emasculation of spikelets in female parents was done late in the afternoon (after 3pm). Panicles that have emerged fifty to sixty per cent out of the flag leaf were used for emasculation. The leaf sheath from the panicle was slightly detached to expose the spikelets and for easiness of emasculation. Very young spikelets from the bottom of the panicle where the height of the anthers is less than half the spikelet were cut away. Spikelets likely to open the next day (where the height of the anthers equal or more than half the spikelets) were selected for emasculation. The top one-third of each selected spikelet to be emasculated was clipped with scissors to expose the anthers. The anthers were removed with the tip of the forceps prong by pressing them against the side of the spikelet and lifting out. The emasculated panicles were bagged in butter paper bags and its bottom edge folded against the peduncle to hold the bag securely in place. Tagging and labeling of the emasculated panicle was done.

#### 3.3.1.2.2 Pollination

Although the stigma remained receptive for three to seven days, pollination on the subsequent day of emasculation gave maximum seed set. At about 8am, panicles from the desired male parent ready to dehisce were selected. The panicles were enclosed in petridish and the top of the petridish gently tapped to release the pollen grains. Pollen grains collected in the petridish were then transferred to the stigma with the help of thin camel brush. The pollinated panicles were re-bagged to avoid contamination by foreign pollen. The pollinated spikelets were checked for seed set on the fifth day after hybridization and the bag was removed.

A total of six cross combinations were made involving three female parents (most susceptible rice genotypes) and two male parents (most tolerant rice genotypes) in a Line x Tester mating design and the hybrid seeds were collected separately. Around ninety hybrid seeds were collected in each cross combination. The cross combinations are detailed in the table 3.

**Table 3. Six crosses from crossing block**

| Sl. No. | Female parent | Male parent | Cross |
|---|---|---|---|
| 1 | Cul-8709 | Tulasi | Cul-8709/ Tulasi |
| 2 | IR-1552 | Tulasi | IR-1552/ Tulasi |
| 3 | Cul-90-03 | Tulasi | Cul-90-03/ Tulasi |
| 4 | Cul-8709 | Cul-18716 | Cul-8709/ Cul-18716 |
| 5 | IR-1552 | Cul-18716 | IR-1552/ Cul-18716 |
| 6 | Cul-90-03 | Cul-18716 | Cul-90-03/ Cul-18716 |

### 3.3.2. Experiment 2: Parental polymorphism study using molecular markers

Bulk segregant analysis warrants the study of segregating generation ($F_2$) developed from hybridization between the two extreme genotypes for the trait to be mapped. Hence, the genotypes PGC 14 (Thulasi) and PGC 31(Cul-8709) respectively that were found to be most tolerant and most susceptible to iron stress were selected for parental polymorphism study. Polymorphism at molecular level between the parents [PGC 14 (Tulasi) and PGC 31 (Cul-8709)] was ascertained by genotyping their DNA with simple sequence repeats (SSR). From the rice microsatellite (RM) markers available at www.gramene.org, a set of 338 RM markers (Appendix I) were selected based on their mapped locations at an average distance of 6 cM between two consecutive markers so as to cover all twelve linkage groups in rice (Venuprasad *et al.*, 2009).

#### 3.3.2.1. Isolation of DNA

Total cellular DNA (Deoxyribo nucleic acid) of two parents [PGC 14 (Thulasi) and PGC 31(Cul-8709)] was extracted by following the protocol described for CTAB method (Dellaporta *et al.*, 1983).

**The procedure used for extraction of the DNA is presented below:**

1. 400 mg of tender leaves of rice was weighed into a pre-chilled mortar and pestle.
2. The leaves were ground by adding 50 µl of β-mercapto ethanol and pinch of PVP (Poly vinyl pyrrolidine) along with liquid nitrogen and made it into fine powder.
3. This was transferred to sterile 2ml tube containing 1ml of pre-warmed CTAB (5X) extraction buffer and mixed well.

4. This mixture was incubated at 65°C for 20-30 min with occasional mixing by gentle inversion.

5. After incubation, 1ml of pre-chilled chloroform: Isoamyl alcohol (24:1) was added and mixed by inversion to emulsion.

6. The tube was centrifuged at 12,000 rpm for 15 minutes at 4°c.

7. The aqueous phase was transferred with a wide bore pipette to a clean tube.

8. Equal volume of chloroform: Isoamyl alcohol (24:1) was added to the tube.

9. The tube was mixed gently by inversion and gently centrifuged at 12,000 rpm for 15 minutes at 4°c.

10. Aqueous phage was then removed with a pipette out into clean tube and 0.6 ml of ice cold isopropanol was added and mixed well until the DNA precipitated and kept at 4°c for 2 hours.

11. The tube was centrifuged at 12,000 rpm for 15 minutes and the supernatant gently poured out by inverting tube.

12. The pellet was washed with 70 per cent ethanol and centrifuged at 10000 rpm for 10min.

13. Supernatant was removed and pellet was dried.

14. After drying, the DNA was dissolved in the sterile distilled water (100ml) and stored at a temperature of -20°c.

### 3.3.2.2. Determination of quantity and quality of isolated DNA

The genomic DNA extracted from individual plant was quantified spectrophotometrically (Nanodrop® ND-1000 UV-visible spectrophotometer) both at 260 nm and 280 nm. The absorbance at 260 nm enables the calculation of DNA concentration in the sample. An OD of 1 at 260 nm corresponds to 50 µg per ml of double stranded DNA. A pure sample of DNA shows the ratio of OD260/280 as 1.8. Ratios less than 1.8 indicated contamination in the preparation either with phenol or with proteins. The values higher than this indicate presence of RNA in the preparation. Ratios of OD at 260 nm over OD at 280 nm were calculated to separate the contaminants from the sample DNA. Computed OD values were used to dilute the DNA samples to working concentrations of 20 ng/ µl.

### 3.3.2.3. Normalization of DNA concentration for PCR

Normalization of DNA was done to bring all DNA concentrations to a relatively equal level (20ng/µl) by appropriate dilutions. Dilutions were done with distilled water.

### 3.3.2.4. Polymerase chain reaction (PCR)

Amplification reaction mixture was prepared in 0.2 ml thin walled flat cap PCR tubes containing following components as enlisted in the table 4.

**Table 4. List of components for polymerase chain reaction (PCR)**

| Sl. No. | Components | Amount (µl) |
|---|---|---|
| 1 | Template DNA (15 ng/µl) | 3 |
| 2 | Primer (5ng/µl) (both forward and reverse) | 4 |
| 3 | PCR master mix | 8 |

The total volume of each reaction mixture was 15µl. Amplification was carried out on Master Cycler Gradient Eppendorf PCR. The amplification profile was as follows:

a) Initial denaturation at 94°C temperature for 5 min.

⇩

b) Denaturation at 94°C for 1min.

⇩

c) Primer annealing at 54°C for 30sec

⇩

d) Primer extension at 72°C for 45sec

⇩

Later, steps b) to d) were repeated 35 times.

⇩

e) Complete primer extension at 72°C for 10 min.

⇩

f) Hold at 4°C till removal

### 3.3.2.5. Separation of amplified products by agarose gel electrophoresis

The gel tray was prepared by sealing the ends with tape. Comb was placed in gel tray about 1 inch from one end of the tray and positioned the comb vertically such that

the teeth are about 1 to 2 mm above the surface of the tray. Agarose of 1.5 per cent (1.5 g in 100ml) was prepared in a glass beaker or conical flask with 100 ml 1X TAE buffer. One litre of 10X TBE buffer was prepared from following components (Table 5).

**Table 5. List of components for preparation of 1l 10X TBE buffer**

| Sl. No. | Components | Quantity of sample (g) | Final concentration of sample |
|---|---|---|---|
| 1 | Tris base | 108g | 890mM |
| 2 | Boric acid | 55g | 890mM |
| 3 | EDTA (pH 8.0) | 3.72g | 2mM |

Agarose was micro waved for 45 to 60 seconds until it was dissolved and solution was clear. Solution was allowed to cool to about 40 to 45°C and 5µl of Ethidium bromide was added at this point and mixed well. This warm gel solution was poured into the gel tray to a depth of about 5 mm and allowed to solidify for about 30 minutes at room temperature. The comb was subsequently removed and the tape used for sealing removed. The tray was placed in electrophoresis chamber and filled (just until wells are submerged) with TAE buffer. To prepare samples for electrophoresis, 6X gel loading dye (1 µl) were added for every 5µl of DNA solution. Mixed well and loaded 10µl DNA sample per well. Electrophoresis was carried out at 70 volts and 400 amp until the dye has migrated two third the length of the gel. On completion of the electrophoresis, the gel was viewed under UV light and the DNA banding pattern was recorded directly in documentation unit (gel doc).

### 3.3.2.6. Analysis of bands for parental polymorphism

The banding pattern itself was noted from the digital image of the gels and scored for polymorphism. Polymorphic primers were selected based on polymorphic bands.

### 3.3.3. Experiment 3: Raising of $F_1$s

A non-replicated $F_1$ block was laid out during Oct, 2014 to Jan, 2015 to raise the hybrids from cross combination PGC 14 (Tulasi) / PGC 31 (Cul-8709). Seeds were sown in tubs containing sterile sand. The 14 day old seedlings were transplanted to earthen pots. Usual agronomic practices to ensure good crop growth were adopted. At time of flowering stage, $F_1$ plants were allowed to self pollination and produce $F_2$ seeds.

Care was taken to cover the panicles with butter bags to prevent unwanted pollination. A total of 1000 $F_2$ seeds were harvested for conduct of Bulk Segregant Analysis (BSA).

### 3.3.4. Experiment 4: Bulk Segregant Analysis (BSA):

#### 3.3.4.1. Phenotyping of $F_2$ plants for iron toxicity tolerance:

Screening of $F_2$ plants (300 nos.) and the parents for their response to iron at 800 ppm was done between March to April, 2015 as per the method enumerated under 3.3.1.1.1.

#### 3.3.4.2. Genotyping parents, susceptible and resistant bulks:

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).

Markers found to be polymorphic between the parents (Experiment 2) were used for genotyping parents, susceptible and resistant bulks. The DNA bulks (RB and SB) were assayed for SSR polymorphisms alongside parental DNA using these polymorphic markers. The banding pattern was noted from the digital image of the gels and scored for polymorphism. Based on the evaluation of DNA bulks, selective genotyping of individual plants in the bulks were done along with parents using co-segregating markers.

#### 3.3.4.3. Confirmation of putative markers:

To confirm the linkage of SSR markers to target locus DNA from selected $F_2$ plants of resistant bulk and susceptible bulk were analyzed with co-segregating polymorphic markers identified from BSA.

### 3.4. Observations recorded

#### 3.4.1. Laboratory screening for iron toxicity tolerance (on 30$^{th}$ day)

**1. Shoot length (cm)**

Measured from the base of the shoot to the tip of the tallest leaf blade and expressed in centimeters.

**2. Root length (cm)**

Measured from the base of the root to the tip of the longest root and expressed in centimeters.

**3. Total number of roots**

The total number of roots including dead and fresh roots in each plant was counted after washing the root zone thoroughly.

**4. Number of fresh roots**

The number of fresh roots in each plant was counted after washing the root zone thoroughly.

**5. Shoot weight (g)**

After 30 days of screening, shoot portion and root portion of each $F_2$ plant was separated, and weight was taken separately for shoot and expressed in grams.

**6. Root weight (g)**

After 30 days of screening, shoot portion and root portion of each $F_2$ plant was separated and weight was taken separately for root and expressed in grams.

**7. Iron reversibly adsorbed on root surface (mg $L^{-1}$)**

The root zone was washed thoroughly with deionised water taking care not to dislodge the iron plaque. The roots were then immersed in 25 ml of 0.01 M Calcium chloride for 5 minutes (Piper, 1996). Iron content in the Calcium chloride solution was then estimated by atomic absorption spectrophotometer (Model: Analyst-400 Perkin-Elmer).

**8. Iron content in root (mg $kg^{-1}$)**

Accurately weighed samples of roots (0.5g) each entry/ individual $F_2$ plant were properly dried at 60°C for seventy-two hours followed by diacid digestion using nitric acid - perchloric acid mixture in 2:1 ratio. After digestion the mixture was diluted with distilled water and made upto to 100 ml before filtration (Piper, 1996). The filtrate was collected and analysed for iron content using atomic absorption spectrophotometer (Model: Analyst-400 Perkin- Elmer).

**9. Iron content in leaf (mg $kg^{-1}$)**

Accurately weighed samples of leaves (0.5g) from each entry/ individual $F_2$ plant was collected and oven dried at 60°C for seventy-two hours followed by diacid digestion using nitric acid - perchloric acid mixture in 2:1 ratio. After digestion the mixture was diluted with distilled water and made upto to 100 ml before filtration (Piper, 1996). The filtrate was collected and analysed for iron content using atomic absorption spectrophotometer (Model: Analyst-400 Perkin- Elmer).

**10. Visual scoring for iron-toxicity symptoms (IRRI, 1996)**

Scoring for iron toxicity symptoms in parents and $F_2$ plants was done (at 30 days after transplanting) using the visual scoring system for iron toxicity according to Standard Evaluation Scale (IRRI, 1996) as detailed in table 6.

**Table 6. Visual scoring for iron-toxicity symptoms (IRRI, 1996)**

| Scale | Description | Category |
|---|---|---|
| 0 | Growth and tillering near normal | Highly resistant |
| 1 | Growth and tillering near normal; reddish-brown spots of orange discoloration on tips of old leaves | Resistant |
| 3 | Growth and tillering near normal; older leaves reddish-brown, purple or orange yellow | Moderately resistant |
| 5 | Growth and tillering delayed; many leaves discolored | Moderately susceptible |
| 7 | Growth and tillering ceased; most leaves discoloured or dead | Susceptible |
| 9 | Almost all plants dead or drying | Highly susceptible |

### 3.4.2. Genotyping of parents and BSA

**1. Quality and quantity of DNA isolated**

Nanodrop® ND-1000 spectrophotometer was used for analyzing purity of DNA. It measures absorbance from 2µl sample with high accuracy and reproducibility. It estimates the concentration of nucleic acid in the sample based on Beer-Lambert law. The purity of DNA was assessed by OD260/OD280. A ratio of 1.8 to 2.0 indicates pure DNA.

The quantity of DNA in the sample was calculated using the formula OD 260 = 1 is equivalent to 50 µg of double stranded DNA

1 OD at 260nm = 50µg/ml DNA

Therefore OD 260 X 50 gives the quantity of DNA in µg/ml

**2. Nature of amplification**

Nature of amplification is identified either monomorphic or polymorphic based on banding pattern of Uvitech Fire reader software (Gel documentation system).

**3. Number of amplicons**

Number of amplicons is identified based on number of bands observed through banding pattern of Uvitech Fire reader software (Gel documentation system).

**4. Size of amplicons**

Uvitech Fire reader software (Gel documentation system) estimates the size of amplicons in base pairs (bp).

**5. Uniqueness of amplicons**

Special feature of amplicons which deviated from normal observation is noted as uniqueness of amplicons.

### 3.5. Statistical Analysis

#### 3.5.1. Laboratory screening for iron toxicity tolerance for parental selection

The data recorded under Experiment I was analyzed using completely randomized design so as to estimate the effect of both varieties and varying levels of iron in the

solution culture on observed variables. The mean squares due to different sources of variation were worked out using software SPSS (Statistical Package for the Social Sciences). The mean squares due to different sources of variation were worked out using the following analysis of variance (Table 7) (Gomez and Gomez, 1984).

**Table 7. Analysis of variance for completely randomized design (CRD)**

| Sources of variation | d.f. | SS | MSS | F ratio |
|---|---|---|---|---|
| Between treatments | t-1 | TSS | TMS | TMS/ EMS |
| Within treatments (Error) | t(r-1) | ESS | EMS | |
| Total | rt-1 | | | |

Where,

t = Number of genotypes

r = number of replications

### 3.5.1.1. Comparison of rice genotypes using Duncan multiple range test

Mean values of visual bronzing scores (toxicity) and biomass were subjected to analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT) by using software SPSS (Statistical Package for the Social Sciences).

### 3.5.1.2. Normalized visual scores for leaf bronzing at 600ppm and 800ppm of Fe

The leaf bronzing at 600ppm and 800ppm of Fe for each treatment were normalized for negate the effect of leaf bronzing observed under 0 ppm Fe and enable unbiased control between varieties as enumerated below:

$$\text{Normalized visual scores for leaf bronzing} = \frac{\text{Leaf bronzing at 600ppm or 800ppm of Fe}}{\text{Leaf bronzing at 0ppm of Fe}}$$

### 3.5.2. Bulk Segregant Analysis (BSA)

### 3.5.2.1. Phenotyping of $F_2$ plants for iron toxicity tolerance

#### 3.5.2.1.1 Parameters of variability

**Mean**

The mean value of each observation was worked out by dividing the totals by corresponding number of observation:

$$\mathbf{X} = \frac{\sum X_i}{N}$$

Where,

$X_i$ - any observation in $i^{th}$ treatment

N - Total number of observations

**Range**

Range of each observation was worked out as the difference between the lowest and highest values present in the observations of a sample.

**Standard Deviation**

The positive square root of mean of squared deviations from arithmetic mean, so called root mean square deviation. The standard deviation is a measure of how widely values are dispersed from the average value (the mean). Standard deviation uses the following formula:

$$SD = \frac{\sqrt{\sum X^2 - (\sum X)^2/N}}{N-1}$$

N- Number of observations (sample size)

**Coefficient of variation**

The ratio of standard deviation of a sample to its mean expressed in percentage is called coefficient of variation.

$$C.V = \frac{SD}{X} \times 100$$

**Skewness**

Skewness characterizes the degree of asymmetry of a distribution around its mean. Positive skewness indicates a distribution with an asymmetric tail extending towards more positive values. Negative skewness indicates a distribution with an asymmetric tail extending towards more negative values". While that definition is accurate, it isn't 100 per cent helpful because it doesn't explain what the resulting number actually means.

The skewness statistic is sometimes also called the skewedness statistic. Normal distributions produce a skewness statistic of about zero. Small variations can occur by chance alone. So a skewness statistic of -0.01819 would be an acceptable skewness

value for a normally distributed set of test scores because it is very close to zero and is probably just a chance fluctuation from zero. As the skewness statistic departs further from zero, a positive value indicates the possibility of a positively skewed distribution (that is, with scores bunched up on the low end of the score scale) or a negative value indicates the possibility of a negatively skewed distribution (that is, with scores bunched up on the high end of the scale). Values of 2 standard errors of skewness (*ses*) or more (regardless of sign) are probably skewed to a significant degree (Cisar, 2010).

$$\text{Skewness (X)} = \frac{1}{N}\sum_{i=1}^{N}\left[\frac{X_i - \overline{X}}{\sigma}\right]^3$$

Where,

$\overline{X}$ - mean of observations

$\sigma$ – Standard Deviation

N - Total number of observations

Skewness = 0: data perfectly symmetrical distribution

= b/w -0.5 and +0.5 : approximately symmetrical distribution

= b/w -1 and – 0.5 or b/w + 0.5 and +1: moderately skewed distribution

= < -1 or > +1: highly skewed distribution

**Kurtosis**

Kurtosis characterizes the relative peakedness or flatness of a distribution compared to the normal distribution. Positive kurtosis indicates a relatively peaked distribution. Negative kurtosis indicates a relatively flat distribution". And, once again, that definition doesn't really help us understand the meaning of the numbers resulting from this statistic.

Normal distributions produce a kurtosis statistic of about zero. Small variations can occur by chance alone. So a kurtosis statistic of 0.09581 would be an acceptable kurtosis value for a mesokurtic (that is, normally high) distribution because it is close to zero. As the kurtosis statistic departs further from zero, a positive value indicates the possibility of a leptokurtic distribution (that is, too tall) or a negative value indicates the possibility of a platykurtic distribution (that is, too flat, or even concave if the value is

large enough). Values of 2 standard errors of kurtosis (*sek*) or more (regardless of sign) probably differ from mesokurtic to a significant degree (Cisar, 2010).

$$\text{Kurtosis} = \left[\frac{1}{N}\sum_{i=1}^{N}\left[\frac{X_i-\bar{X}}{\sigma}\right]^4\right] - 3$$

Where,

$\bar{X}$ - mean of observations

$\sigma$ – Standard Deviation

N - Total number of observations

Kurtosis= (Explained w.r.to normal distribution, Normal distribution has a kurtosis of 3)

Any distribution with kurtosis ~3 = mesokurtic

k< 3= platykurtic *i.e*, compared to normal distribution, its tails are shorter and thinner and often its central peak is lower and broader

k>3= leptokurtic *i.e*, compared to normal distribution, its tails are longer and fatter and often its central peak is higher and sharper

**Histogram**

The purpose of a histogram is to graphically summarize the distribution of a univariate data set. The most common form of the histogram is obtained by splitting the range of the data into equal-sized bins (called classes). Then for each bin, the number of points from the data set that fall into each class is counted (Cisar, 2010).

**3.5.2.1.2. Correlation coefficient analysis**

The simple correlation coefficient was used to determine the degree of association of different characters with dependent character (iron toxicity tolerance) and also among independent components in each of the populations separately. Correlation coefficients were compared against table 'r' values (Fisher and Yates, 1963) at (n-2) df at probability levels of 0.05 and 0.01 to test their significance. Simple phenotypic correlations were computed by using the formula given by Weber and Moorthy (1952) as given below.

$$r = \frac{\text{Cov } xy}{\sqrt{Vx \, Vy}}$$

Where,

Cov xy = Variance between the characters x and y

$V_x$ = Variance of the character x

$V_y$ = Variance of the character y

### 3.5.2.2. Genotyping of $F_2$ plants for iron toxicity tolerance

The data obtained on genotyping the $F_2$ individuals were analyzed using the free software 'Win QTL Cart' (v.2.5) developed by Genetic Bioinformatics Research Center, North Carolina State University. WinQTLCart implements single marker analysis, interval mapping, composite interval mapping, bayesian interval mapping, multiple interval mapping, multiple trait analysis and category trait analysis. It also has provision for estimating confidence intervals by resampling. It can handle data from Backcross, doubled haploid lines, Recombinant inbred line derived by selfing and by sib mating, $F_2$ population, Randomly mated intercross line, test cross, with genotyping done on an intercross and phenotyping on a cross derived from that intercross. It can import data from mapmaker QTL, QTL cartographer and excel.

The analysis using this programme requires preparation of five different Text data files *viz.*, (1) data file of chromosome label and marker number, (2) data file of marker label, (3) data file of marker position and (4) data file of genotype and (5) data file of phenotype (Wang *et al.*, 2012).

#### 3.5.2.2.1 Single marker analysis

Single marker analysis fits the data to the simple linear regression model.

$$y = b0 + b1\ x + e$$

Linkage of the marker to a QTL was determined, if b1 is significantly different from zero. The F statistic compares the hypothesis $H_0$: b1 = 0 to an alternative $H_1$: b1 not 0. The pr(F) is a measure of how much support there is for $H_0$. A smaller pr(F) indicates less support for $H_0$ and thus more support for $H_1$. Significance at the 5 per cent, 1 per cent, 0.1 per cent and 0.01 per cent levels are indicated by *, **, *** and **** respectively.

Logarithm of the odds ratio (LOD score):

$$\text{LOD} = \frac{\text{Probability of data occuring with a QTL}}{\text{Probability of data occuring with no QTL}}$$

LOD of 2 indicates that it is 100 times more likely that a QTL exists in the interval than that there is no QTL.

LOD of 3 indicates that it is 1000 times more likely that a QTL exists in the interval than that there is no QTL.

# Results

# IV. RESULTS

Thirty rice genotypes were selected based on their reaction to iron at varying toxic levels. Their tolerance to iron toxicity was reconfirmed through laboratory experiments. Hybridization of genotypes found to be most susceptible to iron stress was done with the most tolerant genotype. Subsequently the $F_2$ population was generated. Bulk segregant analysis to identify SSR markers linked to leaf bronzing under stress was done with the $F_2$ generation. The results of the investigation are presented in detail under the following headings:

4.1. Experiment 1: Hybridization programme

4.1.1. Parental selection

4.1.2. Hybridization

4.2. Experiment 2: Study of parental polymorphism using molecular markers

4.3. Experiment 3: Raising of $F_1$'s

4.4. Experiment 4: Bulk Segregant Analysis (BSA)

4.4.1. Phenotyping of $F_2$ plants for iron toxicity tolerance

4.4.2. Genotyping parents, susceptible and resistant bulks

4.4.3. Confirmation of putative markers

## 4.1. Experiment 1: Hybridization programme

### 4.1.1. Parental selection

#### 4.1.1.1. Confirmation test-1

Thirty rice genotypes were selected from the KSCSTE project: 'Donor identification for tolerance to iron toxicity in rice (*Oryza sativa* L.)' based on their tolerance reaction to iron stress. These were screened further under varying concentrations of iron (0ppm, 600ppm and 800 ppm) (Confirmation test 1) to confirm their reaction to iron toxicity (Plate 1) as per the method advocated by Shimizu *et al.* (2005).

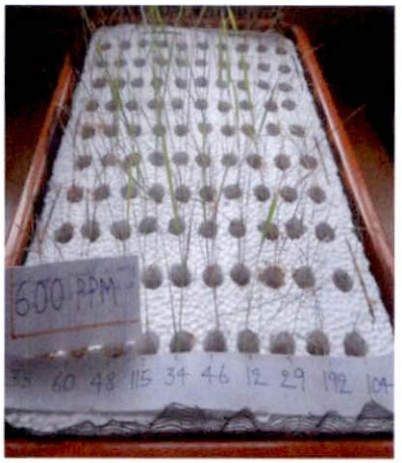
##### 4.1.1.1.1. Analysis of variance

The analysis of variance (Table 8) revealed that there existed high significant differences among the genotypes with respect to leaf bronzing and biomass production under all the three concentrations of iron (0 ppm, 600ppm and 800ppm of Fe).

**Plate 1. Screening of rice genotypes for tolerance to iron (Fe) toxicity - Confirmation test-1**

**Genotypes: 1-10**

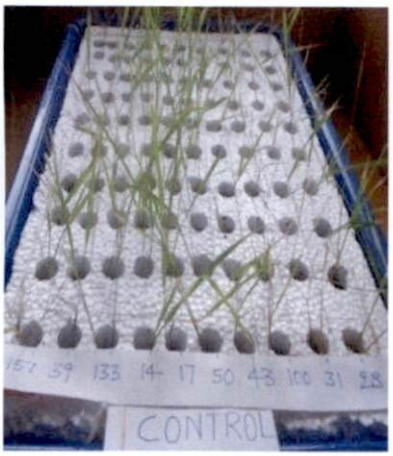

At 0 ppm Fe (Control)




At 600ppm of Fe

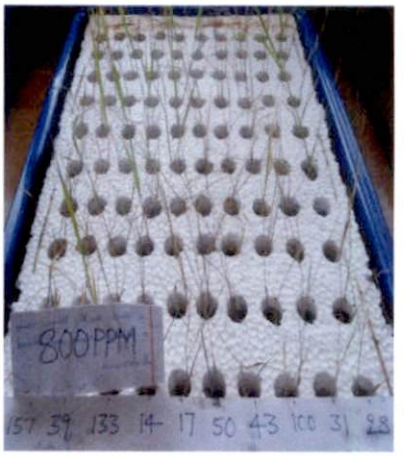

At 800ppm of Fe

**Genotypes: 11-20**




At 0 ppm Fe (Control)


At 600ppm of Fe

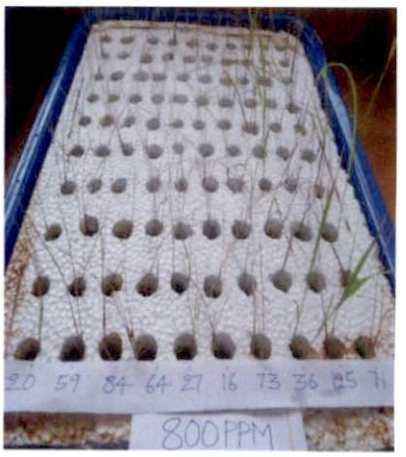



At 800ppm of Fe

**Genotypes: 21-30**


At 0 ppm Fe (Control)


At 600ppm of Fe




At 800ppm of Fe

**Table 8. Analysis of variance for leaf bronzing score and biomass at various iron concentrations (Confirmation test -1)**

| Source of variation | Degrees of freedom | Biomass (g) | | | Leaf bronzing score | | |
|---|---|---|---|---|---|---|---|
| | | 0ppm of Fe | 600ppm of Fe | 800ppm of Fe | 0 ppm of Fe | 600 ppm of Fe | 800 ppm of Fe |
| Treatments | 29 | 0.010** | 0.016** | 0.017** | 7.445** | 5.848** | 2.735** |
| Error | 30 | 0.002 | 0.001 | 0.001 | 0.088 | 0.042 | 0.016 |

*significant at 5% level; **significant at 1% level

**4.1.1.1.2. Variability in performance of genotypes (Confirmation test-1)**

Mean performance of thirty genotypes screened under Confirmation test-1 are given in table 9 and 10.

**4.1.1.1.2.1. Visual scoring for iron-toxicity symptoms**

**a) At 0ppm of Fe**

Leaf bronzing score ranged from 2.0 [T(N)-1 and Abhaya] to 7.8 [ASD (Peringotukurussi)] with a mean of 4.2 under non stressed condition. AM-10-07(2.2) and Cul 8709 (2.3) were found to be on par with Abhaya (2.0), while Supriya (7.7) and PM-706 (7.7) were found to be on par with ASD (Peringotukurussi) (7.8).

b) **At 600 ppm of Fe**

The range of leaf bronzing values was from 4.1 (IVT-33) to 9.0 (PM-706, Cul-8709 and ASD-18) at 600 ppm of Fe and mean is 7.1. Genotype IVT-33 exhibiting the least score of 4.1 was found to be on par with Cul-8723 (4.4) and Cul-18716 (4.5). The highest score of 9.0 was observed in genotypes PM-706, Cul-8709 and ASD-18. Lower normalized score values were observed in genotypes Supriya (1.01), Kargi (1.02) and Karangi (1.04) while higher values were observed in Cul-8709 (3.78), Cul-210-29 (3.70), T(N)-1(3.60) and Abhaya (3.60).

**c) At 800 ppm of Fe**

Leaf bronzing score ranged from 4.7 to 9.0 with a mean of 8.2 under 800 ppm of Fe. Genotype Cul-8723 exhibiting the least score of 4.7 was found to be on par with Tulasi (5.9) and Cul-18716 (5.9). The highest score of 9.0 was observed in genotypes Cul-8709, IR-1552, ASD-18, T(N)-1, CSR 13, Cul-210-29, Cul-3, AM-10-7, ASD (Peringotukurussi), PM-706, PM-717, Karangi and JM-10-31. Lower normalized score

**Table 9. Leaf bronzing scores of rice genotypes at various iron concentrations (Confirmation test -1)**

| Sl. No. | PGC No. | Genotype | Leaf bronzing score | | | Normalized score | |
|---|---|---|---|---|---|---|---|
| | | | 0 ppm of Fe | 600 ppm of Fe | 800ppm of Fe | 600ppm of Fe | 800ppm of Fe |
| 1 | 33 | Cul-18714 | $6.9^{ab}$ | $7.5^{abc}$ | $8.5^{abc}$ | 1.09 | 1.23 |
| 2 | 60 | PM-709 | $2.7^{def}$ | $8.1^{abc}$ | $8.6^{abc}$ | 3.00 | 3.19 |
| 3 | 48 | ASD-16 | $2.6^{def}$ | $7.4^{abc}$ | $8.7^{abc}$ | 2.85 | 3.35 |
| 4 | 115 | IVT-33 | $3.6^{bcd}$ | $4.1^{f}$ | $6.0^{f}$ | 1.14 | 1.67 |
| 5 | 34 | Cul-18716 | $4.0^{bcd}$ | $4.5^{def}$ | $5.9^{f}$ | 1.13 | 1.48 |
| 6 | 46 | Abhaya | $2.0^{gh}$ | $7.2^{bcd}$ | $8.5^{abc}$ | 3.60 | 4.25 |
| 7 | 12 | Kanchana | $5.8^{abc}$ | $6.6^{bcd}$ | $8.4^{abc}$ | 1.14 | 1.45 |
| 8 | 29 | Cul-8759 | $4.4^{abc}$ | $7.1^{bcd}$ | $7.6^{cd}$ | 1.61 | 1.73 |
| 9 | 192 | CSR 13 | $4.9^{abc}$ | $8.0^{abc}$ | $9.0^{a}$ | 1.63 | 1.84 |
| 10 | 104 | Cul-210-29 | $2.3^{fgh}$ | $8.5^{ab}$ | $9.0^{a}$ | 3.70 | 3.91 |
| 11 | 157 | Moncompu-519 | $5.7^{abc}$ | $7.4^{abc}$ | $8.2^{bcd}$ | 1.30 | 1.44 |
| 12 | 39 | Cul-3 | $2.6^{def}$ | $6.9^{bcd}$ | $9.0^{a}$ | 2.65 | 3.46 |
| 13 | 133 | AM-10-7 | $2.2^{fgh}$ | $7.7^{abc}$ | $9.0^{a}$ | 3.50 | 4.09 |
| 14 | 14 | Tulasi | $4.1^{bcd}$ | $5.6^{bcd}$ | $5.9^{f}$ | 1.37 | 1.44 |
| 15 | 17 | IR-36 | $2.4^{efg}$ | $7.5^{abc}$ | $8.6^{abc}$ | 3.13 | 3.58 |
| 16 | 50 | PTB-10 | $4.2^{abc}$ | $7.1^{bcd}$ | $7.8^{bcd}$ | 1.69 | 1.86 |
| 17 | 43 | ASD-18 | $2.8^{cde}$ | $9.0^{a}$ | $9.0^{a}$ | 3.21 | 3.21 |
| 18 | 100 | Cul-90-03 | $2.6^{def}$ | $8.3^{abc}$ | $8.6^{abc}$ | 3.19 | 3.31 |
| 19 | 31 | Cul-8709 | $2.3^{fgh}$ | $9.0^{a}$ | $9.0^{a}$ | 3.78 | 3.91 |
| 20 | 28 | T(N)-1 | $2.0^{gh}$ | $7.2^{bcd}$ | $9.0^{a}$ | 3.60 | 4.50 |
| 21 | 20 | IR-1552 | $2.6^{def}$ | $8.9^{a}$ | $9.0^{a}$ | 3.42 | 3.46 |
| 22 | 84 | ASD(Peringotukurussi) | $7.8^{a}$ | $8.6^{ab}$ | $9.0^{a}$ | 1.10 | 1.15 |
| 23 | 59 | PM-706 | $7.7^{a}$ | $9.0^{a}$ | $9.0^{a}$ | 1.17 | 1.17 |
| 24 | 64 | PM-717 | $5.0^{abc}$ | $6.4^{bcd}$ | $9.0^{a}$ | 1.28 | 1.80 |
| 25 | 27 | Cul-8755 | $4.5^{abc}$ | $4.9^{cde}$ | $8.5^{abc}$ | 1.09 | 1.89 |
| 26 | 16 | Supriya | $7.7^{a}$ | $7.8^{abc}$ | $8.7^{abc}$ | 1.01 | 1.13 |
| 27 | 73 | Karangi | $4.8^{abc}$ | $5.0^{bcd}$ | $9.0^{a}$ | 1.04 | 1.88 |
| 28 | 36 | Cul-8723 | $3.3^{cde}$ | $4.4^{ef}$ | $4.7^{g}$ | 1.33 | 1.42 |
| 29 | 125 | JM-10-31 | $5.8^{abc}$ | $6.7^{bcd}$ | $9.0^{a}$ | 1.16 | 1.55 |
| 30 | 71 | Kargi | $5.4^{abc}$ | $5.5^{bcd}$ | $6.5^{ef}$ | 1.02 | 1.20 |
| | **Mean** | | **4.2** | **7.1** | **8.2** | | |
| | **CD(0.01)** | | **0.81** | **0.57** | **0.35** | | |
| | **CD(0.05)** | | **0.61** | **0.42** | **0.26** | | |

**Table 10. Biomass of rice genotypes at various iron concentrations (Confirmation test -1)**

| Sl.No. | PGC No. | Genotype | Biomass (g) | | | Reduction in biomass compared to 0 ppm of Fe (%) | |
|---|---|---|---|---|---|---|---|
| | | | 0 ppm of Fe | 600 ppm of Fe | 800 ppm of Fe | 600 ppm of Fe | 800 ppm of Fe |
| 1 | 33 | Cul-18714 | $0.59^{ij}$ | $0.57^{jkl}$ | $0.49^{klm}$ | 3.39 | 16.95 |
| 2 | 60 | PM-709 | $0.76^{bcd}$ | $0.59^{jkl}$ | $0.53^{ijk}$ | 22.37 | 30.26 |
| 3 | 48 | ASD-16 | $0.73^{efg}$ | $0.58^{jkl}$ | $0.54^{hij}$ | 20.55 | 26.03 |
| 4 | 115 | IVT-33 | $0.74^{def}$ | $0.71^{ijk}$ | $0.64^{cde}$ | 4.05 | 13.51 |
| 5 | 34 | Cul-18716 | $0.88^{a}$ | $0.84^{ab}$ | $0.73^{ab}$ | 4.76 | 13.10 |
| 6 | 46 | Abhaya | $0.76^{bcd}$ | $0.62^{hij}$ | $0.53^{ijk}$ | 18.42 | 30.26 |
| 7 | 12 | Kanchana | $0.64^{ij}$ | $0.59^{jkl}$ | $0.48^{klm}$ | 7.81 | 25.00 |
| 8 | 29 | Cul-8759 | $0.74^{def}$ | $0.73^{cde}$ | $0.64^{cde}$ | 1.35 | 13.51 |
| 9 | 192 | CSR 13 | $0.55^{j}$ | $0.53^{kl}$ | $0.45^{lm}$ | 3.64 | 18.18 |
| 10 | 104 | Cul-210-29 | $0.84^{abc}$ | $0.60^{ijk}$ | $0.58^{fgh}$ | 28.57 | 30.95 |
| 11 | 157 | Moncompu-519 | $0.72^{fgh}$ | $0.68^{efg}$ | $0.50^{jkl}$ | 5.56 | 30.56 |
| 12 | 39 | Cul-3 | $0.83^{abc}$ | $0.67^{efg}$ | $0.67^{bcd}$ | 19.28 | 19.28 |
| 13 | 133 | AM-10-7 | $0.75^{cde}$ | $0.54^{jkl}$ | $0.55^{ghi}$ | 28.00 | 26.67 |
| 14 | 14 | Tulasi | $0.87^{ab}$ | $0.86^{a}$ | $0.75^{a}$ | 1.15 | 13.79 |
| 15 | 17 | IR-36 | $0.85^{abc}$ | $0.69^{def}$ | $0.64^{cde}$ | 18.82 | 24.71 |
| 16 | 50 | PTB-10 | $0.73^{efg}$ | $0.67^{efg}$ | $0.62^{def}$ | 8.22 | 15.07 |
| 17 | 43 | ASD-18 | $0.78^{bcd}$ | $0.63^{ghi}$ | $0.60^{efg}$ | 19.23 | 23.08 |
| 18 | 100 | Cul-90-03 | $0.65^{hij}$ | $0.52^{l}$ | $0.40^{m}$ | 20.00 | 38.46 |
| 19 | 31 | Cul-8709 | $0.84^{abc}$ | $0.59^{jkl}$ | $0.57^{fgh}$ | 29.76 | 32.14 |
| 20 | 28 | T(N)-1 | $0.76^{bcd}$ | $0.63^{ghi}$ | $0.62^{def}$ | 17.11 | 18.42 |
| 21 | 20 | IR-1552 | $0.76^{cde}$ | $0.63^{ghi}$ | $0.53^{ijk}$ | 17.11 | 30.26 |
| 22 | 84 | ASD(Peringotuk urussi) | $0.75^{cde}$ | $0.64^{cde}$ | $0.56^{ghi}$ | 14.67 | 25.33 |
| 23 | 59 | PM-706 | $0.87^{ab}$ | $0.76^{bcd}$ | $0.63^{def}$ | 12.64 | 27.59 |
| 24 | 64 | PM-717 | $0.81^{bcd}$ | $0.77^{abc}$ | $0.65^{bcd}$ | 4.94 | 19.75 |
| 25 | 27 | Cul-8755 | $0.71^{ghi}$ | $0.67^{efg}$ | $0.53^{ijk}$ | 5.63 | 25.35 |
| 26 | 16 | Supriya | $0.75^{def}$ | $0.70^{def}$ | $0.64^{cde}$ | 6.67 | 14.67 |
| 27 | 73 | Karangi | $0.86^{abc}$ | $0.81^{abc}$ | $0.65^{bcd}$ | 5.81 | 24.42 |
| 28 | 36 | Cul-8723 | $0.80^{bcd}$ | $0.75^{bcd}$ | $0.72^{abc}$ | 6.25 | 10.00 |
| 29 | 125 | JM-10-31 | $0.74^{def}$ | $0.69^{def}$ | $0.52^{jkl}$ | 6.76 | 29.73 |
| 30 | 71 | Kargi | $0.86^{abc}$ | $0.74^{cde}$ | $0.72^{abc}$ | 13.95 | 16.27 |
| | **Mean** | | **0.76** | **0.67** | **0.59** | **12.55** | **22.78** |
| | **CD(0.01)** | | **0.110** | **0.085** | **0.093** | | |
| | **CD(0.05)** | | **0.082** | **0.063** | **0.069** | | |

values were observed in genotypes Supriya (1.13), ASD (Peringotukurussi) (1.15) and PM-706 (1.17) while the higher values were observed in T(N)-1 (4.50), Abhaya (4.25) and AM-10-7 (4.09).

**4.1.1.1.2.2. Biomass (g)**

**a) At 0ppm of Fe**

Biomass content ranged from 0.55g (CSR-13) to 0.88g (Cul-18716) with a mean of 0.76g under non stressed condition. Cul-18714 (0.59g) and Kanchana (0.64g) were found to be on par with CSR-13 (0.55g), while Tulasi (0.87g), PM-706 (0.87g), Karangi (0.86g) and Kargi (0.86g) were found to be on par with Cul-18716 (0.88g).

**b) At 600 ppm of Fe**

The range of biomass values was from 0.52g (Cul-90-03) to 0.86g (Tulasi) at 600 ppm of Fe and mean is 0.67g. Genotype Cul-90-03 exhibiting the least biomass of 0.52g was found to be on par with CSR 13 (0.53g) and AM-10-7 (0.54g). Genotype Tulasi exhibiting the highest biomass of 0.86g was found to be on par with Cul-18716 (0.84g) and Karangi (0.81g). Lower reduction of biomass in percentage over control at 600 ppm of Fe were observed in genotypes Tulasi (1.15%), Cul-8759 (1.35%) and Cul-18714 (3.39%) while the higher reduction of biomass in percentage over control were observed in Cul-8709 (29.76%), Cul-210-29 (28.57%) and AM-10-7 (28.00%).

**c) At 800 ppm of Fe**

Biomass content ranged from 0.40g (Cul-90-03) to 0.75g (Tulasi) with a mean of 0.59g under 800ppm of Fe. Genotype Cul-90-03 exhibiting the least biomass of 0.40g was found to be on par with CSR 13 (0.45g) and Kanchana (0.48g). Genotype Tulasi exhibiting the highest biomass of 0.75g was found to be on par with Cul-18716 (0.73g), Kargi (0.72g) and Cul-8723 (0.72g). Lower reduction of biomass in percentage over control at 800 ppm of Fe were observed in genotypes Cul-8723 (10.00%), Cul-18716 (13.10%) and IVT-33 (13.51%) while the higher reduction of biomass in percentage over control were observed in Cul-90-03 (38.46%), Cul-8709 (32.14%) and Cul-210-29 (30.95%).

Susceptible rice genotypes among these 30 rice genotypes were selected based on results of screening of these genotypes at 600ppm of Fe treatment. 12 rice genotypes are

showed as higher leaf bronzing score and higher normalized score values at 600 ppm of Fe treatment. These rice genotypes are selected as susceptible rice genotypes. Tolerant or resistant rice genotypes among these 30 rice genotypes were selected based on results of screening of these genotypes at 800ppm of Fe treatment. Five rice genotypes are showed lower leaf bronzing and lower normalized score values at 800 ppm of Fe treatment of 30 rice genotypes. These genotypes were selected as tolerant rice genotypes. Hence, from these 30 rice genotypes, 12 susceptible and 5 tolerant rice genotypes are selected. The 17 rice genotypes were screened (Confirmation test-2) for confirmation of most tolerant and most susceptible rice genotypes to reactions of iron toxicity.

#### 4.1.1.2. Confirmation test-2

Based on the performance of the thirty rice genotypes under Confirmation test-1, seventeen genotypes were selected for reconfirmation of their response to iron stress (0 ppm, 600ppm and 800ppm of Fe). Twelve rice genotypes that recorded high leaf bronzing score (9) and higher normalized score values at 600 ppm of Fe treatment were selected as genotypes susceptible to iron stress. Five genotypes that scored the least normalized leaf bronzing score at 800ppm of Fe treatment among the 30 genotypes in confirmation test-1 were selected for screening in Confirmation test-2 (Plate 2).

##### 4.1.1.2.1. Analysis of variance (Confirmation test-2)

The analysis of variance (Table 11) revealed that there existed high significant differences among the genotypes with respect to leaf bronzing and biomass produced under all the three concentrations of iron (0 ppm, 600ppm and 800ppm of Fe).

**Table 11. Analysis of variance for leaf bronzing scores and biomass at various iron concentrations (Confirmation test -2)**

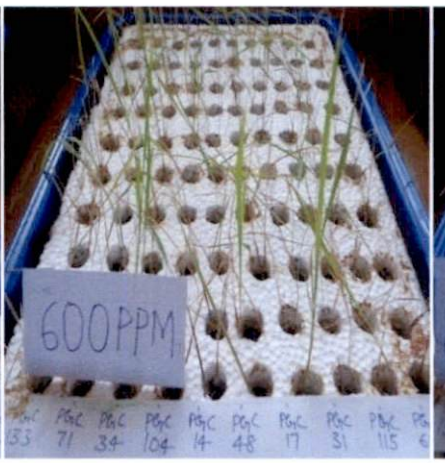
| Source of variation | df | Biomass | | | Leaf bronzing score | | |
|---|---|---|---|---|---|---|---|
| | | 0 ppm of Fe | 600 ppm of Fe | 800 ppm of Fe | 0 ppm of Fe | 600 ppm of Fe | 800ppm of Fe |
| Treatments | 16 | 0.012** | 0.012** | 0.010** | 4.089** | 4.856** | 3.307** |
| Error | 17 | 0.001 | 0.001 | 0.001 | 0.074 | 0.078 | 0.049 |

*significant at 5% level; **significant at 1% level

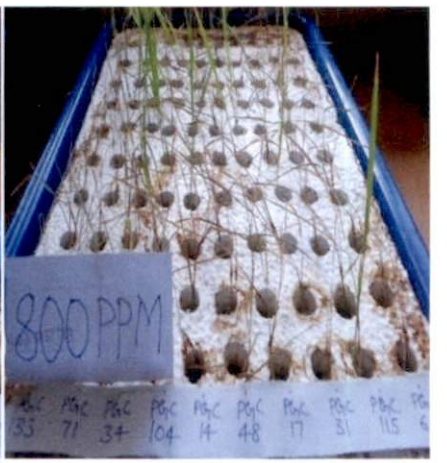
**Plate 2. Screening of rice genotypes for tolerance to iron (Fe) toxicity - Confirmation test-2**

**Genotypes: 1-10**


At 0 ppm Fe (Control)




At 600ppm of Fe




At 800ppm of Fe

**Genotypes: 11-17**


At 0 ppm Fe (Control)


At 600ppm of Fe


At 800ppm of Fe

#### 4.1.1.2.2. Variability in performance of genotypes (Confirmation test-2)

Mean performance of seventeen genotypes screened under Confirmation test-2 are given in table 12 and 13, and detailed below.

##### 4.1.1.2.2.1. Visual scoring for iron-toxicity symptoms

**a) At 0ppm of Fe**

Mean value of leaf bronzing score at 0ppm of Fe was 3.4. Values ranged between 1.2 (ASD-18) and 5.9 (PM-709). Abhaya (1.3) and Cul-8723 (2.5) were found to be on par with ASD-18 (1.2) while Kargi (5.5) and Tulasi (4.6) were found to be on par with PM-709 (5.9).

**b) At 600 ppm of Fe**

The range of leaf bronzing values varied from 3.9 (TN-1) to 9.0 (Cul-8709) with a mean of 6.3 at 600 ppm of Fe. Genotype TN-1 exhibiting the least score of 3.9 was found to be on par with Cul-8723 (4.5) and ASD-18 (4.6). Genotype Cul-8709 exhibiting the highest score of 9.0 was found to be on par with IR-1552 (8.4) and Cul-90-03 (7.8). Lower normalized score values were observed in genotypes Tulasi (1.13), Kargi (1.25) and PM-709 (1.26) while the higher values were observed in Abhaya (4.50), ASD-18 (3.79) and Cul-8709 (3.60).

**c) At 800 ppm of Fe**

Leaf bronzing score ranged from 5.4 to 9.0 with a mean of 8.1 under 800 ppm of Fe. Genotype Tulasi exhibiting the least score of 5.4 was found to be on par with Cul-18716 (5.7) and Cul-210-29 (7.4). The highest score of 9.0 was observed in genotypes Cul-8709, Cul-90-03, Kargi and ASD-18. Lower normalized score values were observed in genotypes Tulasi (1.18), Cul-210-29 (1.43) and PM-709 (1.49) while the higher values were observed in ASD-18 (7.50), Abhaya (6.65) and Cul-8709 (3.60).

##### 4.1.1.2.2.2. Biomass (g)

**a) At 0ppm of Fe**

Biomass content ranged from 0.63g (PM-709 and AM-10-7) to 0.88g (Cul-18716) with a mean of 0.76g under non stressed condition. Kargi (0.68g) and Cul-90-03

**Table 12. Leaf bronzing scores of rice genotypes at various iron concentrations (Confirmation test -2)**

| Sl.No. | PGC No. | Genotype | Leaf bronzing score | | | Normalized score | |
|---|---|---|---|---|---|---|---|
| | | | 0 ppm of Fe | 600 ppm of Fe | 800 ppm of Fe | 600 ppm of Fe | 800 ppm of Fe |
| 1 | 133 | AM-10-7 | $4.0^{d}$ | $7.6^{c}$ | $7.9^{c}$ | 1.90 | 1.98 |
| 2 | 71 | Kargi | $5.5^{ab}$ | $6.9^{de}$ | $9.0^{a}$ | 1.25 | 1.64 |
| 3 | 34 | Cul-18716 | $3.7^{ef}$ | $4.7^{hi}$ | $5.7^{e}$ | 1.27 | 1.54 |
| 4 | 104 | Cul-210-29 | $4.2^{cd}$ | $6.0^{g}$ | $7.4^{d}$ | 1.78 | 1.43 |
| 5 | 14 | Tulasi | $4.6^{c}$ | $5.2^{h}$ | $5.4^{e}$ | 1.13 | 1.18 |
| 6 | 48 | ASD-16 | $3.9^{def}$ | $6.2^{fg}$ | $8.5^{b}$ | 1.61 | 2.19 |
| 7 | 17 | IR-36 | $3.4^{fg}$ | $4.7^{hi}$ | $7.6^{cd}$ | 1.39 | 2.27 |
| 8 | 31 | Cul-8709 | $2.5^{hi}$ | $9.0^{a}$ | $9.0^{a}$ | 3.60 | 3.60 |
| 9 | 115 | IVT-33 | $3.7^{ef}$ | $5.9^{g}$ | $7.5^{cd}$ | 1.60 | 2.04 |
| 10 | 60 | PM-709 | $5.9^{a}$ | $7.5^{cd}$ | $8.8^{ab}$ | 1.26 | 1.49 |
| 11 | 39 | Cul-3 | $3.9^{def}$ | $6.5^{ef}$ | $8.8^{ab}$ | 1.67 | 2.26 |
| 12 | 28 | T(N)-1 | $2.7^{h}$ | $3.9^{j}$ | $8.5^{b}$ | 1.47 | 3.21 |
| 13 | 46 | Abhaya | $1.3^{i}$ | $5.9^{g}$ | $8.7^{ab}$ | 4.50 | 6.65 |
| 14 | 43 | ASD-18 | $1.2^{i}$ | $4.6^{i}$ | $9.0^{a}$ | 3.79 | 7.50 |
| 15 | 36 | Cul-8723 | $2.5^{hi}$ | $4.5^{i}$ | $8.6^{ab}$ | 1.82 | 3.49 |
| 16 | 20 | IR-1552 | $2.9^{gh}$ | $8.4^{ab}$ | $8.8^{ab}$ | 2.89 | 3.03 |
| 17 | 100 | Cul-90-03 | $2.7^{h}$ | $7.8^{bc}$ | $9.0^{a}$ | 2.89 | 3.33 |
| | **Mean** | | **3.4** | **6.3** | **8.1** | | |
| | **CD(0.01)** | | **0.771** | **0.793** | **0.630** | | |
| | **CD(0.05)** | | **0.566** | **0.582** | **0.462** | | |

**Table 13. Biomass of rice genotypes at various iron concentrations (Confirmation test -2)**

| Sl. No. | PGC No. | Genotype | Biomass (g) | | | Reduction in biomass compared to 0 ppm of Fe (%) | |
|---|---|---|---|---|---|---|---|
| | | | 0 ppm of Fe | 600 ppm of Fe | 800 ppm of Fe | 600 ppm of Fe | 800 ppm of Fe |
| 1 | 133 | AM-10-7 | $0.63^{e}$ | $0.57^{hi}$ | $0.55^{ef}$ | 9.52 | 12.70 |
| 2 | 71 | Kargi | $0.68^{de}$ | $0.65^{efg}$ | $0.56^{ef}$ | 3.70 | 17.04 |
| 3 | 34 | Cul-18716 | $0.88^{a}$ | $0.82^{a}$ | $0.81^{a}$ | 6.86 | 8.00 |
| 4 | 104 | Cul-210-29 | $0.73^{cd}$ | $0.68^{cde}$ | $0.60^{cde}$ | 6.90 | 17.24 |
| 5 | 14 | Tulasi | $0.78^{bc}$ | $0.77^{ab}$ | $0.75^{ab}$ | 3.77 | 6.29 |
| 6 | 48 | ASD-16 | $0.72^{cde}$ | $0.64^{efg}$ | $0.63^{cde}$ | 10.49 | 12.59 |
| 7 | 17 | IR-36 | $0.83^{abc}$ | $0.77^{ab}$ | $0.65^{cd}$ | 7.27 | 21.82 |
| 8 | 31 | Cul-8709 | $0.86^{ab}$ | $0.62^{fgh}$ | $0.60^{cde}$ | 27.49 | 29.82 |
| 9 | 115 | IVT-33 | $0.72^{cde}$ | $0.66^{def}$ | $0.62^{cde}$ | 8.33 | 14.58 |
| 10 | 60 | PM-709 | $0.63^{e}$ | $0.59^{ghi}$ | $0.55^{ef}$ | 5.60 | 12.00 |
| 11 | 39 | Cul-3 | $0.83^{abc}$ | $0.75^{abc}$ | $0.67^{bc}$ | 9.70 | 19.39 |
| 12 | 28 | T(N)-1 | $0.83^{abc}$ | $0.76^{ab}$ | $0.66^{c}$ | 9.04 | 21.08 |
| 13 | 46 | Abhaya | $0.69^{de}$ | $0.61^{fgh}$ | $0.57^{def}$ | 12.32 | 17.39 |
| 14 | 43 | ASD-18 | $0.87^{ab}$ | $0.75^{abc}$ | $0.67^{bc}$ | 13.79 | 22.99 |
| 15 | 36 | Cul-8723 | $0.78^{bc}$ | $0.73^{bcd}$ | $0.59^{def}$ | 7.05 | 24.36 |
| 16 | 20 | IR-1552 | $0.86^{ab}$ | $0.70^{bcd}$ | $0.59^{def}$ | 18.60 | 30.99 |
| 17 | 100 | Cul-90-03 | $0.68^{de}$ | $0.55^{i}$ | $0.53^{f}$ | 19.85 | 22.06 |
| | **Mean** | | **0.76** | **0.68** | **0.62** | **10.60** | **18.26** |
| | **CD(0.01)** | | **0.088** | **0.066** | **0.078** | | |
| | **CD(0.05)** | | **0.065** | **0.048** | **0.057** | | |

(0.68g) were found to be on par with PM-709 and AM-10-7 (0.63g), while ASD-18 (0.87g), IR1552 (0.86g) and Cul-8709 (0.86g) were found to be on par with Cul-18716 (0.88g).

**b) At 600 ppm of Fe**

The range of biomass values was from 0.55g (Cul-90-03) to 0.82g (Cul-18716) at 600 ppm of Fe and mean is 0.68g. Genotype Cul-90-03 exhibiting the least biomass of 0.55g was found to be on par with AM-10-7 (0.57g) and PM-709 (0.59g). Genotype Cul-18716 exhibiting the highest biomass of 0.82g was found to be on par with Tulasi (0.77g) and IR-36 (0.77g). Lower per cent reduction of biomass over control at 600 ppm of Fe were observed in genotypes Kargi (3.70%), Tulasi (3.77%) and PM-709 (5.60%) while the higher reduction of biomass in percentage over control were observed in Cul-8709 (27.49%), Cul-90-03 (19.85%) and IR-1552 (18.60%).

**c) At 800 ppm of Fe**

Biomass content ranged from 0.53g (Cul-90-03) to 0.81g (Cul-18716) with a mean of 0.62g under 800ppm of Fe. Genotype Cul-90-03 exhibiting the least biomass of 0.53g was found to be on par with PM-709 (0.55g) and AM-10-7 (0.55g). Genotype Cul-18716 exhibiting the highest biomass of 0.81g was found to be on par with Tulasi (0.75g), ASD-18 (0.67g) and Cul-3 (0.67g). Lower reduction of biomass over control at 800 ppm of Fe were observed in genotypes Tulasi (6.29%), Cul-18716 (8.00%) and PM-709 (12.00%) while higher reduction of biomass was observed in IR-1552 (30.99%), Cul-8709 (29.82%) and Cul-8723 (24.36%).

### 4.1.2. Hybridization

Based on the reaction of genotypes to stress at 600ppm and 800ppm of iron, two most tolerant rice genotypes [PGC 14 (Tulasi) and PGC 34 (Cul-18716)] were identified. Similarly, three rice genotypes that were found most susceptible to iron stress were selected are PGC 31 (Cul-8709), PGC 20 (IR-1552) and PGC 100 (Cul-90-03). The tolerant genotypes were hybridized separately with each of the susceptible genotypes resulting in 6 cross-combinations. The information on seed set is detailed in table 14.

**Table 14. Details of seed set in cross- combinations**

| Female parent | Male parent | Cross | Number of $F_1$ seeds obtained |
|---|---|---|---|
| PGC 31 (Cul-8709) | PGC 14 (Tulasi) | Cul-8709/ Tulasi | 81 |
| PGC 20 (IR-1552) | PGC 14 (Tulasi) | IR-1552/ Tulasi | 92 |
| PGC 100 (Cul-90-03) | PGC 14 (Tulasi) | Cul-90-03/ Tulasi | 93 |
| PGC 31 (Cul-8709) | PGC 34 (Cul-18716) | Cul-8709/ Cul-18716 | 64 |
| PGC 20 (IR-1552) | PGC 34 (Cul-18716) | IR-1552/ Cul-18716 | 78 |
| PGC 100 (Cul-90-03) | PGC 34 (Cul-18716) | Cul-90-03/ Cul-18716 | 95 |

### 4.2. Experiment 2: Study of parental polymorphism using molecular markers

Polymorphism at molecular level between the genotypes PGC (Cul-8709) and PGC (Tulasi) the most susceptible and resistant parents respectively to iron toxicity were ascertained by genotyping them with 338 simple sequence repeats (SSR) markers listed in appendix I under section 3.2 of Chapter 3. Quantity and quality parameters of parental DNA are presented in table 15. The detailed outcome of the study is enumerated in table 16 and Plate no 3 to 9. List of 37 polymorphic markers presented in table 17.

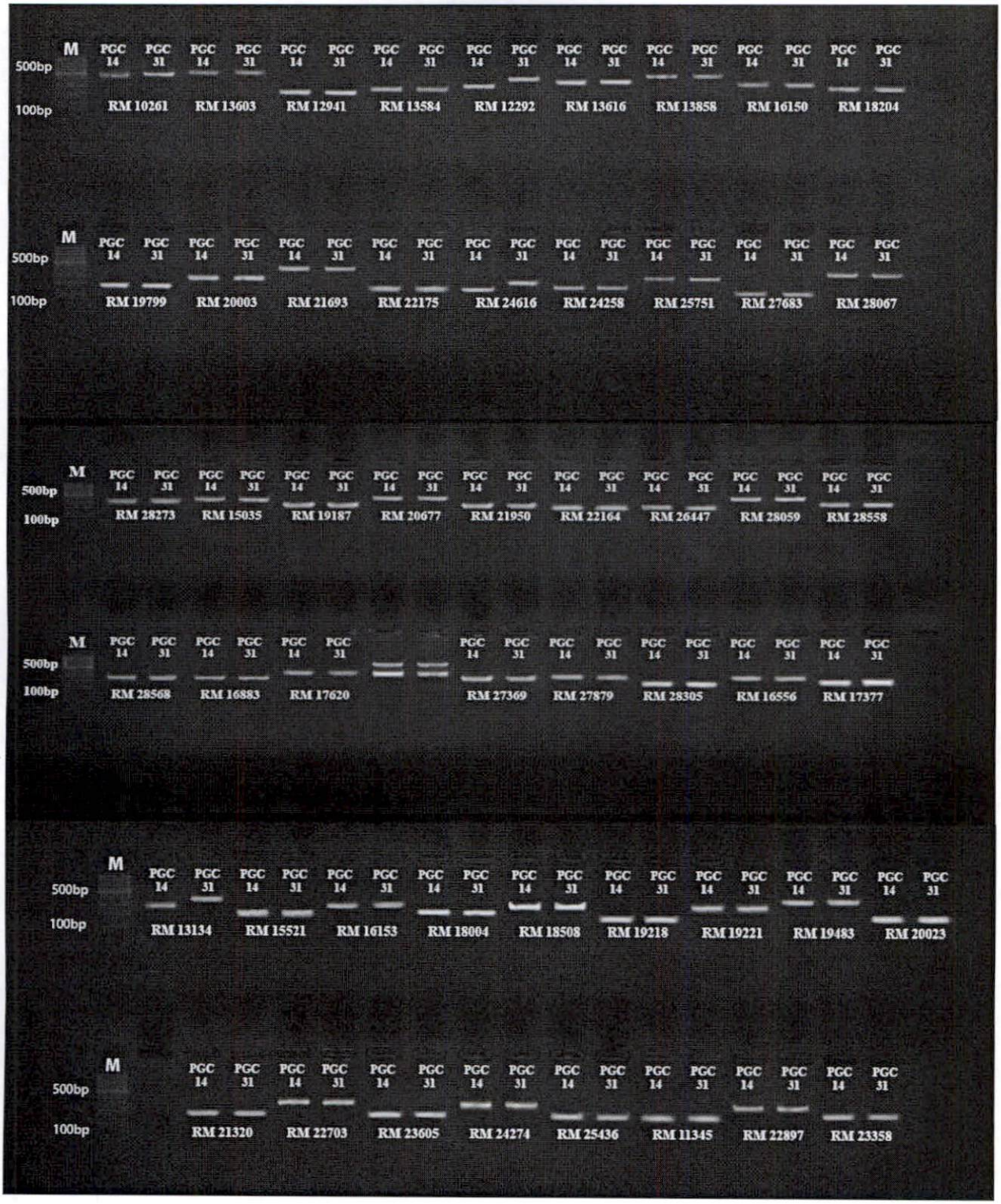
**Table 15. Quantity and quality parameters of parental DNA (Tulasi and Cul-8709)**

| Genotype | Quantity(ng/µl) | Quality (A260/A280) |
|---|---|---|
| PGC 14 (Tulasi) | 2170.52 | 1.87 |
| PGC 31 (Cul 8709) | 3940.19 | 1.82 |

The 338 rice microsatellites markers selected for the parental polymorphism study comprised of 36 markers each distributed on each chromosome of 1 and 2 while 28, each were distributed on the 3rd and 4th linkage group, Twenty nine markers each were linked to chromosome number 5 and 6. Others included 25, 27, 17, 21, 30 and 32 numbers located on Chromosome 7, 8, 9, 10, 11 and 12 respectively.

Out of 338 rice microsatellite markers, 37 were found polymorphic between the resistant parent PGC 14 (Tulasi) and susceptible parent PGC 31 (Cul-8709). Among the 37 polymorphic rice microsatellite markers, one marker was located on chromosome 7, two each were located on chromosome 3, 4, 6, three each were located on chromosome 1, 5, 8, 11 and 12, five each on on chromosome 2, 9 and 10. Single amplicon was observed in all cases. Wide variation was observed in size of amplicons among different RM markers. Amplicon size varied between 65bp (RM439) and 683bp (RM411).

**Plate 3. Parental polymorphism study using microsatellite markers (I)**

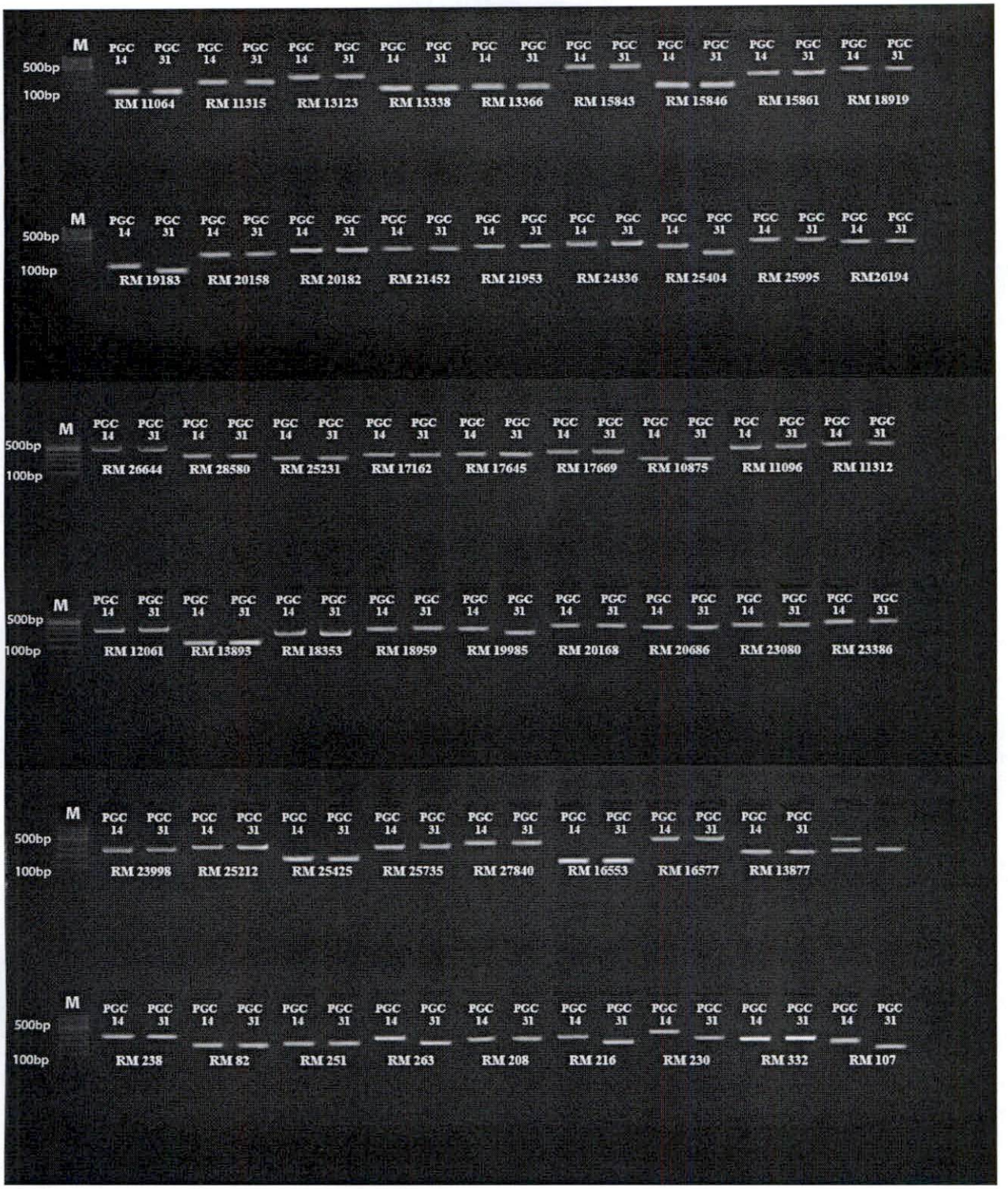



M - Ladder (1kb)

PGC 14 (Tulasi) – Resistant parent to Fe toxicity

PGC 31 (CUL 8709) – Susceptible parent to iron toxicity

**Plate 4. Parental polymorphism study using microsatellite markers (II)**

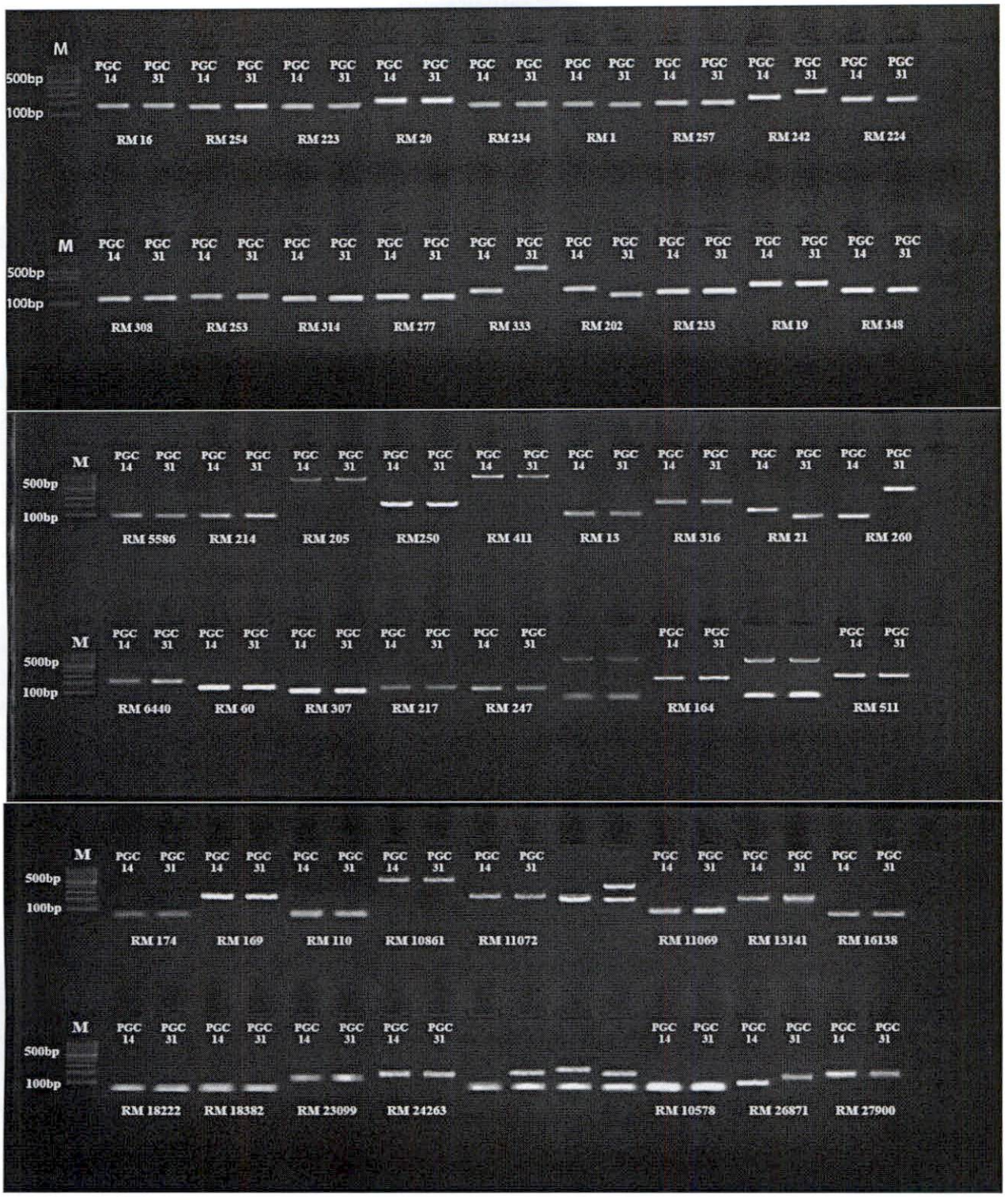



M - Ladder (1kb)

PGC 14 (Tulasi) – Resistant parent to Fe toxicity

PGC 31 (CUL 8709) – Susceptible parent to iron toxicity

**Plate 5. Parental polymorphism study using microsatellite markers (III)**

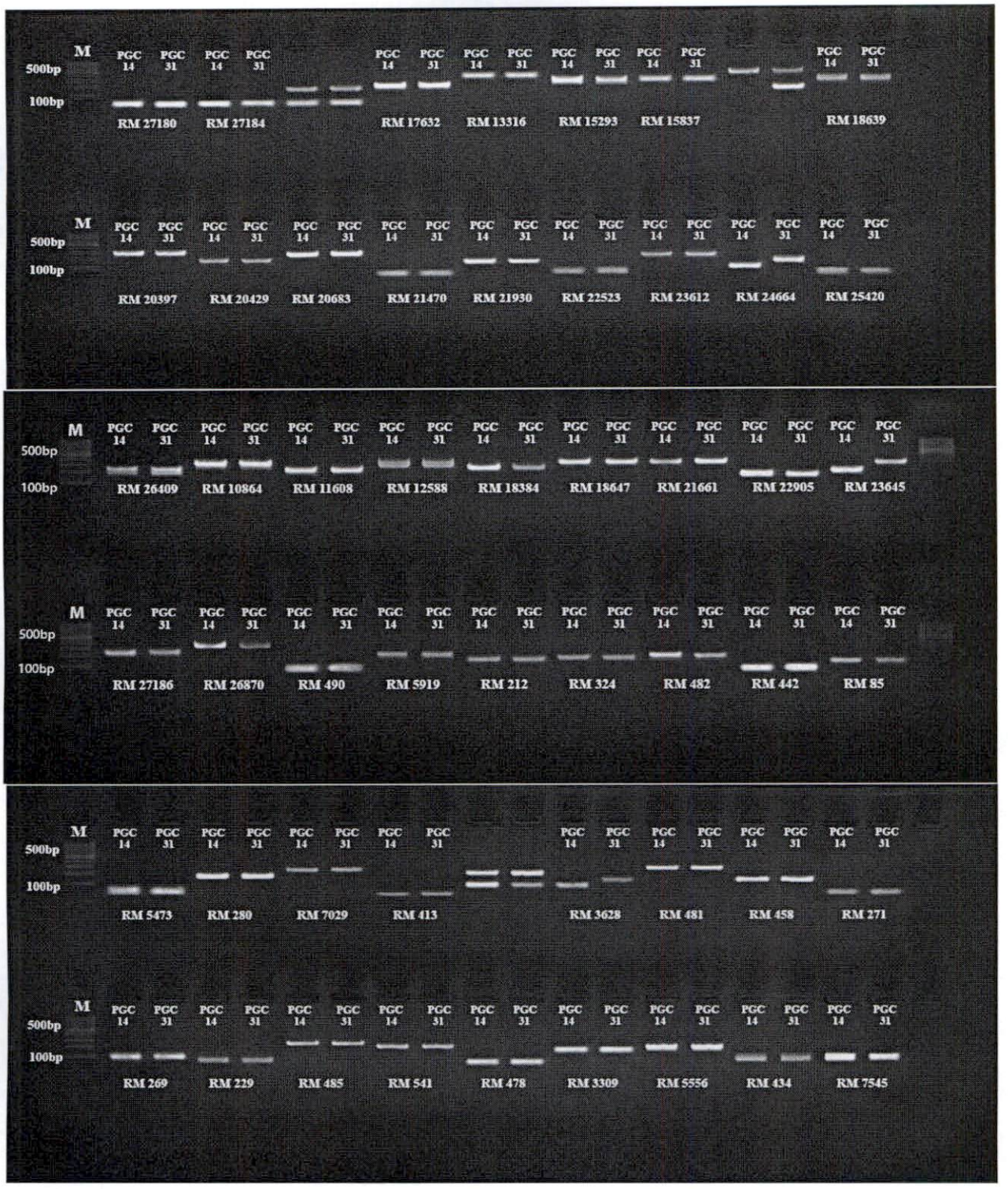



M - Ladder (1kb)

PGC 14 (Tulasi) – Resistant parent to Fe toxicity

PGC 31 (CUL 8709) – Susceptible parent to iron toxicity

**Plate 6. Parental polymorphism study using microsatellite markers (IV)**

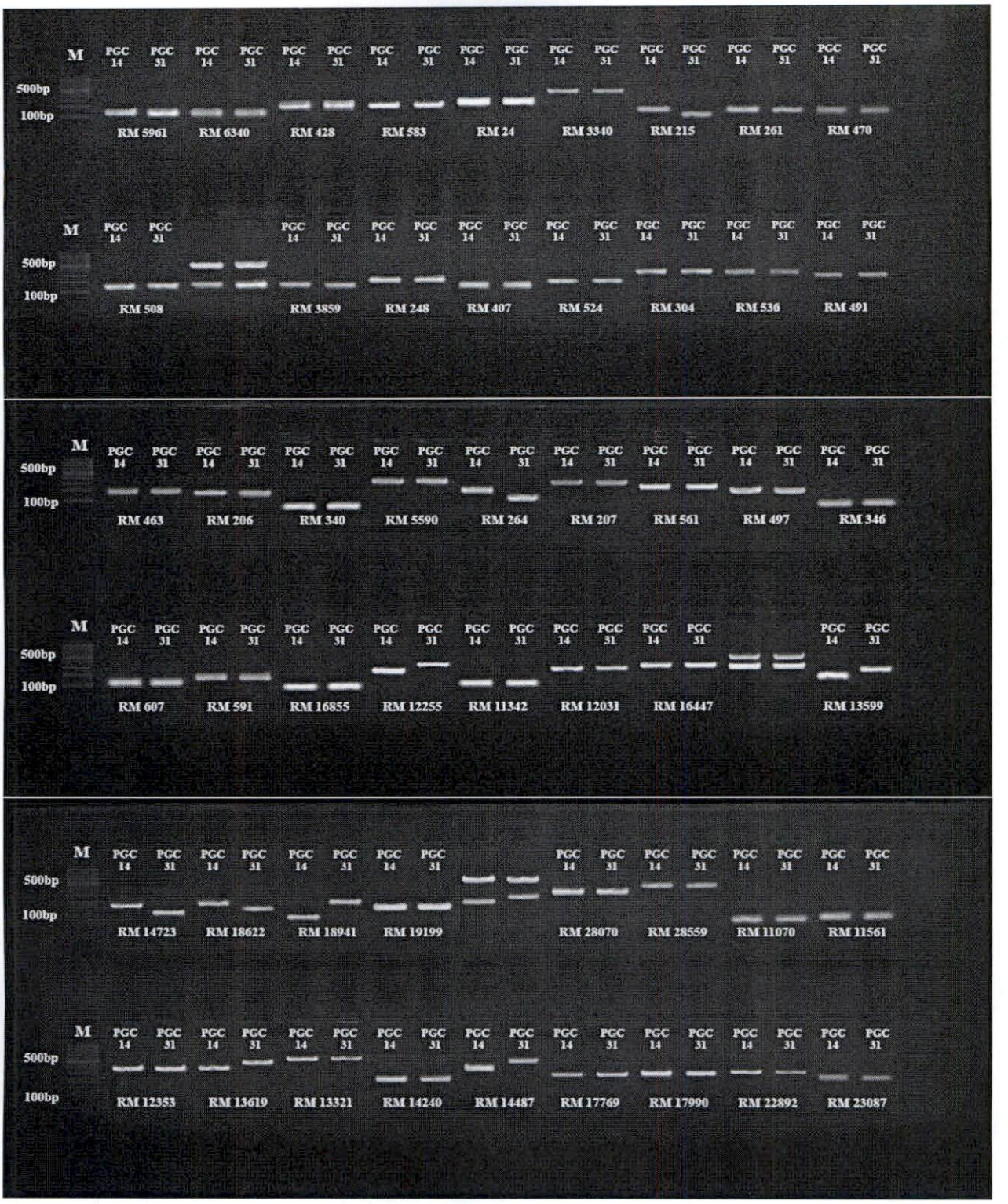



M - Ladder (1kb)

PGC 14 (Tulasi) – Resistant parent to Fe toxicity

PGC 31 (CUL 8709) – Susceptible parent to iron toxicity

**Plate 7. Parental polymorphism study using microsatellite markers (V)**

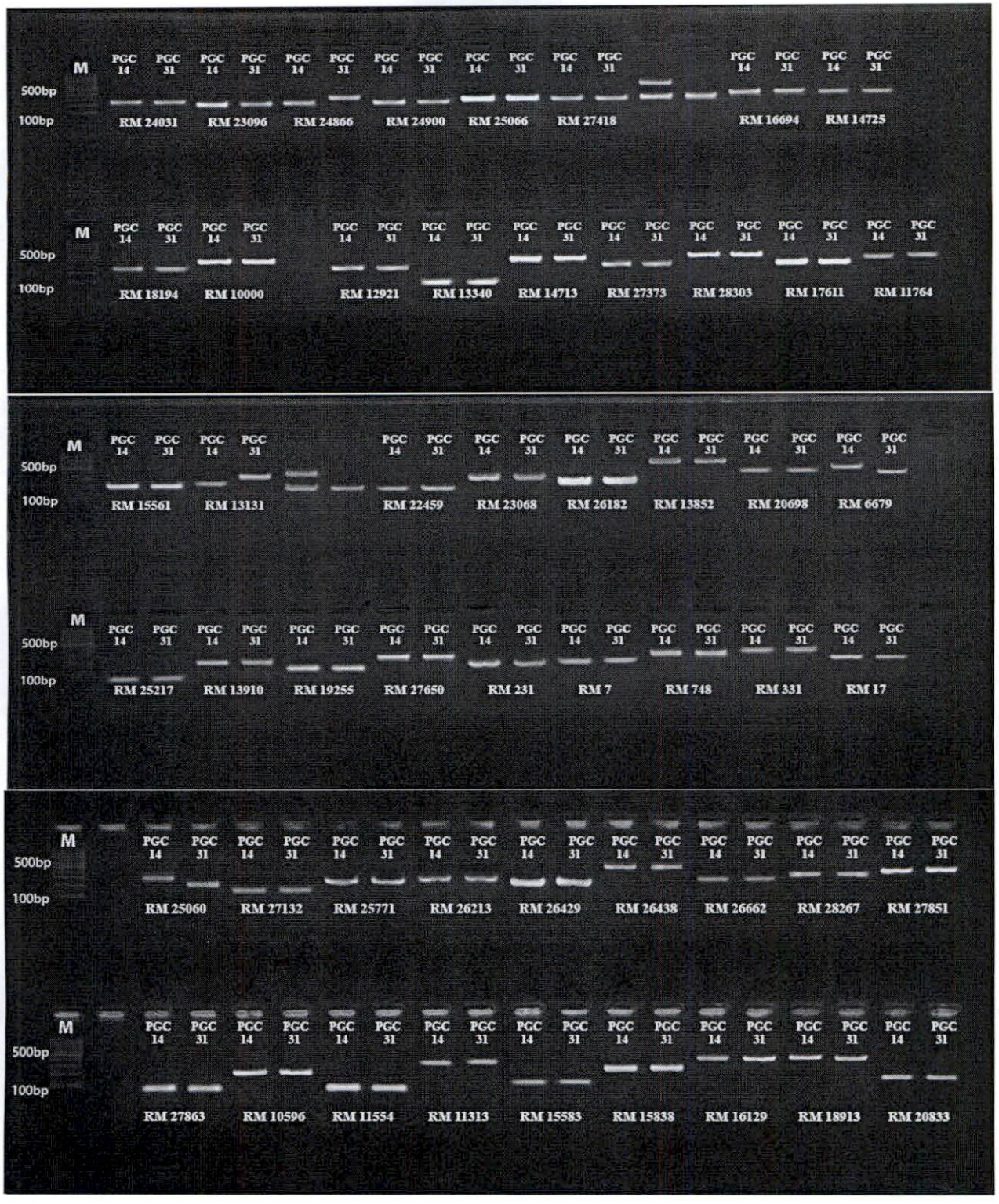



M - Ladder (1kb)

PGC 14 (Tulasi) – Resistant parent to Fe toxicity

PGC 31 (CUL 8709) – Susceptible parent to iron toxicity

**Plate 8. Parental polymorphism study using microsatellite markers (VI)**

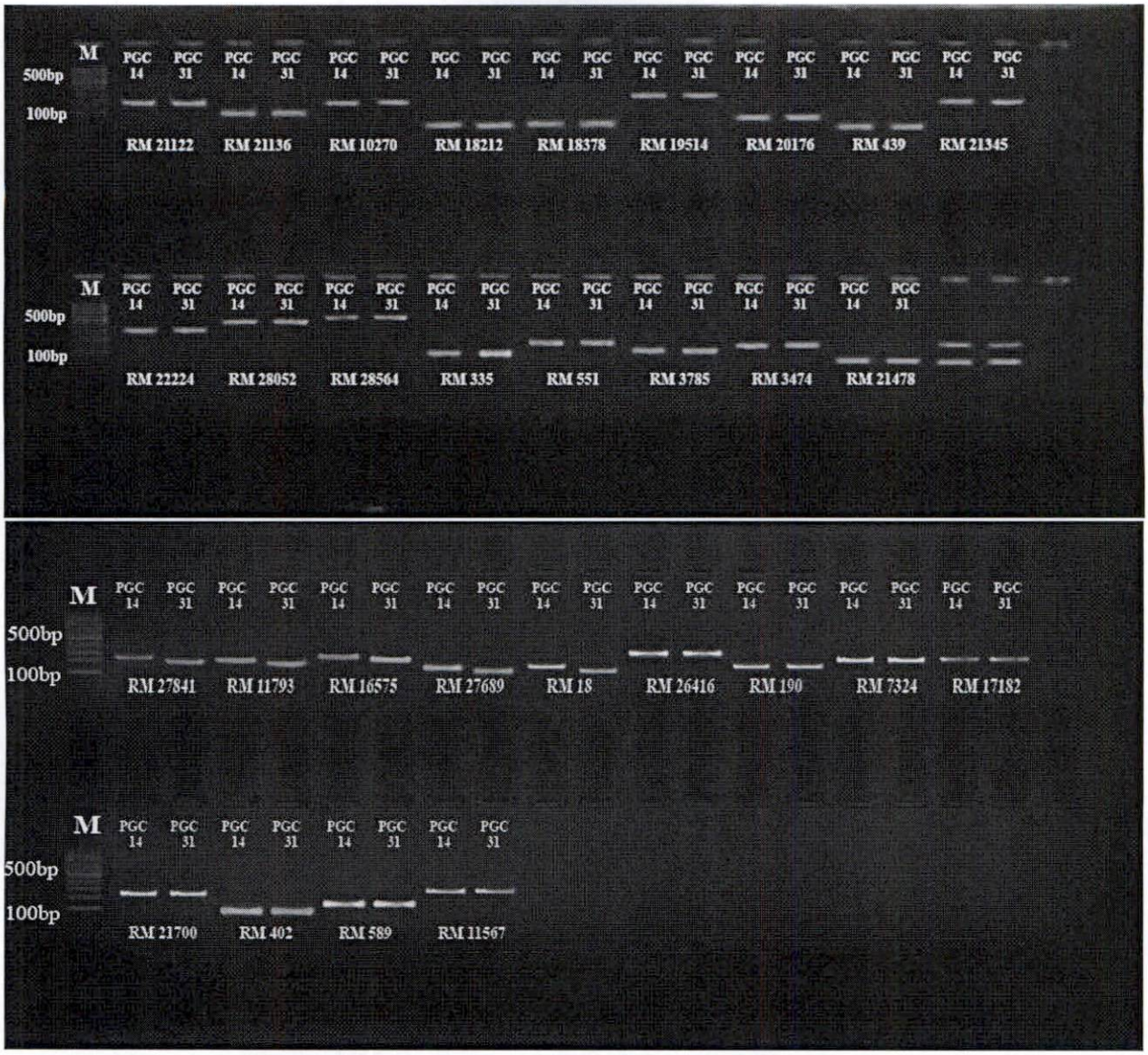



M - Ladder (1kb)

PGC 14 (Tulasi) – Resistant parent to Fe toxicity

PGC 31 (CUL 8709) – Susceptible parent to iron toxicity

**Plate 9. Parental polymorphism study using microsatellite markers (VII)**




M - Ladder (1kb)

PGC 14 (Tulasi) – Resistant parent to Fe toxicity

PGC 31 (CUL 8709) – Susceptible parent to iron toxicity

**Table 16. Details of parental polymorphism study**

| Sl. No | Rice microsatellite | Nature of amplification | Number of amplicon | Size of amplicon (bp) | |
|---|---|---|---|---|---|
| | | | | Monomorphic band | Polymorphic band |
| 1 | RM 10261 | Monomorphic | 1 | 458 | |
| 2 | RM 13603 | Monomorphic | 1 | 520 | |
| 3 | RM 12941 | Monomorphic | 1 | 210 | |
| 4 | RM 13584 | Monomorphic | 1 | 252 | |
| 5 | RM 12292 | Polymorphic | 2 | | 287 & 400 |
| 6 | RM 13616 | Monomorphic | 1 | 341 | |
| 7 | RM 13858 | Monomorphic | 1 | 438 | |
| 8 | RM 16150 | Monomorphic | 1 | 313 | |
| 9 | RM 18204 | Monomorphic | 1 | 252 | |
| 10 | RM 19799 | Monomorphic | 1 | 166 | |
| 11 | RM 20003 | Monomorphic | 1 | 240 | |
| 12 | RM 21693 | Monomorphic | 1 | 385 | |
| 13 | RM 22175 | Monomorphic | 1 | 142 | |
| 14 | RM 24616 | Polymorphic | 2 | | 135 & 191 |
| 15 | RM 24258 | Monomorphic | 1 | 142 | |
| 16 | RM 25751 | Monomorphic | 1 | 240 | |
| 17 | RM 27683 | Monomorphic | 1 | 100 | |
| 18 | RM 28067 | Monomorphic | 1 | 287 | |
| 19 | RM 28273 | Monomorphic | 1 | 319 | |
| 20 | RM 15035 | Monomorphic | 1 | 359 | |
| 21 | RM 19187 | Monomorphic | 1 | 230 | |
| 22 | RM 20677 | Monomorphic | 1 | 400 | |
| 23 | RM 21950 | Monomorphic | 1 | 230 | |
| 24 | RM 22164 | Monomorphic | 1 | 200 | |
| 25 | RM 26447 | Monomorphic | 1 | 230 | |
| 26 | RM 28059 | Monomorphic | 1 | 400 | |
| 27 | RM 28558 | Monomorphic | 1 | 281 | |
| 28 | RM 28568 | Monomorphic | 1 | 200 | |
| 29 | RM 16883 | Monomorphic | 1 | 170 | |
| 30 | RM 17620 | Monomorphic | 1 | 300 | |
| 31 | RM 27369 | Monomorphic | 1 | 170 | |
| 32 | RM 27879 | Monomorphic | 1 | 218 | |
| 33 | RM 28305 | Monomorphic | 1 | 123 | |
| 34 | RM 16556 | Monomorphic | 1 | 200 | |
| 35 | RM 17377 | Monomorphic | 1 | 138 | |
| 36 | RM 13134 | Polymorphic | 2 | | 246 & 359 |
| 37 | RM 15521 | Monomorphic | 1 | 147 | |
| 38 | RM 16153 | Monomorphic | 1 | 229 | |
| 39 | RM 18004 | Monomorphic | 1 | 156 | |
| 40 | RM 18508 | Monomorphic | 1 | 229 | |

**Table 16. Continued.....**

| Sl. No | Rice microsatellite | Nature of amplification | Number of amplicon | Size of amplicon (bp) | |
|---|---|---|---|---|---|
| | | | | Monomorphic band | Polymorphic band |
| 41 | RM 19218 | Monomorphic | 1 | 115 | |
| 42 | RM 19221 | Monomorphic | 1 | 229 | |
| 43 | RM 19483 | Monomorphic | 1 | 339 | |
| 44 | RM 20023 | Monomorphic | 1 | 120 | |
| 45 | RM 21320 | Monomorphic | 1 | 181 | |
| 46 | RM 22703 | Monomorphic | 1 | 328 | |
| 47 | RM 23605 | Monomorphic | 1 | 172 | |
| 48 | RM 24274 | Monomorphic | 1 | 300 | |
| 49 | RM 25436 | Monomorphic | 1 | 163 | |
| 50 | RM 11345 | Monomorphic | 1 | 156 | |
| 51 | RM 22897 | Monomorphic | 1 | 273 | |
| 52 | RM 23358 | Monomorphic | 1 | 172 | |
| 53 | RM 11064 | Monomorphic | 1 | 93 | |
| 54 | RM 11315 | Monomorphic | 1 | 169 | |
| 55 | RM 13123 | Monomorphic | 1 | 224 | |
| 56 | RM 13338 | Monomorphic | 1 | 100 | |
| 57 | RM 13366 | Monomorphic | 1 | 114 | |
| 58 | RM 15843 | Monomorphic | 1 | 424 | |
| 59 | RM 15846 | Monomorphic | 1 | 121 | |
| 60 | RM 15861 | Monomorphic | 1 | 251 | |
| 61 | RM 18919 | Monomorphic | 1 | 338 | |
| 62 | RM 19183 | Polymorphic | 2 | | 94 & 87 |
| 63 | RM 20158 | Monomorphic | 1 | 154 | |
| 64 | RM 20182 | Monomorphic | 1 | 200 | |
| 65 | RM 21452 | Monomorphic | 1 | 215 | |
| 66 | RM 21953 | Monomorphic | 1 | 265 | |
| 67 | RM 24336 | Monomorphic | 1 | 300 | |
| 68 | RM 25404 | Polymorphic | 2 | | 247 & 154 |
| 69 | RM 25995 | Monomorphic | 1 | 367 | |
| 70 | RM 26194 | Monomorphic | 1 | 334 | |
| 71 | RM 26644 | Monomorphic | 1 | 460 | |
| 72 | RM 28580 | Monomorphic | 1 | 319 | |
| 73 | RM 25231 | Monomorphic | 1 | 263 | |
| 74 | RM 17162 | Monomorphic | 1 | 339 | |
| 75 | RM 17645 | Monomorphic | 1 | 339 | |
| 76 | RM 17669 | Monomorphic | 1 | 380 | |
| 77 | RM 10875 | Monomorphic | 1 | 229 | |
| 78 | RM 11096 | Monomorphic | 1 | 480 | |
| 79 | RM 11312 | Monomorphic | 1 | 530 | |
| 80 | RM 12061 | Monomorphic | 1 | 334 | |

**Table 16. Continued.....**

| Sl. No | Rice microsatellite | Nature of amplification | Number of amplicon | Size of amplicon (bp) | |
|---|---|---|---|---|---|
| | | | | Monomorphic band | Polymorphic band |
| 81 | RM 13893 | Monomorphic | 1 | 135 | |
| 82 | RM 18353 | Monomorphic | 1 | 283 | |
| 83 | RM 18959 | Monomorphic | 1 | 367 | |
| 84 | RM 19985 | Polymorphic | 2 | | 367 & 303 |
| 85 | RM 20168 | Monomorphic | 1 | 433 | |
| 86 | RM 20686 | Monomorphic | 1 | 384 | |
| 87 | RM 23080 | Monomorphic | 1 | 433 | |
| 88 | RM 23386 | Monomorphic | 1 | 495 | |
| 89 | RM 23998 | Monomorphic | 1 | 285 | |
| 90 | RM 25212 | Monomorphic | 1 | 329 | |
| 91 | RM 25425 | Monomorphic | 1 | 157 | |
| 92 | RM 25735 | Monomorphic | 1 | 329 | |
| 93 | RM 27840 | Monomorphic | 1 | 400 | |
| 94 | RM 16553 | Monomorphic | 1 | 131 | |
| 95 | RM 16577 | Monomorphic | 1 | 500 | |
| 96 | RM 13877 | Monomorphic | 1 | 241 | |
| 97 | RM 238 | Monomorphic | 1 | 316 | |
| 98 | RM 82 | Monomorphic | 1 | 188 | |
| 99 | RM 251 | Monomorphic | 1 | 200 | |
| 100 | RM 263 | Polymoprhic | 2 | | 284 & 212 |
| 101 | RM 208 | Monomorphic | 1 | 269 | |
| 102 | RM 216 | Polymorphic | 1 | | 300 & 226 |
| 103 | RM 230 | Polymorphic | 1 | | 390 & 284 |
| 104 | RM 332 | Monomorphic | 1 | 284 | |
| 105 | RM 107 | Polymorphic | 2 | | 239 & 167 |
| 106 | RM 16 | Monomorphic | 1 | 183 | |
| 107 | RM 254 | Monomorphic | 1 | 175 | |
| 108 | RM 223 | Monomorphic | 1 | 166 | |
| 109 | RM 20 | Monomorphic | 1 | 226 | |
| 110 | RM 234 | Monomorphic | 1 | 175 | |
| 111 | RM 1 | Monomorphic | 1 | 175 | |
| 112 | RM 257 | Monomorphic | 1 | 183 | |
| 113 | RM 242 | Polymorphic | 2 | | 226 & 300 |
| 114 | RM 224 | Monomorphic | 1 | 209 | |
| 115 | RM 308 | Monomorphic | 1 | 135 | |
| 116 | RM 253 | Monomorphic | 1 | 174 | |
| 117 | RM 314 | Monomorphic | 1 | 135 | |
| 118 | RM 277 | Monomorphic | 1 | 143 | |
| 119 | RM 333 | Polymorphic | 2 | | 191 & 559 |
| 120 | RM 202 | Polymorphic | 2 | | 208 & 151 |

**Table 16. Continued.....**

| Sl. No | Rice microsatellite | Nature of amplification | Number of amplicon | Size of amplicon (bp) | |
|---|---|---|---|---|---|
| | | | | Monomorphic band | Polymorphic band |
| 121 | RM 233 | Monomorphic | 1 | 200 | |
| 122 | RM 19 | Monomorphic | 1 | 331 | |
| 123 | RM 348 | Monomorphic | 1 | 200 | |
| 124 | RM 5586 | Monomorphic | 1 | 100 | |
| 125 | RM 214 | Monomorphic | 1 | 83 | |
| 126 | RM 205 | Monomorphic | 1 | 648 | |
| 127 | RM 250 | Monomorphic | 1 | 292 | |
| 128 | RM 411 | Monomorphic | 1 | 683 | |
| 129 | RM 13 | Monomorphic | 1 | 100 | |
| 130 | RM 316 | Monomorphic | 1 | 315 | |
| 131 | RM 21 | Polymorphic | 2 | | 135 & 94 |
| 132 | RM 260 | Polymorphic | 2 | | 93 & 480 |
| 133 | RM 6440 | Monomorphic | 1 | 213 | |
| 134 | RM 60 | Monomorphic | 1 | 143 | |
| 135 | RM 307 | Monomorphic | 1 | 123 | |
| 136 | RM 217 | Monomorphic | 1 | 136 | |
| 137 | RM 247 | Monomorphic | 1 | 123 | |
| 138 | RM 164 | Monomorphic | 1 | 240 | |
| 139 | RM 511 | Monomorphic | 1 | 285 | |
| 140 | RM 174 | Monomorphic | 1 | 82 | |
| 141 | RM 169 | Monomorphic | 1 | 240 | |
| 142 | RM 110 | Monomorphic | 1 | 86 | |
| 143 | RM 10861 | Monomorphic | 1 | 561 | |
| 144 | RM 11072 | Monomorphic | 1 | 240 | |
| 145 | RM 11069 | Monomorphic | 1 | 98 | |
| 146 | RM 13141 | Monomorphic | 1 | 210 | |
| 147 | RM 16138 | Monomorphic | 1 | 73 | |
| 148 | RM 18222 | Monomorphic | 1 | 73 | |
| 149 | RM 18382 | Monomorphic | 1 | 76 | |
| 150 | RM 23099 | Monomorphic | 1 | 161 | |
| 151 | RM 24263 | Monomorphic | 1 | 190 | |
| 152 | RM 10578 | Monomorphic | 1 | 71 | |
| 153 | RM 26871 | Polymorphic | 2 | | 96 & 152 |
| 154 | RM 27900 | Monomorphic | 1 | 190 | |
| 155 | RM 27180 | Monomorphic | 1 | 69 | |
| 156 | RM 27184 | Monomorphic | 1 | 73 | |
| 157 | RM 17632 | Monomorphic | 1 | 209 | |
| 158 | RM 13316 | Monomorphic | 1 | 348 | |
| 159 | RM 15293 | Monomorphic | 1 | 238 | |
| 160 | RM 15837 | Monomorphic | 1 | 273 | |

**Table 16. Continued.....**

| Sl. No | Rice microsatellite | Nature of amplification | Number of amplicon | Size of amplicon (bp) | |
|---|---|---|---|---|---|
| | | | | Monomorphic band | Polymorphic band |
| 161 | RM 18639 | Monomorphic | 1 | 260 | |
| 162 | RM 20397 | Monomorphic | 1 | 255 | |
| 163 | RM 20429 | Monomorphic | 1 | 157 | |
| 164 | RM 20683 | Monomorphic | 1 | 230 | |
| 165 | RM 21470 | Monomorphic | 1 | 71 | |
| 166 | RM 21930 | Monomorphic | 1 | 157 | |
| 167 | RM 22523 | Monomorphic | 1 | 88 | |
| 168 | RM 23612 | Monomorphic | 1 | 220 | |
| 169 | RM 24664 | Polymorphic | 2 | | 100 & 183 |
| 170 | RM 25420 | Monomorphic | 1 | 82 | |
| 171 | RM 26409 | Monomorphic | 1 | 210 | |
| 172 | RM 10864 | Monomorphic | 1 | 300 | |
| 173 | RM 11608 | Monomorphic | 1 | 191 | |
| 174 | RM 12588 | Monomorphic | 1 | 247 | |
| 175 | RM 18384 | Monomorphic | 1 | 210 | |
| 176 | RM 18647 | Monomorphic | 1 | 300 | |
| 177 | RM 21661 | Monomorphic | 1 | 312 | |
| 178 | RM 22905 | Monomorphic | 1 | 150 | |
| 179 | RM 23645 | Polymorphic | 2 | | 191 & 285 |
| 180 | RM 27186 | Monomorphic | 1 | 200 | |
| 181 | RM 26870 | Monomorphic | 1 | 319 | |
| 182 | RM 490 | Monomorphic | 1 | 80 | |
| 183 | RM 5919 | Monomorphic | 1 | 176 | |
| 184 | RM 212 | Monomorphic | 1 | 123 | |
| 185 | RM 324 | Monomorphic | 1 | 141 | |
| 186 | RM 482 | Monomorphic | 1 | 156 | |
| 187 | RM 442 | Monomorphic | 1 | 93 | |
| 188 | RM 85 | Monomorphic | 1 | 118 | |
| 189 | RM 5473 | Monomorphic | 1 | 85 | |
| 190 | RM 280 | Monomorphic | 1 | 152 | |
| 191 | RM 7029 | Monomorphic | 1 | 219 | |
| 192 | RM 413 | Monomorphic | 1 | 67 | |
| 193 | RM 3628 | Polymorphic | 2 | | 94 & 115 |
| 194 | RM 481 | Monomorphic | 1 | 242 | |
| 195 | RM 458 | Monomorphic | 1 | 131 | |
| 196 | RM 271 | Monomorphic | 1 | 72 | |
| 197 | RM 269 | Monomorphic | 1 | 110 | |
| 198 | RM 229 | Monomorphic | 1 | 98 | |
| 199 | RM 485 | Monomorphic | 1 | 209 | |
| 200 | RM 541 | Monomorphic | 1 | 175 | |

**Table 16. Continued.....**

| Sl. No | Rice microsatellite | Nature of amplification | Number of amplicon | Size of amplicon (bp) | |
|---|---|---|---|---|---|
| | | | | Monomorphic band | Polymorphic band |
| 201 | RM 478 | Monomorphic | 1 | 82 | |
| 202 | RM 3309 | Monomorphic | 1 | 148 | |
| 203 | RM 5556 | Monomorphic | 1 | 175 | |
| 204 | RM 434 | Monomorphic | 1 | 95 | |
| 205 | RM 7545 | Monomorphic | 1 | 110 | |
| 206 | RM 5961 | Monomorphic | 1 | 120 | |
| 207 | RM 6340 | Monomorphic | 1 | 120 | |
| 208 | RM 428 | Monomorphic | 1 | 178 | |
| 209 | RM 583 | Monomorphic | 1 | 189 | |
| 210 | RM 24 | Monomorphic | 1 | 225 | |
| 211 | RM 3340 | Monomorphic | 1 | 459 | |
| 212 | RM 215 | Polymorphic | 2 | | 132 & 100 |
| 213 | RM 261 | Monomorphic | 1 | 126 | |
| 214 | RM 470 | Monomorphic | 1 | 126 | |
| 215 | RM 508 | Monomorphic | 1 | 150 | |
| 216 | RM 3859 | Monomorphic | 1 | 163 | |
| 217 | RM 248 | Monomorphic | 1 | 200 | |
| 218 | RM 407 | Monomorphic | 1 | 157 | |
| 219 | RM 524 | Monomorphic | 1 | 185 | |
| 220 | RM 304 | Monomorphic | 1 | 347 | |
| 221 | RM 536 | Monomorphic | 1 | 322 | |
| 222 | RM 491 | Monomorphic | 1 | 264 | |
| 223 | RM 463 | Monomorphic | 1 | 196 | |
| 224 | RM 206 | Monomorphic | 1 | 188 | |
| 225 | RM 340 | Monomorphic | 1 | 78 | |
| 226 | RM 5590 | Monomorphic | 1 | 359 | |
| 227 | RM 264 | Polymorphic | 2 | | 206 & 128 |
| 228 | RM 207 | Monomorphic | 1 | 300 | |
| 229 | RM 561 | Monomorphic | 1 | 214 | |
| 230 | RM 497 | Monomorphic | 1 | 156 | |
| 231 | RM 346 | Monomorphic | 1 | 89 | |
| 232 | RM 607 | Monomorphic | 1 | 106 | |
| 233 | RM 591 | Monomorphic | 1 | 132 | |
| 234 | RM 16855 | Monomorphic | 1 | 94 | |
| 235 | RM 12255 | Polymorphic | 2 | | 210 & 300 |
| 236 | RM 11342 | Monomorphic | 1 | 106 | |
| 237 | RM 12031 | Monomorphic | 1 | 211 | |
| 238 | RM 16447 | Monomorphic | 1 | 251 | |
| 239 | RM 13599 | Polymorphic | 2 | | 165 & 213 |
| 240 | RM 14723 | Polymorphic | 2 | | 144 & 93 |

**Table 16. Continued.....**

| Sl. No | Rice microsatellite | Nature of amplification | Number of amplicon | Size of amplicon (bp) | |
|---|---|---|---|---|---|
| | | | | Monomorphic band | Polymorphic band |
| 241 | RM 18622 | Polymorphic | 2 | | 164 & 125 |
| 242 | RM 18941 | Polymorphic | 2 | | 178 & 86 |
| 243 | RM 19199 | Monomorphic | 1 | 131 | |
| 244 | RM 28070 | Monomorphic | 1 | 284 | |
| 245 | RM 28559 | Monomorphic | 1 | 436 | |
| 246 | RM 11070 | Monomorphic | 1 | 67 | |
| 247 | RM 11561 | Monomorphic | 1 | 73 | |
| 248 | RM 12353 | Monomorphic | 1 | 355 | |
| 249 | RM 13619 | Polymorphic | 2 | | 255 & 379 |
| 250 | RM 13321 | Monomorphic | 1 | 561 | |
| 251 | RM 14240 | Monomorphic | 1 | 182 | |
| 252 | RM 14487 | Polymorphic | 2 | | 368 & 481 |
| 253 | RM 17769 | Monomorphic | 1 | 282 | |
| 254 | RM 17990 | Monomorphic | 1 | 291 | |
| 255 | RM 22892 | Monomorphic | 1 | 310 | |
| 256 | RM 23087 | Monomorphic | 1 | 257 | |
| 257 | RM 24031 | Monomorphic | 1 | 251 | |
| 258 | RM 23096 | Monomorphic | 1 | 186 | |
| 259 | RM 24866 | Polymorphic | 2 | | 192 & 297 |
| 260 | RM 24900 | Monomorphic | 1 | 181 | |
| 261 | RM 25066 | Monomorphic | 1 | 234 | |
| 262 | RM 27418 | Monomorphic | 1 | 222 | |
| 263 | RM 16694 | Monomorphic | 1 | 316 | |
| 264 | RM 14725 | Monomorphic | 1 | 328 | |
| 265 | RM 18194 | Monomorphic | 1 | 245 | |
| 266 | RM 10000 | Monomorphic | 1 | 365 | |
| 267 | RM 12921 | Monomorphic | 1 | 258 | |
| 268 | RM 13340 | Monomorphic | 1 | 84 | |
| 269 | RM 14713 | Monomorphic | 1 | 439 | |
| 270 | RM 27373 | Monomorphic | 1 | 315 | |
| 271 | RM 28303 | Monomorphic | 1 | 500 | |
| 272 | RM 17611 | Monomorphic | 1 | 348 | |
| 273 | RM 11764 | Monomorphic | 1 | 459 | |
| 274 | RM 15561 | Monomorphic | 1 | 200 | |
| 275 | RM 13131 | Polymorphic | 2 | | 219 & 331 |
| 276 | RM 22459 | Monomorphic | 1 | 163 | |
| 277 | RM 23068 | Monomorphic | 1 | 300 | |
| 278 | RM 26182 | Monomorphic | 1 | 261 | |
| 279 | RM 13852 | Monomorphic | 1 | 555 | |
| 280 | RM 20698 | Monomorphic | 1 | 389 | |

**Table 16. Continued.....**

| Sl. No | Rice microsatellite | Nature of amplification | Number of amplicon | Size of amplicon (bp) | |
|---|---|---|---|---|---|
| | | | | Monomorphic band | Polymorphic band |
| 281 | RM 6679 | Polymorphic | 2 | | 400 & 375 |
| 282 | RM 25217 | Monomorphic | 1 | 80 | |
| 283 | RM 13910 | Monomorphic | 1 | 200 | |
| 284 | RM 19255 | Monomorphic | 1 | 143 | |
| 285 | RM 27650 | Monomorphic | 1 | 273 | |
| 286 | RM 231 | Monomorphic | 1 | 164 | |
| 287 | RM 7 | Monomorphic | 1 | 222 | |
| 288 | RM 748 | Monomorphic | 1 | 328 | |
| 289 | RM 331 | Monomorphic | 1 | 386 | |
| 290 | RM 17 | Monomorphic | 1 | 259 | |
| 291 | RM 25060 | Polymorphic | 2 | | 257 & 191 |
| 292 | RM 27132 | Monomorphic | 1 | 166 | |
| 293 | RM 25771 | Monomorphic | 1 | 253 | |
| 294 | RM 26213 | Monomorphic | 1 | 280 | |
| 295 | RM 26429 | Monomorphic | 1 | 220 | |
| 296 | RM 26438 | Monomorphic | 1 | 479 | |
| 297 | RM 26662 | Monomorphic | 1 | 274 | |
| 298 | RM 28267 | Monomorphic | 1 | 354 | |
| 299 | RM 27851 | Monomorphic | 1 | 396 | |
| 300 | RM 27863 | Monomorphic | 1 | 107 | |
| 301 | RM 10596 | Monomorphic | 1 | 245 | |
| 302 | RM 11554 | Monomorphic | 1 | 115 | |
| 303 | RM 11313 | Monomorphic | 1 | 355 | |
| 304 | RM 15583 | Monomorphic | 1 | 152 | |
| 305 | RM 15838 | Monomorphic | 1 | 288 | |
| 306 | RM 16129 | Monomorphic | 1 | 458 | |
| 307 | RM 18913 | Monomorphic | 1 | 479 | |
| 308 | RM 20833 | Monomorphic | 1 | 200 | |
| 309 | RM 21122 | Monomorphic | 1 | 159 | |
| 310 | RM 21136 | Monomorphic | 1 | 105 | |
| 311 | RM 10270 | Monomorphic | 1 | 159 | |
| 312 | RM 18212 | Monomorphic | 1 | 68 | |
| 313 | RM 18378 | Monomorphic | 1 | 75 | |
| 314 | RM 19514 | Monomorphic | 1 | 243 | |
| 315 | RM 20176 | Monomorphic | 1 | 92 | |
| 316 | RM 439 | Monomorphic | 1 | 65 | |
| 317 | RM 21345 | Monomorphic | 1 | 168 | |
| 318 | RM 22224 | Monomorphic | 1 | 268 | |
| 319 | RM 28052 | Monomorphic | 1 | 424 | |
| 320 | RM 28564 | Monomorphic | 1 | 525 | |

**Table 16. Continued.....**

| Sl. No | Rice microsatellite | Nature of amplification | Number of amplicon | Size of amplicon (bp) | |
|---|---|---|---|---|---|
| | | | | Monomorphic band | Polymorphic band |
| 321 | RM 335 | Monomorphic | 1 | 100 | |
| 322 | RM 551 | Monomorphic | 1 | 159 | |
| 323 | RM 3785 | Monomorphic | 1 | 112 | |
| 324 | RM 3474 | Monomorphic | 1 | 138 | |
| 325 | RM 21478 | Monomorphic | 1 | 84 | |
| 326 | RM 27841 | Polymorphic | 2 | | 254 & 180 |
| 327 | RM 11793 | Polymorphic | 2 | | 200 & 180 |
| 328 | RM 16575 | Polymorphic | 2 | | 247 & 210 |
| 329 | RM 27689 | Polymorphic | 2 | | 144 & 107 |
| 330 | RM 18 | Polymorphic | 2 | | 157 & 111 |
| 331 | RM 26416 | Monomorphic | 1 | 283 | |
| 332 | RM 190 | Monomorphic | 1 | 127 | |
| 333 | RM 7324 | Monomorphic | 1 | 186 | |
| 334 | RM 17182 | Monomorphic | 1 | 200 | |
| 335 | RM 21700 | Monomorphic | 1 | 326 | |
| 336 | RM 402 | Monomorphic | 1 | 179 | |
| 337 | RM 589 | Monomorphic | 1 | 228 | |
| 338 | RM 11567 | Monomorphic | 1 | 370 | |

**Table 17. List of markers found polymorphic between the resistant and susceptible parent**

| Sl. No | Rice microsatellite | Nature of amplification | Number of amplicon | Size of amplicon (bp) | |
|---|---|---|---|---|---|
| | | | | Polymorphic band | |
| 1 | RM 12292 | Polymorphic | 2 | 287 | 400 |
| 2 | RM 24616 | Polymorphic | 2 | 135 | 191 |
| 3 | RM 13134 | Polymorphic | 2 | 246 | 359 |
| 4 | RM 19183 | Polymorphic | 2 | 94 | 87 |
| 5 | RM 25404 | Polymorphic | 2 | 247 | 154 |
| 6 | RM 19985 | Polymorphic | 2 | 367 | 303 |
| 7 | RM 263 | Polymoprhic | 2 | 284 | 212 |
| 8 | RM 216 | Polymorphic | 2 | 300 | 226 |
| 9 | RM 230 | Polymorphic | 2 | 390 | 284 |
| 10 | RM 107 | Polymorphic | 2 | 239 | 167 |
| 11 | RM 242 | Polymorphic | 2 | 226 | 300 |
| 12 | RM 333 | Polymorphic | 2 | 191 | 559 |
| 13 | RM 202 | Polymorphic | 2 | 208 | 151 |
| 14 | RM 21 | Polymorphic | 2 | 135 | 94 |
| 15 | RM 260 | Polymorphic | 2 | 93 | 480 |
| 16 | RM 26871 | Polymorphic | 2 | 96 | 152 |
| 17 | RM 24664 | Polymorphic | 2 | 100 | 183 |
| 18 | RM 23645 | Polymorphic | 2 | 191 | 285 |
| 19 | RM 3628 | Polymorphic | 2 | 94 | 115 |
| 20 | RM 215 | Polymorphic | 2 | 132 | 100 |
| 21 | RM 264 | Polymorphic | 2 | 206 | 128 |
| 22 | RM 12255 | Polymorphic | 2 | 210 | 300 |
| 23 | RM 13599 | Polymorphic | 2 | 165 | 213 |
| 24 | RM 14723 | Polymorphic | 2 | 144 | 93 |
| 25 | RM 18622 | Polymorphic | 2 | 164 | 125 |
| 26 | RM 18941 | Polymorphic | 2 | 178 | 86 |
| 27 | RM 13619 | Polymorphic | 2 | 255 | 379 |
| 28 | RM 14487 | Polymorphic | 2 | 368 | 481 |
| 29 | RM 24866 | Polymorphic | 2 | 192 | 297 |
| 30 | RM 13131 | Polymorphic | 2 | 219 | 331 |
| 31 | RM 6679 | Polymorphic | 2 | 400 | 375 |
| 32 | RM 25060 | Polymorphic | 2 | 257 | 191 |
| 33 | RM 27841 | Polymorphic | 2 | 254 | 180 |
| 34 | RM 11793 | Polymorphic | 2 | 200 | 180 |
| 35 | RM 16575 | Polymorphic | 2 | 247 | 210 |
| 36 | RM 27689 | Polymorphic | 2 | 144 | 107 |
| 37 | RM 18 | Polymorphic | 2 | 157 | 111 |

### 4.3. Experiment 3: Raising of $F_1$'s

The $F_1$ seeds of the cross [PGC (Cul-8709/ PGC (Tulasi)] were raised and selfed to generate sufficient seeds of $F_2$ generation, for conduct of Bulk segregant analysis (BSA).

### 4.4. Experiment 4: Bulk Segregant Analysis (BSA)

#### 4.4.1. Phenotyping of $F_2$ plants for iron toxicity tolerance

Three hundred $F_2$ plants produced under Experiment 3 were screened in hydroponics system to ascertain their reaction to iron stress (800 pm Fe) as per the method advocated by Shimizu *et al.* (2005).

##### 4.4.1.1. Variability in performance of genotypes

Wide variability (Table 18, Plate 10 &11 and Fig 1 & 2) was observed among the $F_2$ plants with respect to shoot length, root length, total number of roots, number of fresh roots, shoot weight, root weight and visual scoring for iron-toxicity symptoms, iron reversibly adsorbed on root surface, iron content in root and iron content in leaf of $F_2$ plants. Skewness and kurtosis values of screening observations presented in the table 19. The results have been detailed below.

###### 4.4.1.1.1. Shoot length (cm)

Mean shoot length of 300 $F_2$ plants after 6 weeks of 800ppm of Fe treatment was 55.3cm. Shoot length of 300 $F_2$ plants ranged from 49.0cm to 62.8cm. Shoot length values of PGC 14 (Tulasi) and PGC 31 (Cul-8709) were 61.2cm and 49.7cm respectively.

The least shoot length values were observed in plant numbers 109 (49cm), 18 (49.4cm), 202(49.5cm), 194(49.9cm), 12 (50.1cm), 122 (50.3cm), 66 (50.6cm), 334 (51.1cm), 113 (51.2cm) and 183 (51.2cm) respectively. The highest shoot length values were observed in plant numbers 248 (62.8cm), 309 (62.8cm), 156 (62.6cm), 52 (62.5cm), 111 (62.3cm), 320 (62.3), 287 (62.1cm), 20 (61.8cm), 268 (61.8cm) and 246 (61.6cm) respectively.

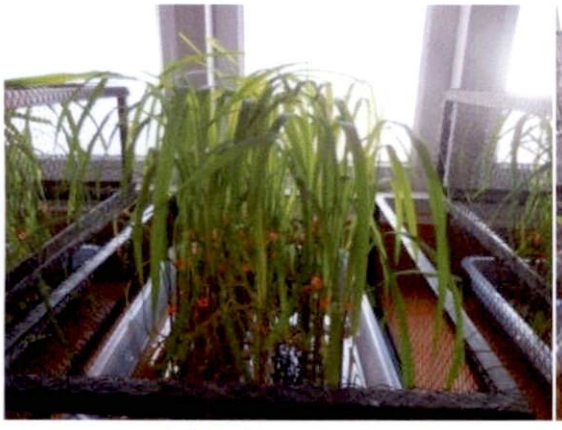
**Plate 10. Phenotyping of $F_2$ plants for iron toxicity tolerance at 800 ppm Fe**

**Plant No. 1 to 60**

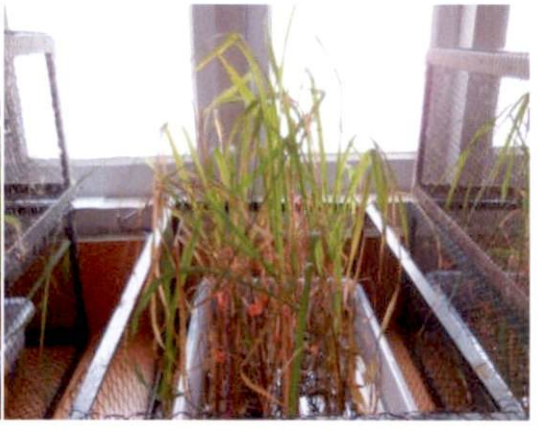
At initiation of screening

After 4 weeks of Fe stress (800ppm)

**Plant No. 61 to 120**



At initiation of screening



After 4 weeks of Fe stress (800ppm)

**Plant No. 121 to 180**

At initiation of screening

After 4 weeks of Fe stress (800ppm)

**Plate 11. Phenotyping of $F_2$ plants for iron toxicity tolerance at 800 ppm Fe (II)**

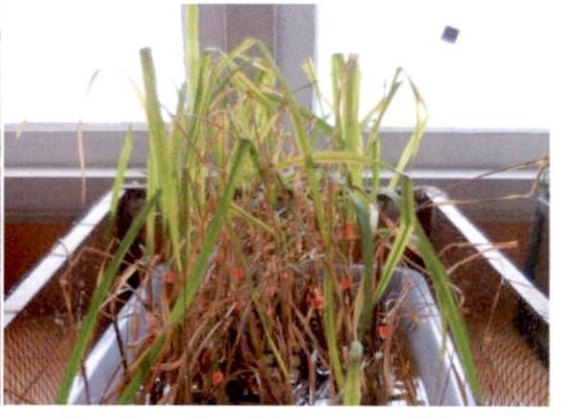
**Plant No. 181 to 240**

At initiation of screening

After 4 weeks of Fe stress (800ppm)

**Plant No. 240 to 300**

At initiation of screening



After 4 weeks of Fe stress (800ppm)

**Table 18. Variability in $F_2$ population screened for response at 800 ppm of iron**

| Sl. No. | Trait | Mean | Range | Standard deviation | Coefficient of variation | Skewness | Kurtosis |
|---|---|---|---|---|---|---|---|
| 1 | Leaf bronzing after 4 weeks | 5.43 | 8.00 | 2.97 | 54.69 | -0.14 | -1.41 |
| 2 | Leaf bronzing after 6 weeks | 7.65 | 8.00 | 2.37 | 30.90 | -1.67 | 1.55 |
| 3 | Root length (cm) | 19.52 | 10.30 | 2.25 | 11.51 | 0.39 | -0.48 |
| 4 | Shoot length (cm) | 55.29 | 13.80 | 2.69 | 4.87 | 0.73 | 0.45 |
| 5 | Root weight (g) | 4.24 | 5.35 | 1.25 | 29.43 | 1.42 | 1.20 |
| 6 | Shoot weight (g) | 6.02 | 9.95 | 2.13 | 35.44 | 1.44 | 1.28 |
| 7 | Total number of roots | 25.32 | 14.00 | 2.68 | 10.58 | 0.99 | 1.03 |
| 8 | Number of fresh roots | 5.24 | 32.00 | 8.20 | 156.53 | 1.56 | 1.41 |
| 9 | Iron adsorbed on root surface (g) | 5.02 | 12.48 | 2.75 | 54.81 | 1.67 | 2.10 |
| 10 | Iron content in root (g) | 8746.52 | 7772.31 | 1813.20 | 20.73 | 0.70 | -0.37 |
| 11 | Leaf iron content (g) | 1633.15 | 2667.97 | 614.40 | 37.62 | 0.19 | -0.84 |

**Table 19. Skewness and kurtosis of leaf bronzing score and growth traits in $F_2$ population**

| Sl. No. | Trait | skewness | kurtosis |
|---|---|---|---|
| 1 | Leaf bronzing after 4 weeks | -0.14 | -1.44 |
| 2 | Leaf bronzing after 6 weeks | -1.67 | 1.55 |
| 3 | Root length (cm) | 0.39 | -0.48 |
| 4 | Shoot length (cm) | 0.73 | 0.45 |
| 5 | Root weight (g) | 1.42 | 1.20 |
| 6 | Shoot weight (g) | 1.44 | 1.28 |
| 7 | Total number of roots | 0.99 | 1.03 |
| 8 | Number of fresh roots | 1.56 | 1.41 |
| 9 | Iron adsorbed on root surface (g) | 1.67 | 2.10 |
| 10 | Iron content in root (g) | 0.70 | -0.37 |
| 11 | Leaf iron content (g) | 0.19 | -0.84 |

Skewness and kurtosis of this observation is 0.73 and 0.45 respectively. Frequency distribution (Fig. 1) was used to determine the number of individuals in the segregating $F_2$ population that had shoot length values close to parent PGC 14 (Tulasi) (61.2cm) and PGC 31 (Cul-8709) (49.7cm) as well as intermediate between the two. $F_2$ individuals with a shoot length $\geq$ 61.0 cm were designated as having higher shoot length; those with values between 50.1 to 60.9cm as intermediate and individuals with shoot length $\leq$ 50.0cm as low. Results indicated that out of the 300 $F_2$ plants, fourteen possessed high shoot length (4.67%), 282 $F_2$ plants had intermediate values (94%) while shoot length was low in four $F_2$ individuals (1.33%).

#### 4.4.1.1.2. Root length (cm)

Mean root length of 300 $F_2$ plants after 6 weeks of 800ppm of Fe treatment was 19.5cm. Root length of 300 $F_2$ plants ranged from 15.6cm to 25.9cm. Root length values of PGC 14 (Tulasi) and PGC 31 (Cul-8709) were 23.4cm and 16.3cm respectively.

Least root length values were observed in plant numbers 18, 109, 12, 202, 194, 231, 334, 113, 8 and 41 and their values are 15.6cm, 15.7cm, 15.8cm, 15.8cm, 15.9cm, 15.9 cm, 15.9cm, 16.0cm, 16.1cm and 16.1cm respectively. Highest root length values were observed in plant numbers 248,111, 246, 268, 156, 20, 309, 110, 287 and 308 and their values are 25.9cm, 25.6cm, 25.3cm, 25.1cm, 24.9cm, 24.4cm, 24.3cm, 23.9cm, 23.6cm and 23.6cm respectively.

Skewness and kurtosis of this observation is 0.39 and -0.48 respectively. Frequency distribution (Fig. 1) was used to determine the number of individuals in the segregating $F_2$ population that had root length values close to parent PGC 14 (Tulasi) (23.4cm) and PGC 31 (Cul-8709) (16.3cm) as well as intermediate between the two. $F_2$ individuals with a root length $\geq$ 23.0 cm were designated as having higher root length; those with values between 17.1cm to 22.9 cm as intermediate and individuals with root length $\leq$ 17.0 cm as low. Results indicated that out of the 300 $F_2$ plants, twenty four $F_2$ plants possessed high root length (8%), 221 $F_2$ plants had intermediate values (74.67%) while root length was low in fifty five $F_2$ individuals (18.33%).

#### 4.4.1.1.3. Total number of roots

Mean total number of roots of 300 $F_2$ plants after 6 weeks of 800ppm of Fe treatment was 25. Total number of roots of 300 $F_2$ plants ranged from 20 to 34. Total number of roots of PGC 14 (Tulasi) and PGC 31 (Cul-8709) were 31 roots and 22 roots respectively. Skewness and kurtosis of this observation was 0.99 and 1.03 respectively.

Least number of roots (20 roots) was observed in plant number 173 followed by plant number 18 (21roots), 19 (21roots), 41 (21roots), 115 (21roots), 124 (21 roots), 172 (21roots), 194 (21 roots), 3 (22 roots) and 12 (22roots) while the high root number (34 roots) was observed in plant number 20, 111 and 309 followed by 52, 248, 320 (33roots), 82, 110, 156 and 246 (32 roots).

Skewness and kurtosis of this observation is 0.99 and 1.03 respectively. Frequency distribution (Fig. 1) was used to determine the number of individuals in the segregating $F_2$ population that had total number of roots close to parent PGC 14 (Tulasi) (31roots) and PGC 31 (Cul-8709) (22roots) as well as intermediate between the two. $F_2$ individuals with a total number of roots $\geq 31$ were designated as having higher total number of roots; those with values between 23 to 30 roots as intermediate and individuals with total number of roots $\leq 22$ as low. Results indicated that out of the 300 $F_2$ plants, 19 $F_2$ plants possessed high total number of roots (6.33%), 246 $F_2$ plants had intermediate values (82%) while total number of roots was low in thirty five $F_2$ individuals (11.67%).

#### 4.4.1.1.4. Number of fresh roots

Mean number of fresh roots of 300 $F_2$ plants after 6 weeks of 800ppm of Fe treatment was 5. Number of fresh roots ranged from 0 to 32. Number of fresh roots of PGC 14 (Tulasi) was 27 roots and none of fresh roots were observed in PGC 31 (Cul-8709).

None of fresh roots were observed in 155 $F_2$ plants out of 300 $F_2$ plants at the end of 6 weeks of exposure to iron stress (800ppm of Fe). High number of fresh roots were observed in plant number 248 (32 nos.) followed by plant number 20, 111 (31

nos.), 246 (30 nos.), 309 (29 nos.), 52, 110, 287, 320 (28 nos. each), and plant no. 156 and 268 with 27 fresh roots each.

Skewness and kurtosis of this observation is 1.56 and 1.41 respectively. Frequency distribution (Fig. 1) was used to determine the number of individuals in the segregating $F_2$ population that had number of fresh roots close to parent PGC 14 (Tulasi) (27roots) and PGC 31 (Cul-8709) (no fresh roots) as well as intermediate between the two. $F_2$ individuals with a number of fresh roots $\geq$ 27 were designated as having higher number of fresh roots; those with values between 1 to 26 roots as intermediate and individuals without fresh roots as low. Results indicated that out of the 300 $F_2$ plants, eleven $F_2$ plants possessed high number of fresh roots (3.67%), 134 $F_2$ plants had intermediate values (44.67%) while number of fresh roots was low in 155 $F_2$ individuals (51.67%).

#### 4.4.1.1.5. Shoot weight (g)

Mean shoot weight of 300 $F_2$ plants after 6 weeks of 800ppm of Fe treatment was 6.02g. Shoot weight ranged from 3.50g to 13.40g. Shoot weight values of PGC 14 (Tulasi) and PGC 31 (Cul-8709) were 11.30g and 3.95g respectively.

Plant numbers 5 (3.50g), 3 (3.60g), 202 (3.80g), 18 (3.90g), 249 (3.90g), 109 (3.95g), 12 (4.00g), 71 (4.05g), 185 (4.10g) and 48 (4.15g) recorded lower shoot weight among the 300 $F_2$ plants tested. Highest shoot weight values were observed in plant numbers 309 (13.40g), 308 (12.95g), 320 (12.60g), 287 (12.30g), 111 (12.25g), 20 (12.05g), 52 (11.95g), 319 (11.85g), 248 (11.6g) and 110 (11.55g) respectively.

Skewness and kurtosis of this observation is 1.44 and 1.27 respectively. Frequency distribution (Fig. 2) was used to determine the number of individuals in the segregating $F_2$ population that had shoot weight values close to parent PGC 14 (Tulasi) (11.30g) and PGC 31 (Cul-8709) (3.95g) as well as intermediate between the two. $F_2$ individuals with a shoot weight $\geq$ 11.30g were designated as having higher shoot weight; those with values between 4.00 to 11.25g as intermediate and individuals with shoot weight $\leq$ 3.95g as low. Results indicated that out of the 300 $F_2$ plants, 12 $F_2$ plants possessed high shoot weight (4%), 282 $F_2$ plants had intermediate values (94%) while shoot weight was low in six $F_2$ individuals (2%).

#### 4.4.1.1.6. Root weight (g)

Mean root weight of 300 $F_2$ plants after 6 weeks of 800ppm of Fe treatment was 4.24g. Root weight ranged from 2.85g to 8.20g. Root weight values of PGC 14 (Tulasi) and PGC 31 (Cul-8709) were 7.80g and 2.95g respectively.

Lowest root weight values were observed in plant numbers 12, 18, 249, 202, 66, 109, 1, 10, 113 and 213 and their values are 2.85g, 2.85g, 2.85g, 2.90g, 3.00g, 3.00g, 3.05g, 3.05g, 3.05g and 3.05g respectively. Higher root weight values were observed in plant numbers 111, 156, 32, 20, 309, 248, 378, 268, 176 and 246 their values are 8.20g, 8.20g, 7.95g, 7.85g, 7.80g, 7.55g, 7.50g, 7.45g, 7.40g and 7.40g respectively.

Skewness and kurtosis of this observation is 1.42 and 1.20 respectively. Frequency distribution (Fig. 2) was used to determine the number of individuals in the segregating $F_2$ population that had root weight values close to parent PGC 14 (Tulasi) (7.80g) and PGC 31 (Cul-8709) (2.95g) as well as intermediate between the two. $F_2$ individuals with a root weight $\geq$ 7.80g were designated as having higher root weight; those with values between 3.00 to 7.75g as intermediate and individuals with root weight $\leq$ 2.95g as low. Results indicated that out of the 300 $F_2$ plants, five $F_2$ plants possessed high root weight (1.67%), 291 $F_2$ plants had intermediate values (97%) while root weight was low in four $F_2$ individuals (1.33%).

#### 4.4.1.1.7. Iron reversibly adsorbed on root surface (mg $L^{-1}$)

Mean iron reversibly adsorbed on root surface of 300 $F_2$ plants after 6 weeks of 800 mg $L^{-1}$ of Fe treatment was 5.02mg $L^{-1}$. Iron reversibly adsorbed on root surface ranged from 2.65 mg $L^{-1}$ to 15.13 mg $L^{-1}$. Iron reversibly adsorbed on root surface of PGC 14 (Tulasi) and PGC 31 (Cul-8709) was 12.19 mg $L^{-1}$ and 3.36 mg $L^{-1}$ respectively.

Lowest iron content (2.65 mg $L^{-1}$) adsorbed on root surface observed in plant number 108 followed by plant number 75 (2.68 mg $L^{-1}$), 66 (2.71 mg $L^{-1}$), 303 (2.74 mg $L^{-1}$), 168 (2.75 mg $L^{-1}$), 78 (2.77 mg $L^{-1}$), 182 (2.77 mg $L^{-1}$), 73 (2.78 mg $L^{-1}$), 12 (2.80 mg $L^{-1}$) and 205 (2.80 mg $L^{-1}$). Highest iron content (15.13 mg $L^{-1}$) adsorbed on root surface was observed in plant number 156 followed by plant number 320 (14.87 mg $L^{-}$

$^{-1}$), 52 (14.35 mg $L^{-1}$), 287 (13.46 mg $L^{-1}$), 111 (13.38 mg $L^{-1}$), 319 (13.04 mg $L^{-1}$), 354 (12.85 mg $L^{-1}$), 248 (12.81 mg $L^{-1}$), 300 (12.62 mg $L^{-1}$) and 20 (12.60 mg $L^{-1}$).

Skewness and kurtosis of this observation is 1.67 and 2.10 respectively. Frequency distribution (Fig. 2) was used to determine the number of individuals in the segregating $F_2$ population that had iron reversibly adsorbed on root surface close to parent PGC 14 (Tulasi) (12.19 mg $L^{-1}$) and PGC 31 (Cul-8709) (3.36 mg $L^{-1}$) as well as intermediate between the two. $F_2$ individuals with iron reversibly adsorbed on root surface $\geq$ 12.10 mg $L^{-1}$ were designated as having higher iron reversibly adsorbed on root surface; those with values between 3.41 to 12.09 mg $L^{-1}$ as intermediate and individuals with iron reversibly adsorbed on root surface $\leq$ 3.40 mg $L^{-1}$ as low. Results indicated that out of the 300 $F_2$ plants, 13 $F_2$ plants possessed high iron reversibly adsorbed on root surface (4.33%), 177 $F_2$ plants had intermediate values (59%) while iron reversibly adsorbed on root surface was low in 110 $F_2$ individuals (36.67%).

#### 4.4.1.1.8. Iron content in root (mg $kg^{-1}$)

Mean iron content in root of 300 $F_2$ plants after 6 weeks of 800ppm of Fe treatment was 8746.52mg $kg^{-1}$. Iron content in root ranged from 6160.38 mg $kg^{-1}$ to 13932.69 mg $kg^{-1}$. Iron content in root of PGC 14 (Tulasi) and PGC 31 (Cul-8709) was 11918.52 mg $kg^{-1}$ and 6889.42 mg $kg^{-1}$ respectively.

Lowest iron content in roots of $F_2$ plants were observed in plant numbers 122 (6160.38 mg $kg^{-1}$), 269 (6323.25 mg $kg^{-1}$), 270 (6335.99 mg $kg^{-1}$), 105 (6337.06 mg $kg^{-1}$), 24 (6357.14 mg $kg^{-1}$), 35 (6383.93 mg $kg^{-1}$), 301 (6386.65 mg $kg^{-1}$), 12 (6391.67 mg $kg^{-1}$), 282 (6412.83 mg $kg^{-1}$) and 336 (6436.96 mg $kg^{-1}$) respectively. Highest iron content in roots of $F_2$ plants were observed in plant numbers 248 (13932.69 mg $kg^{-1}$), 300 (13762.20 mg $kg^{-1}$), 156 (13723.96 mg $kg^{-1}$), 320 (13685.57 mg $kg^{-1}$), 287 (13360.82 mg $kg^{-1}$), 309 (12979.04 mg $kg^{-1}$), 251 (12944.21 mg $kg^{-1}$), 110 (12691.29 mg $kg^{-1}$), 319 (12468.93 mg $kg^{-1}$) and 364 (12343.25 mg $kg^{-1}$) respectively.

Skewness and kurtosis of this observation is 0.70 and -0.37 respectively. Frequency distribution (Fig. 2) was used to determine the number of individuals in the segregating $F_2$ population that had iron content in root close to parent PGC 14 (Tulasi) (11918.52 mg $kg^{-1}$) and PGC 31 (Cul-8709) (6889.42 mg $kg^{-1}$) as well as intermediate

between the two. $F_2$ individuals with iron content in root $\geq$ 11918 mg $kg^{-1}$ were designated as having higher iron content in root; those with values between 6890.01 to 11917.99 mg $kg^{-1}$ as intermediate and individuals with iron content in root $\leq$ 6890 mg $kg^{-1}$ as low. Results indicated that out of the 300 $F_2$ plants, sixteen $F_2$ plants possessed high iron content in root (5.33%), 239 $F_2$ plants had intermediate values (79.67%) while iron content in root was low in forty five $F_2$ individuals (15%).

#### 4.4.1.1.9. Iron content in leaf (mg $kg^{-1}$)

Mean iron content in leaf of 300 $F_2$ plants after 6 weeks of 800ppm of Fe treatment was 1633.15 mg $kg^{-1}$. Iron content in leaf ranged from 656.25 mg $kg^{-1}$ of Fe to 3324.22 mg $kg^{-1}$ of Fe of Fe. Iron content in leaf of PGC 14 (Tulasi) and PGC 31 (Cul-8709) was 731.25 mg $kg^{-1}$ and 2258.75 mg $kg^{-1}$ respectively.

Lowest iron content in leaf were observed in plant numbers 320 (656.25 mg $kg^{-1}$), plant number 309 (658.75 mg $kg^{-1}$), 319 (665.00 mg $kg^{-1}$), 268 (678.75 mg $kg^{-1}$), 248 (688.75 mg $kg^{-1}$), 300 (691.25 mg $kg^{-1}$), 111 (692.50 mg $kg^{-1}$), 354 (706.25 mg $kg^{-1}$), 246 (716.25 mg $kg^{-1}$) and 156 (726.25 mg $kg^{-1}$) respectively. Highest leaf iron content were observed in plant numbers 39 (3324.22 mg $kg^{-1}$), 173 (3177.78 mg $kg^{-1}$), 164 (3153.74 mg $kg^{-1}$), 10 (3068.66 mg $kg^{-1}$), 291 (2952.50 mg $kg^{-1}$), 148 (2821.25 mg $kg^{-1}$), 65 (2816.58 mg $kg^{-1}$), 166 (2730.00 mg $kg^{-1}$), 50 (2691.25 mg $kg^{-1}$) and 87 (2682.50 mg $kg^{-1}$) respectively.

Skewness and kurtosis of this observation is 0.19 and -0.84 respectively. Frequency distribution (Fig. 2) was used to determine the number of individuals in the segregating $F_2$ population that had iron content in leaf close to parent PGC 14 (Tulasi) (731.25 mg $kg^{-1}$) and PGC 31 (Cul-8709) (2258.75 mg $kg^{-1}$) as well as intermediate between the two. $F_2$ individuals with iron content in leaf $\geq$ 2258 mg $kg^{-1}$ were designated as having higher iron content in leaf; those with values between 732.01 to 2257.99 mg $kg^{-1}$ as intermediate and individuals with iron content in leaf $\leq$ 732 mg $kg^{-1}$ as low. Results indicated that out of the 300 $F_2$ plants, 53 $F_2$ plants possessed high iron content in leaf (17.67%), 236 $F_2$ plants had intermediate values (78.67%) while iron content in leaf was low in eleven $F_2$ individuals (3.67%).

#### 4.4.1.1.10. Visual scoring for iron-toxicity symptoms (IRRI, 1996)

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.

Skewness and kurtosis of visual scoring for iron-toxicity symptoms after 4 weeks of 800ppm of Fe treatment is -0.14 and -1.41 respectively. Frequency distribution (Fig. 1) was used to determine the number of individuals in the segregating $F_2$ population that had visual scoring for iron-toxicity symptoms close to parent PGC 14 (Tulasi) (1) and PGC 31 (Cul-8709) (9) as well as intermediate between the two. $F_2$ individuals with visual scoring for iron-toxicity symptoms $\geq 9$ were designated as having higher visual scoring for iron-toxicity symptoms; those with values between 3 and 7 as intermediate and individuals with visual scoring for iron-toxicity symptoms $\leq 1$ as low. Results indicated that out of the 300 $F_2$ plants, 87 $F_2$ plants possessed high visual scoring for iron-toxicity symptoms (29%), 163 $F_2$ plants had intermediate values (54.33%) while visual scoring for iron-toxicity symptoms was low in 50 $F_2$ individuals (16.67%).

Skewness and kurtosis of visual scoring for iron-toxicity symptoms after 6 weeks of 800ppm of Fe treatment is -1.67 and 1.55 respectively. Frequency distribution (Fig. 1) was used to determine the number of individuals in the segregating $F_2$ population that had visual scoring for iron-toxicity symptoms close to parent PGC 14 (Tulasi) (1) and PGC 31 (Cul-8709) (9) as well as intermediate between the two. $F_2$ individuals with visual scoring for iron-toxicity symptoms $\geq 9$ were designated as having higher visual scoring for iron-toxicity symptoms; those with values between 3 and 7 as intermediate and individuals with visual scoring for iron-toxicity symptoms $\leq 1$ as low. Results indicated that out of the 300 $F_2$ plants, 206 $F_2$ plants possessed high visual scoring for iron-toxicity symptoms (68.67%), 79 $F_2$ plants had intermediate values (54.33%) while visual scoring for iron-toxicity symptoms was low in 15 $F_2$ individuals (5%).

#### 4.4.1.2. Correlation coefficient analysis

Iron toxicity tolerance is a complex character and is influenced by various other characters therefore it is essential to understand the association of other characters with Iron toxicity tolerance in addition to the information on genetic variability (Dufey *et al.*, 2015). Hence, association analysis was undertaken to determine the direction of selection and number of characteristics to be considered in improving iron toxicity tolerance. Data on correlation analysis and simple regression analysis of $F_2$ plants is presented in the table 20.

##### 4.4.1.2.1 Association between leaf bronzing and traits influenced under iron toxic condition

Results indicated the presence high significant positive correlation (0.71) between leaf bronzing score (visual scoring for iron-toxicity symptoms) and iron content in the leaf while the correlation between leaf bronzing score and traits root length (-0.66), shoot length (-0.76), total number of roots (-0.81), number of fresh roots (-0.98), root weight (-0.72), shoot weight (-0.83), iron reversibly adsorbed on root surface (-0.94) and iron content in the root (-0.61) was high significant and negative.

##### 4.4.1.2.2. Inter-correlation among traits influenced under iron toxic condition

###### 4.4.1.2.2.1. Shoot length (cm)

Shoot length exhibited high significant positive correlation with root 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 with values 0.79, 0.85, 0.78, 0.75, 0.82, 0.78 and 0.54 respectively. However, it recorded high significant negative correlation with leaf bronzing score (-0.76) and iron content in leaf (-0.63).

###### 4.4.1.2.2.2. Root length (cm)

The correlation between root length was high significant and positive with 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 with values 0.79, 0.80, 0.68, 0.71, 0.70, 0.68 and 0.40 respectively. However, it recorded high significant negative correlation with leaf bronzing score (-0.66) and iron content in leaf (-0.55).

**Table 20. Correlation coefficients among leaf bronzing score and growth traits influenced under iron toxic condition (800 ppm Fe)**

| Character | Leaf bronzing after 6 weeks | Root length | Shoot length | Total number of roots | Number of fresh roots | Root weight | Shoot weight | Iron adsorbed on root surface | Iron content in root | Leaf iron content |
|---|---|---|---|---|---|---|---|---|---|---|
| Leaf bronzing after 6 weeks | 1.00 | | | | | | | | | |
| Root length (cm) | -0.66** | 1.00 | | | | | | | | |
| Shoot length (cm) | -0.76** | 0.79** | 1.00 | | | | | | | |
| Total number of roots | -0.81** | 0.80** | 0.85** | 1.00 | | | | | | |
| Number of fresh roots | -0.98** | 0.68** | 0.78** | 0.84** | 1.00 | | | | | |
| Root weight (g) | -0.72** | 0.71** | 0.75** | 0.78** | 0.74** | 1.00 | | | | |
| Shoot weight (g) | -0.83** | 0.70** | 0.82** | 0.82** | 0.84** | 0.85** | 1.00 | | | |
| Iron adsorbed on root surface (mg $L^{-1}$) | -0.94** | 0.68** | 0.78** | 0.83** | 0.96** | 0.75** | 0.83** | 1.00 | | |
| Iron content in root (mg $kg^{-1}$) | -0.61** | 0.40** | 0.54** | 0.53** | 0.64** | 0.41** | 0.52** | 0.67** | 1.00 | |
| Iron content in oldest leaf (mg $kg^{-1}$) | 0.71** | -0.55** | -0.63** | -0.69** | -0.76** | -0.57** | -0.64** | -0.79** | -0.62** | 1.00 |

*significant at 5% level; **significant at 1% level

#### 4.4.1.2.2.3. Total number of roots

Total number of roots showed high significant positive correlation with root length, shoot length, number of fresh roots, root weight, shoot weight, iron reversibly adsorbed on root surface and iron content in the root with values 0.80, 0.85, 0.84, 0.78, 0.82, 0.83 and 0.53 respectively. However, it recorded high significant negative correlation with leaf bronzing score (-0.81) and iron content in leaf (-0.69).

#### 4.4.1.2.2.4. Number of fresh roots

The number of fresh roots showed high significant positive correlation with root length, shoot length, total number of roots, root weight, shoot weight, iron reversibly adsorbed on root surface and iron content in the root with values 0.68, 0.78, 0.84, 0.74, 0.84, 0.96 and 0.64 respectively. However, it recorded high significant negative correlation with leaf bronzing score (-0.98) and iron content in leaf (-0.76).

#### 4.4.1.2.2.5. Shoot weight (g)

Shoot weight showed high significant positive correlation with root length, shoot length, total number of roots, number of fresh roots, root weight, iron reversibly adsorbed on root surface and iron content in the root with values 0.70, 0.82, 0.82, 0.84, 0.85, 0.83 and 0.52 respectively. However, it recorded high significant negative correlation with leaf bronzing score (-0.83) and iron content in leaf (-0.64).

#### 4.4.1.2.2.6. Root weight (g)

Root weight showed high significant positive correlation with root length, shoot length, total number of roots, number of fresh roots, shoot weight, iron reversibly adsorbed on root surface and iron content in the root with values 0.71, 0.75, 0.78, 0.74, 0.85, 0.75 and 0.41 respectively. However, it recorded high significant negative correlation with leaf bronzing score (-0.72) and iron content in leaf (-0.57).

#### 4.4.1.2.2.7. Iron reversibly adsorbed on root surface (mg $L^{-1}$)

Iron reversibly adsorbed on root surface showed high significant positive correlation with root length, shoot length, total number of roots, number of fresh roots, root weight, shoot weight and iron content in the root with values 0.68, 0.78, 0.83, 0.96, 0.75, 0.83 and 0.67 respectively. However, it recorded high significant negative correlation with leaf bronzing score (-0.94) and iron content in leaf (-0.79).

#### 4.4.1.2.2.8. Iron content in root (mg $kg^{-1}$)

Iron content in the root showed high significant positive correlation with root length, shoot length, total number of roots, number of fresh roots, root weight, shoot weight and iron reversibly adsorbed on root surface with values 0.40, 0.54, 0.53, 0.64, 0.41, 0.52 and 0.67 respectively. However, it recorded high significant negative correlation with leaf bronzing score (-0.61) and iron content in leaf (-0.62).

#### 4.4.1.2.2.9. Iron content in leaf (mg $kg^{-1}$)

Iron content in leaf showed high significant negative correlation with 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 with values -0.55, -0.63, -0.69, -0.76, -0.57, -0.64, -0.79 and -0.62 respectively. However, it recorded high significant negative correlation with leaf bronzing score (0.71).

### 4.4.1.3. Selection of plants to constitute the resistant bulk (RB) and susceptible bulk (SB)

From phenotypic screening of 300 $F_2$ plants for iron toxicity tolerance, ten plants each were selected to constitute the resistant bulk (RB) and susceptible bulk (SB) based on their reaction to iron stress as per the method applied by Shimizu *et al.* (2005). The most tolerant $F_2$ plants selected to constitute the RB had recorded a leaf bronzing score of 1 after 6 weeks of exposure to iron stress (800ppm). However the ten susceptible $F_2$ plants selected to constitute the SB had exhibited a leaf bronzing score of 9 at 4 weeks of exposure to 800ppm of Fe.

A total of 15 $F_2$ plants (Table 21 and Appendix II) 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 (Table 21). 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 21 and Table 28).

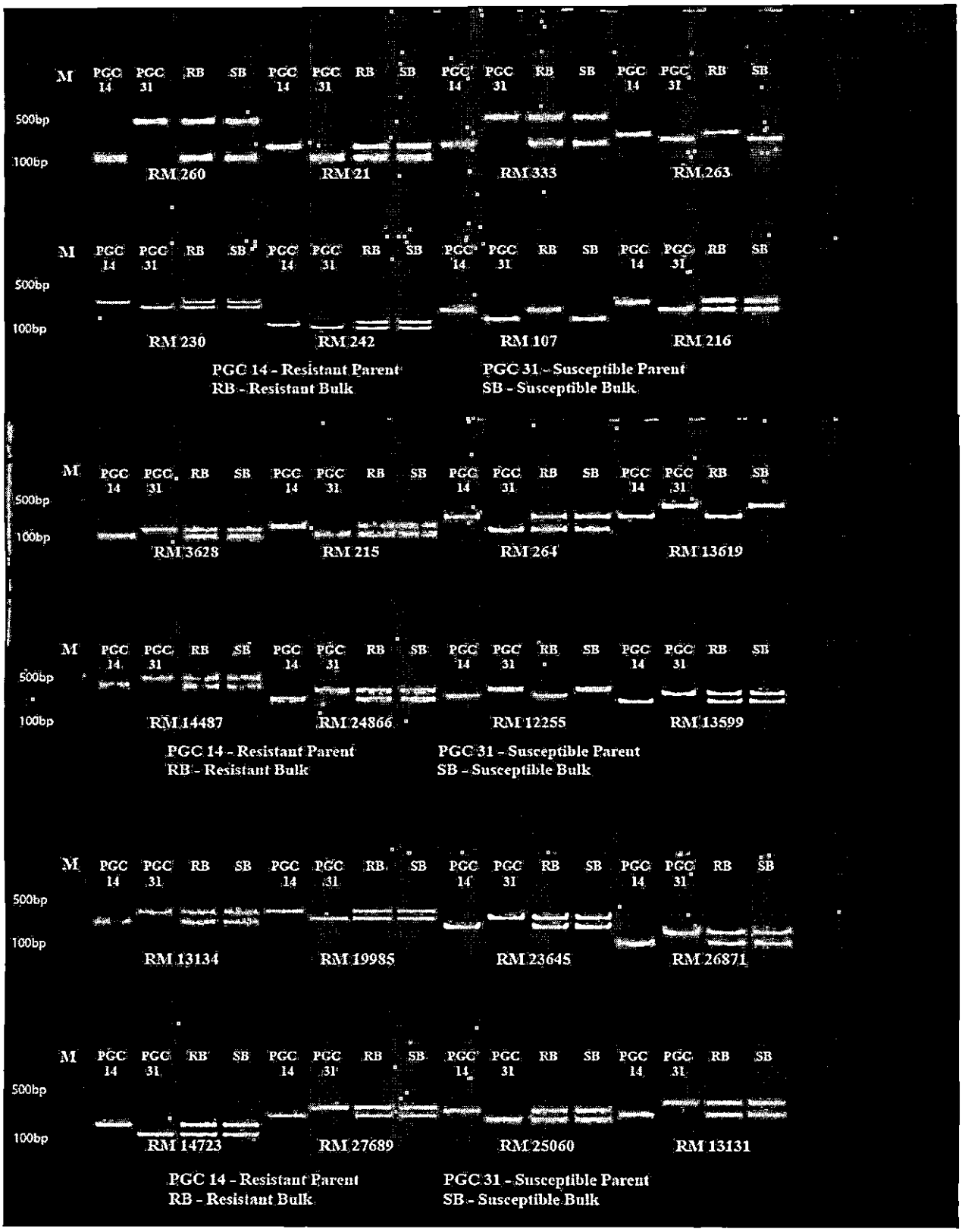
Through a similar exercise, out of the 87 $F_2$ plants (Appendix II) that recorded a leaf bronzing score of 9 on four weeks of exposure to iron stress (800ppm of Fe), ten most susceptible $F_2$ plants were selected. In this instance unlike in the above case, the ten plants with the least total score 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 21, Table 29 and Fig 3).

### 4.4.2. Genotyping parents, susceptible and resistant bulks

Thirty-seven RM markers that were observed to be polymorphic between parents PGC 14 (Tulasi) and PGC 31 (Cul-8709) under Experiment 2 (Table 18) were used for genotyping parents, susceptible bulk (SB) and resistant bulks (RB) through bulk segregant analysis. The score sheet of banding pattern in parents, SB and RB is detailed in plates 12 & 13 and table 22.

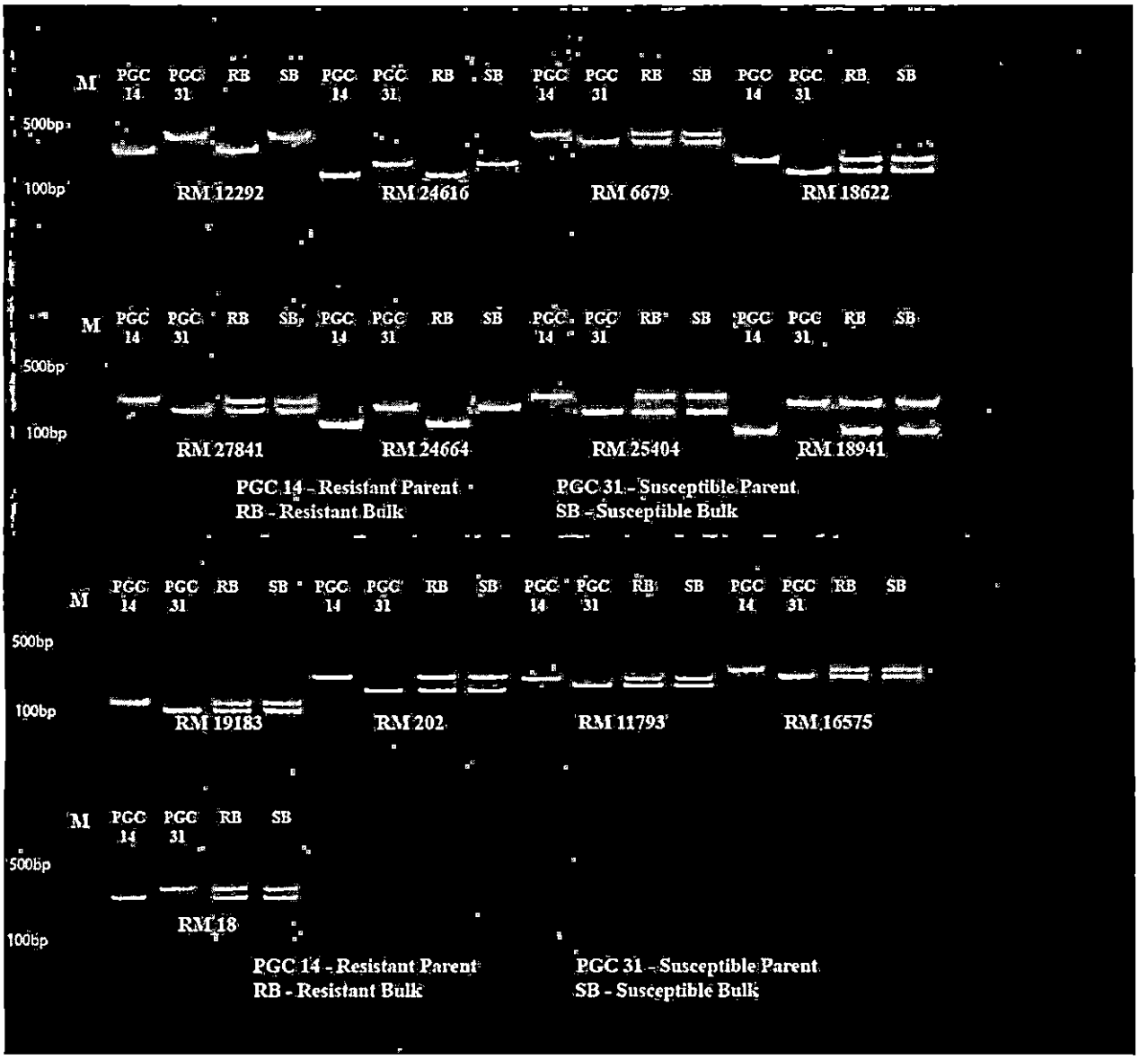
Seven markers out of thirty-seven RM markers were found to co-segregate with the resistant parent and resistant bulk and with the susceptible parent and susceptible bulk. The seven markers that were found to be co-segregating are RM 12292, RM 12255, RM 13619, RM 263, RM 107, RM 24616 and RM 24664. Of these markers, RM 12292 and RM 12255 markers were present on chromosome number 1 while, markers RM 13619 and RM 263 were present on chromosome 2. Markers RM 107, RM 24616 and RM 24664 were presented on chromosome 9.

**Plate 12. Genotyping resistant bulk (RB), susceptible bulk (SB) bulk and parents with 37 RM markers through Bulk segregant analysis (I)**




M – Ladder (1kb)

Plate 13. Genotyping resistant bulk (RB), susceptible bulk (SB) bulk and parents with 37 RM markers through Bulk segregant analysis (II)




M – Ladder (1kb)

**Table 21. Phenotypic data of individual $F_2$ plants and parents used for selective genotyping at 800ppm of iron in BSA**

| $F_2$ plant no | Leaf bronzing score ($4^{th}$ week) | Leaf bronzing score ($6^{th}$ week) | Number of roots | Root length (cm) | Shoot length (cm) | Root weight (g) | Shoot weight (g) | Number 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 |
| 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 |
| *PGC14 | 1 | 1 | 31 | 23.4 | 61.2 | 7.80 | 11.30 | 27 | 12.19 | 11918.52 | 731.25 |
| **PGC31 | 9 | 9 | 22 | 16.3 | 49.7 | 2.95 | 3.950 | 0 | 3.36 | 6889.42 | 2258.75 |

* PGC 14 (Tulasi) – Resistant parent **PGC 31 (Cul-8709) – Susceptible parent

**Table 22. Data on genotyping resistant bulk (RB), susceptible bulk (SB) and parents using 37 markers found polymorphic between parents.**

| Rice microsatellite | PGC 14 - Tulasi (Resistant parent) | Resistant bulk | PGC 31-Cul-8709 (Susceptible parent ) | Susceptible bulk |
|---|---|---|---|---|
| RM 12292 | 0 | 0 | 1 | 1 |
| RM 24616 | 0 | 0 | 1 | 1 |
| RM 13134 | 0 | 2 | 1 | 2 |
| RM 19183 | 0 | 2 | 1 | 2 |
| RM 25404 | 0 | 2 | 1 | 2 |
| RM 19985 | 0 | 2 | 1 | 2 |
| RM 263 | 0 | 0 | 1 | 1 |
| RM 216 | 0 | 2 | 1 | 2 |
| RM 230 | 0 | 2 | 1 | 2 |
| RM 107 | 0 | 0 | 1 | 1 |
| RM 242 | 0 | 2 | 1 | 2 |
| RM 333 | 0 | 2 | 1 | 2 |
| RM 202 | 0 | 2 | 1 | 2 |
| RM 21 | 0 | 2 | 1 | 2 |
| RM 260 | 0 | 2 | 1 | 2 |
| RM 26871 | 0 | 2 | 1 | 2 |
| RM 24664 | 0 | 0 | 1 | 1 |
| RM 23645 | 0 | 2 | 1 | 2 |
| RM 3628 | 0 | 2 | 1 | 2 |
| RM 215 | 0 | 2 | 1 | 2 |
| RM 264 | 0 | 2 | 1 | 2 |
| RM 12255 | 0 | 0 | 1 | 1 |
| RM 13599 | 0 | 2 | 1 | 2 |
| RM 14723 | 0 | 2 | 1 | 2 |
| RM 18622 | 0 | 2 | 1 | 2 |
| RM 18941 | 0 | 2 | 1 | 2 |
| RM 13619 | 0 | 0 | 1 | 1 |
| RM 14487 | 0 | 2 | 1 | 2 |
| RM 24866 | 0 | 2 | 1 | 2 |
| RM 13131 | 0 | 2 | 1 | 2 |
| RM 6679 | 0 | 2 | 1 | 2 |
| RM 25060 | 0 | 2 | 1 | 2 |
| RM 27841 | 0 | 2 | 1 | 2 |
| RM 11793 | 0 | 2 | 1 | 2 |
| RM 16575 | 0 | 2 | 1 | 2 |
| RM 27689 | 0 | 2 | 1 | 2 |
| RM 18 | 0 | 2 | 1 | 2 |

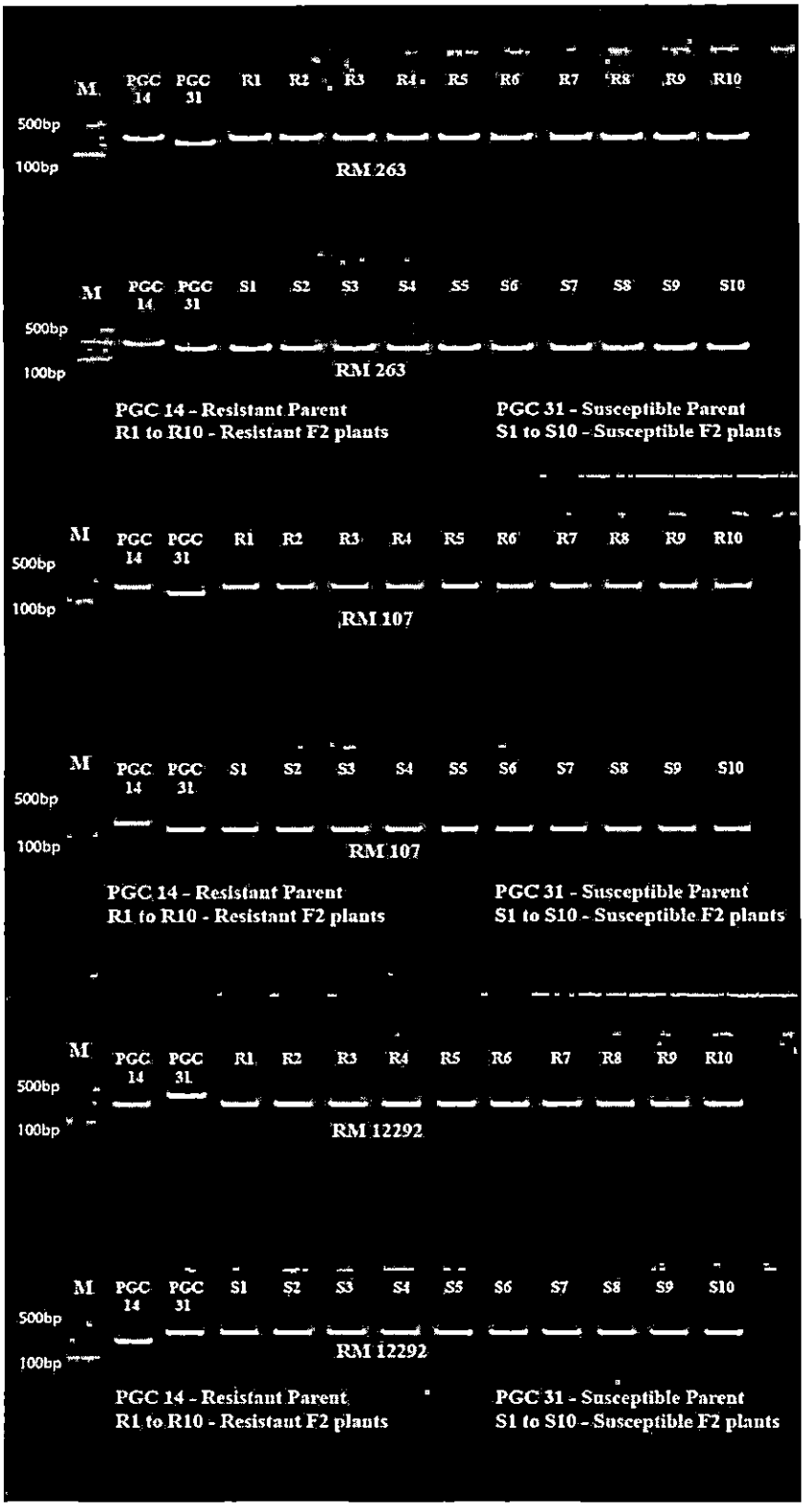
0: Monomorphic band as in PGC 14 - Tulasi (Resistant parent)
1: Monomorphic band as in PGC 31-Cul-8709 (Susceptible parent)
2: Bands of two parents present (heterozygote)

Analysis of the individual $F_2$ plants in both the susceptible bulk (SB) and resistant bulks (RB) with the seven RM markers (Table 23 and Plates 14 to 16) indicated that all the individuals in the resistant bulk exhibited the resistant parent allele, while, in all the individuals constituting the susceptible bulk, the allele for the seven markers were the same as in the susceptible parent.

### 4.4.3. Confirmation of putative markers

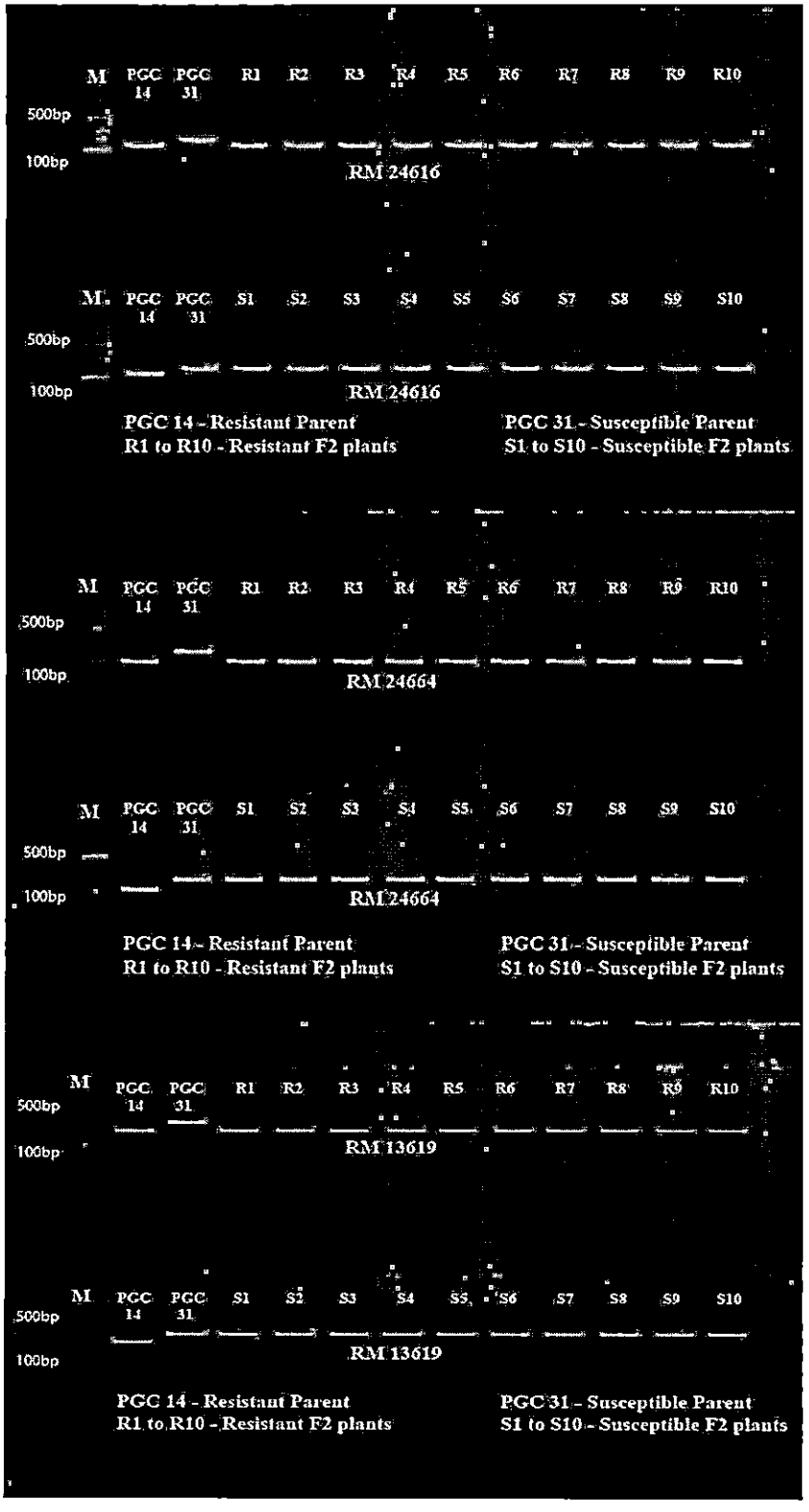
Seven RM markers like RM 263, RM 107, RM 12292, RM 24616, RM 24664, RM 13619 and RM 12255 were significantly ($P < 0.001$) associated with the variation of visual scoring for iron-toxicity symptoms (leaf bronzing index). RM 12255 and RM 12292 markers were significantly ($P < 0.001$) associated with the variation of leaf bronzing index on chromosome number 1. Similarly, RM 263 and RM 13619 markers on chromosome number 2 and RM 107, RM 24616 and RM 24664 markers on chromosome number 9 were significantly ($P < 0.001$) associated with the variation of leaf bronzing index. Remaining RM markers were not significantly associated with the variation of the visual scoring for iron-toxicity symptoms (leaf bronzing index). A total of three QTLs with LOD values of 8.0, 4.5 and 6.9 respectively were mapped on chromosome 1, 2, and 9 respectively (Fig 4).

**Plate 14. Selective genotyping of individual $F_2$ plants of bulks and parents with RM 263, RM 107 and RM 12292**



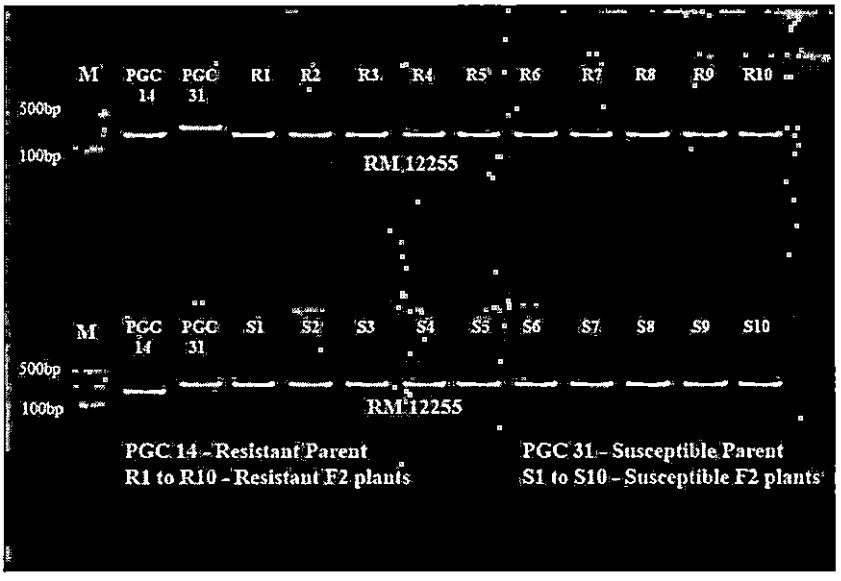

M – Ladder (1kb)

**Plate 15. Selective genotyping of individual $F_2$ plants of bulks and parents with RM 24616, RM 24664 and RM 13619**




M – Ladder (1kb)

**Plate 16. Selective genotyping of individual $F_2$ plants of bulks and parents with RM 12255**




M – Ladder (1kb)

**Table 23. Genotypic data of individual $F_2$ plants and parents used for selective genotyping in BSA**

| Plant number | RM 263 | RM 107 | RM 13619 | RM 12255 | RM 12292 | RM 24616 | RM 24664 |
|---|---|---|---|---|---|---|---|
| 248 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 320 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 309 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 111 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 156 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 287 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 268 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 52 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 300 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 202 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 66 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 18 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 109 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 113 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 122 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 334 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 231 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 213 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| *PGC14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **PGC31 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

0: Monomorphic band as in PGC 14 - Tulasi (Resistant parent)

1: Monomorphic band as in PGC 31-Cul-8709 (Susceptible parent)

# Discussion

# V. DISCUSSION

Rice grown on flooded acid soils is often subjected to iron (Fe) toxicity. Iron an essential element in plants is found to be involved in many crucial physiological processes. However, when provided in excess, iron can also be toxic. In well-aerated soils, Fe is present as ferric hydroxides with low plant availability (Conte and Walker 2011). Under anaerobic soils and at low redox potential (Eh), Fe is reduced from its less available from ferric form ($Fe^{3+}$) to its soluble form $Fe^{2+}$ (Ferrous ion) which is taken up excessively by plants. In plant tissues, $Fe^{2+}$ participates in Fenton reactions, catalyzing the generation of hydroxyl radicals (-OH) and other reactive oxygen species (ROS) (Thongbai and Goodman 2000).

The typical symptoms associated with iron toxicity are leaf discoloration (bronzing) and reddish spots (Ponnamperuma *et al.,* 1955; Sahrawat, 2010). Yield losses associated with iron toxicity usually range from 30 per cent to 60 per cent (Majerus *et al.,* 2007; Sahrawat, 2010). In the case of severe toxicity at younger stage, complete crop failure can occur (Audebert and Sahrawat, 2000).

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). Since resistance to iron toxicity is a complex trait, controlled by several genes, QTL mapping combined with marker-assisted selection appears as a viable approach for improving tolerance to iron stress (Mackill *et al.*, 1999). Hence, the present study aimed to identify markers linked to the genomic area conferring tolerance to iron toxicity by analyzing the genotyping and phenotyping data through bulk segregant analysis.

The results of the present investigation have been discussed under the following headings.

5.1. Experiment 1: Hybridization programme

5.1.1. Parental selection

5.1.1.1 Confirmation test – 1

5.1.1.2 Confirmation test – 2

5.1.2. Hybridization

5.2. Experiment 2: Study of parental polymorphism study using molecular markers

5.3. Experiment 3: Raising of $F_1$'s

5.4. Experiment 4: Bulk Segregant Analysis (BSA)

5.4.1. Phenotyping of $F_2$ plants for iron toxicity tolerance

5.4.2. Genotyping parents, susceptible and resistant bulks

5.4.3. Confirmation of putative markers

## 5.1. Experiment 1: Hybridization programme

### 5.1.1. Parental selection

#### 5.1.1.1 Confirmation test-1

Thirty rice genotypes were selected from the KSCSTE project: 'Donor identification for tolerance to iron toxicity in rice (*Oryza sativa* L.)' and screened for their tolerance to iron toxicity. The extent of tolerance was assessed based on the degree of leaf bronzing on exposure to iron stress.

Variance due to genotypes (Table 8) at all three levels of iron (control, 600ppm of Fe and 800ppm of Fe) was found highly significant for visual bronzing scores (toxicity) which indicated that the genotypes differ significantly for this trait.

At 600 ppm of Fe, genotypes IVT-33, Cul-8723 and Cul-18716 exhibited lower leaf bronzing score (≤5) (Table 9) as per standard evaluation score (IRRI, 2006). Iron toxicity occurs when the rice plant accumulates a toxic concentration of Fe in the leaves (Sahrawat, 2010). The degree of leaf bronzing has been suggested to be a good measure of the severity of Fe toxicity in flooded rice (Fageria *et al.,* 2003). Genotypes Cul-8755, Karangi, Kargi and Tulasi with a LBI between 5.1 and 5.6 were found next best to the genotypes listed earlier in terms of tolerance to iron stress. Existence of such variability at genotypic level across genotypes in response to Fe toxicity has been reported earlier. Several rice cultivars have been reported to be resistant to this constraint (Gunawardena *et al.,* 1982; Fageria and Rabelo, 1987; Fageria *et al.,* 1990; De Datta *et al.*, 1994; Sahrawat and Sika, 2002; Sahrawat, 2004; Shimizu *et al.,* 2005; Balasubramanian *et al.*, 2007; Nozoe *et al.*, 2008; Majerus *et al.,* 2009; Sahrawat, 2010; Samaranayake *et al.,* 2012 and Onaga *et al.,* 2013a)

Among the genotypes listed, at a higher concentration of Fe (800 ppm), Cul-8723, Cul-18716, Tulasi, IVT-33 and Kargi exhibited the lower LBI (<6.0). Cul-8723 exhibited the least leaf bronzing score (4.7) at 800 ppm and was significantly different from all other genotypes while genotypes Tulasi, Cul-18716, IVT 33 and Kargi were on par with each other and found next best to Cul-8723. These genotypes had also exhibited lower scores of leaf bronzing at 600 ppm of Fe. Adequate Fe concentration in the plant tissue is reported to be in the range of 70 to 300 mg $kg^{-1}$ (Welch *et al.,* 1993). A soil solution concentration of 300 mg water soluble Fe $l^{-1}$ is generally considered the critical limit for the cultivation of lowland rice (Lantin and Neue, 1989). Iron toxicity is considered to occur at concentrations above this sufficiency range (Tanaka and Yoshida, 1972). According to Fageria *et al.* (1981) the level of Fe that can be toxic to crop performance is also dependent on rice cultivars. Lower leaf bronzing at higher concentrations of Fe can be considered as a reliable technique to identify genotypes tolerant to Fe stress. Hence, the five genotypes (IVT-33, Cul-8723, Cul-18716, Kargi and Tulasi) that exhibited lower LBI even at higher (800ppm) of iron were selected as genotypes tolerant to iron stress for further studies.

At 600 ppm of Fe, most of the genotypes registered a leaf bronzing score of above 7.5 indicating susceptible reaction to Fe stress. Genotypes ASD 18, Cul 8709, PM 706, IR-1552, ASD (Peringotukurussi), CUL-210-29, CUL-90-03, PM 709 and CSR 13 with a score ranging between 8 and 9 were considered highly susceptible to Fe stress. In lieu with the findings of Fageria *et al.* (1981) mentioned earlier, twelve rice genotypes Cul-8709, IR-1552, ASD-18, Cul-210-29, Cul-90-03, PM-709, AM-10-7, IR-36, ASD-16, Abhaya, T(N)-1 and Cul-3 that exhibited higher leaf bronzing score and higher normalized score values at 600 ppm of Fe treatment were identified as genotypes highly susceptible to iron stress.

In addition to leaf bronzing score, reduction in biomass has been a valid criterion for identifying genotypes tolerant to Fe stress. In the present study, among the 30 genotypes screened, biomass *per se* of Tulasi (0.75g) followed by Cul-18716 (0.73g), Kargi and Cul-8723 (0.72g each) were high at 800ppm of Fe. These genotypes showed higher biomass *per se* at 600ppm of Fe also. The reduction in biomass over control in these genotypes ranged between 1.15 per cent and 29.76 per cent at 600ppm

of Fe and 10.00 per cent to 38.46 per cent at 800ppm of Fe (Table 10). In addition to these four genotypes, Kargi had also registered a lower reduction in biomass at 800ppm Fe. The lower LBI scores coupled with higher biomass recorded by these genotypes emphasized their ability to tolerate Fe stress. This justifies their selection as genotypes tolerant to iron toxicity for further studies. Similar to the present study, Nayak *et al.* (2008) had evaluated 65 genotypes for their tolerance to excessive iron and found that irrespective of their growth duration, tolerant genotypes produced higher biomass than the iron-susceptible cultivars.

Onaga *et al.* (2013a) reported that the genotypes K98, PNA, IR73678-20-1-B and WITA4 found to be tolerant to iron toxicity produced relatively stable biomass levels in iron toxic field. The reduction in biomass was found to be the least in these genotypes. In consonance with this argument, genotypes Cul-8709, Cul-210-29 and AM-10-7 that registered a higher reduction in biomass (29.76%, 28.57% and 28.00% respectively) at 600ppm of Fe were identified as highly susceptible to iron stress. Onaga *et al.* (2013a) had also observed significant biomass reductions in the highly susceptible genotypes SUPA, K85, Kayiso and NS4.

The selection of genotypes for further studies was based on the ranking of individuals considering both LBI and biomass as per the procedure advocated by Arunachalam and Bandopadhyay (1984). Each rice genotype was ranked in serratum based on the magnitude of biomass in consideration of the DMRT test values *i.e.*, individuals with DMRT annotation 'a' were assigned rank 1, 2 for individuals with DMRT annotation 'ab', 3 for 'abc' and so on. Hence, higher the biomass of the genotype lower will be the numerical value of the rank.

For ranking individuals based on LBI, normalized LBI score, and per cent reduction in biomass over control at 600 and 800ppm of Fe treatment, the reverse format was followed *i.e.*, the individuals were ranked in descending order of magnitude. An individual with least score was assigned rank 1, the next 2 and so on. Therefore, individuals with the least LBI score, normalized LBI score, and per cent reduction in biomass over control at 600 and 800ppm of Fe treatment were ranked 1, 2 and so on. The corresponding DMRT annotations of the genotypes were also taken into consideration while ranking genotypes based on LBI score (Table 24). Final ranking of

**Table 24. Ranking of genotypes based on LBI, normalized LBI, biomass *per se* and reduction of biomass (%) at 600ppm of Fe stress (Confirmation test-1)**

| Sl. No. | PGC No | Genotype | Ranking of genotypes | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | LBI (1) | Normalized LBI score (2) | Biomass (3) | Reduction of biomass (%) (4) | Total score (1 + 2+ 3+ 4) | Final ranking |
| 1 | 33 | Cul-18714 | 6 | 4 | 12 | 3 | 25 | 6 |
| 2 | 60 | PM-709 | 6 | 19 | 12 | 26 | 63 | 19 |
| 3 | 48 | ASD-16 | 6 | 18 | 12 | 25 | 61 | 18 |
| 4 | 115 | IVT-33 | 1 | 7 | 5 | 5 | 18 | 2 |
| 5 | 34 | Cul-18716 | 3 | 6 | 2 | 6 | 17 | 1 |
| 6 | 46 | Abhaya | 5 | 25 | 10 | 20 | 60 | 17 |
| 7 | 12 | Kanchana | 5 | 7 | 12 | 14 | 38 | 12 |
| 8 | 29 | Cul-8759 | 5 | 14 | 5 | 2 | 26 | 7 |
| 9 | 192 | CSR 13 | 6 | 15 | 13 | 4 | 38 | 12 |
| 10 | 104 | Cul-210-29 | 6 | 26 | 11 | 28 | 71 | 22 |
| 11 | 157 | Moncompu-519 | 6 | 11 | 7 | 8 | 32 | 9 |
| 12 | 39 | Cul-3 | 5 | 17 | 7 | 23 | 52 | 14 |
| 13 | 133 | AM-10-7 | 6 | 24 | 12 | 27 | 69 | 21 |
| 14 | 14 | Tulasi | 5 | 13 | 1 | 1 | 20 | 3 |
| 15 | 17 | IR-36 | 6 | 20 | 6 | 21 | 53 | 15 |
| 16 | 50 | PTB-10 | 5 | 16 | 7 | 15 | 43 | 13 |
| 17 | 43 | ASD-18 | 7 | 22 | 9 | 22 | 60 | 17 |
| 18 | 100 | Cul-90-03 | 6 | 21 | 14 | 24 | 65 | 20 |
| 19 | 31 | Cul-8709 | 7 | 27 | 12 | 29 | 75 | 23 |
| 20 | 28 | T(N)-1 | 5 | 25 | 9 | 19 | 58 | 16 |
| 21 | 20 | IR-1552 | 7 | 23 | 9 | 19 | 58 | 16 |
| 22 | 84 | ASD (Peringotukurussi) | 6 | 5 | 8 | 18 | 37 | 11 |
| 23 | 59 | PM-706 | 7 | 9 | 4 | 16 | 36 | 10 |
| 24 | 64 | PM-717 | 5 | 10 | 4 | 7 | 26 | 7 |
| 25 | 27 | Cul-8755 | 4 | 4 | 7 | 9 | 24 | 5 |
| 26 | 16 | Supriya | 6 | 1 | 6 | 12 | 25 | 6 |
| 27 | 73 | Karangi | 5 | 3 | 3 | 10 | 21 | 4 |
| 28 | 36 | Cul-8723 | 2 | 12 | 4 | 11 | 29 | 8 |
| 29 | 125 | JM-10-31 | 5 | 8 | 6 | 13 | 32 | 9 |
| 30 | 71 | Kargi | 5 | 2 | 5 | 17 | 29 | 8 |

individuals were done considering the summation of score obtained by the genotype for each of the above criterion (LBI score, normalized LBI score, biomass *per se* and per cent in reduction of biomass). Individuals with the least total score were therefore assigned final rank 1. The lower rank of a genotype is an indication that it is less affected by iron stress. It is an indication that the genotype has registered lower leaf bronzing score and reduction in biomass when exposed to Fe stress.

Ranking of genotypes following the procedure mentioned above revealed that genotypes Cul-8709, Cul-210-29, AM-10-7, Cul-90-03, PM-709, ASD-16, ASD-18, Abhaya, IR-1552, T(N)-1, IR-36 and Cul-3 recorded the highest score values at 600ppm of Fe treatment. Hence, these 12 rice genotypes were considered as highly susceptible to iron toxicity (Table 24).

Similar ranking of genotypes at 800ppm of Fe treatment indicated that Cul-8723, Tulasi, Cul-18716, Kargi and IVT-33 recorded lower total score. Therefore these five were considered as most tolerant to iron stress. Though Supriya had higher total score, it was not considered owing to higher normalized leaf bronzing score (Table 25).

The shortlisted 17 genotypes comprising of 12 highly susceptible and five most tolerant genotypes were selected for further screening for tolerance to iron stress under confirmation test 2.

### 5.1.1.2 Confirmation test – 2

Variance due to genotypes (Table 11) was found highly significant for visual bronzing scores (toxicity) indicating that the genotypes differ significantly for this trait at all three levels of iron (control, 600ppm of Fe and 800ppm of Fe).

At 600 ppm of Fe, most of the genotypes out of the total seventeen except T(N)-1, Cul-8723, ASD-18, Cul-18716 and IR-36, registered a leaf bronzing score of above 6.5 (Table 12) indicating their susceptibility to Fe stress. Cul-8709 recorded the highest leaf bronzing score of 9.0 at 600 ppm. Genotypes IR-1552 and Cul-90-03 also exhibited a high LBI very similar to Cul-8709. These had also exhibited higher normalized score values at 600 ppm of Fe. Considering the above, all three genotypes

**Table 25. Ranking of genotypes based on LBI, normalized LBI, biomass *per se* and reduction of biomass (%) at 800ppm of Fe stress (Confirmation test-1)**

| Sl. No. | PGC No | Genotype | Ranking of genotypes | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | LBI (1) | Normalized LBI score (2) | Biomass (3) | Reduction of biomass (%) (4) | Total score (1 + 2+ 3+ 4) | Final ranking |
| 1 | 33 | Cul-18714 | 6 | 5 | 13 | 8 | 32 | 7 |
| 2 | 60 | PM-709 | 6 | 19 | 11 | 23 | 59 | 21 |
| 3 | 48 | ASD-16 | 6 | 22 | 10 | 19 | 57 | 20 |
| 4 | 115 | IVT-33 | 2 | 11 | 5 | 3 | 21 | 5 |
| 5 | 34 | Cul-18716 | 2 | 9 | 2 | 2 | 15 | 3 |
| 6 | 46 | Abhaya | 6 | 27 | 11 | 23 | 67 | 26 |
| 7 | 12 | Kanchana | 6 | 8 | 13 | 16 | 43 | 12 |
| 8 | 29 | Cul-8759 | 4 | 12 | 5 | 3 | 24 | 6 |
| 9 | 192 | CSR 13 | 7 | 14 | 14 | 9 | 44 | 13 |
| 10 | 104 | Cul-210-29 | 7 | 25 | 8 | 25 | 65 | 24 |
| 11 | 157 | Moncompu-519 | 5 | 7 | 12 | 24 | 48 | 16 |
| 12 | 39 | Cul-3 | 7 | 23 | 4 | 11 | 45 | 14 |
| 13 | 133 | AM-10-7 | 7 | 26 | 9 | 20 | 62 | 22 |
| 14 | 14 | Tulasi | 2 | 7 | 1 | 4 | 14 | 2 |
| 15 | 17 | IR-36 | 6 | 24 | 5 | 15 | 50 | 17 |
| 16 | 50 | PTB-10 | 5 | 15 | 6 | 6 | 32 | 7 |
| 17 | 43 | ASD-18 | 7 | 20 | 7 | 13 | 47 | 15 |
| 18 | 100 | Cul-90-03 | 6 | 21 | 15 | 27 | 69 | 27 |
| 19 | 31 | Cul-8709 | 7 | 25 | 8 | 26 | 66 | 25 |
| 20 | 28 | T(N)-1 | 7 | 28 | 6 | 10 | 51 | 18 |
| 21 | 20 | IR-1552 | 7 | 23 | 11 | 23 | 64 | 23 |
| 22 | 84 | ASD (Peringotukurussi) | 7 | 2 | 9 | 17 | 35 | 8 |
| 23 | 59 | PM-706 | 7 | 3 | 6 | 21 | 37 | 10 |
| 24 | 64 | PM-717 | 7 | 13 | 4 | 12 | 36 | 9 |
| 25 | 27 | Cul-8755 | 6 | 17 | 11 | 18 | 52 | 19 |
| 26 | 16 | Supriya | 6 | 1 | 5 | 5 | 17 | 4 |
| 27 | 73 | Karangi | 7 | 16 | 4 | 14 | 41 | 11 |
| 28 | 36 | Cul-8723 | 1 | 6 | 3 | 1 | 11 | 1 |
| 29 | 125 | JM-10-31 | 7 | 10 | 12 | 22 | 51 | 18 |
| 30 | 71 | Kargi | 3 | 4 | 3 | 7 | 17 | 4 |

(Cul-8709, IR-1552 and Cul-90-03) were selected as susceptible parents for further studies.

At 800 ppm of Fe, almost all genotypes except Tulasi and Cul-18716 had registered a leaf bronzing score of above 7.4. Of the two, Tulasi exhibited the least leaf bronzing score (5.4) at 800 ppm and was significantly different from all other genotypes while Cul-18716 (5.7) was found next best to Tulasi. These genotypes had also exhibited lower scores of leaf bronzing at 600 ppm of Fe (Table 12).

Reduction in biomass has been a valid criterion for identifying genotypes tolerant to Fe stress, in addition to leaf bronzing score. In the present study, among the 17 genotypes screened biomass *per se* (Table 12) of Cul-18716 (0.81g) followed by Tulasi (0.75g), ASD-18 and Cul 3 (0.67g each) were high at both 600 and 800ppm Fe stress. The reduction in biomass over control in these genotypes ranged between 3.77% and 13.79% at 600ppm of Fe while it was between 6.29% and 22.99% at 800ppm of Fe (Table 13). The lower LBI scores coupled with higher biomass recorded by these genotypes emphasized their ability to tolerate Fe stress. Hence, the two genotypes (Tulasi and Cul-18716) were selected as resistant parents for further studies. The selections of these two genotypes as the tolerant parent were further confirmed by ranking of individuals (Table 27) at 800ppm of Fe as enumerated under 5.1.1.2. Confirmation test – 1. Results revealed that these genotypes Tulasi and Cul-18716 recorded the least total score.

Further, a similar ranking of genotypes based on their response at 600ppm of Fe revealed that Cul-8709, Cul-90-03 and IR-1552 had registered the highest total scores. This indicated that the genotypes had exhibited the high LBI score, normalized LBI score and per cent in reduction of biomass but lower biomass *per se* at 600ppm of Fe. Therefore for further studies, Cul-8709, Cul-90-03 and IR-1552 were selected as genotypes highly susceptible to iron stress (Table 26). Considering that among the above three genotypes, Cul 8709 (PGC 31) had registered the highest per cent reduction in biomass at 600ppm of Fe,it was identified to be most susceptible to Fe stress.

**Table 26. Ranking of genotypes based on LBI, normalized LBI, biomass *per se* and reduction of biomass (%) at 600ppm of Fe stress (Confirmation test-2)**

| Sl. No. | PGC No | Genotype | Ranking of genotypes | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | LBI (1) | Normalized LBI score (2) | Biomass (3) | Reduction of biomass (%) (4) | Total score (1 + 2+ 3+ 4) | Final ranking |
| 1 | 133 | AM-10-7 | 10 | 12 | 10 | 10 | 42 | 12 |
| 2 | 71 | Kargi | 8 | 2 | 7 | 1 | 18 | 4 |
| 3 | 34 | Cul-18716 | 3 | 4 | 1 | 4 | 12 | 2 |
| 4 | 104 | Cul-210-29 | 5 | 10 | 5 | 5 | 25 | 7 |
| 5 | 14 | Tulasi | 4 | 1 | 2 | 2 | 9 | 1 |
| 6 | 48 | ASD-16 | 6 | 8 | 7 | 12 | 33 | 10 |
| 7 | 17 | IR-36 | 3 | 5 | 2 | 7 | 17 | 3 |
| 8 | 31 | Cul-8709 | 13 | 14 | 8 | 17 | 52 | 15 |
| 9 | 115 | IVT-33 | 5 | 7 | 6 | 8 | 26 | 8 |
| 10 | 60 | PM-709 | 9 | 3 | 9 | 3 | 24 | 6 |
| 11 | 39 | Cul-3 | 7 | 9 | 3 | 11 | 30 | 9 |
| 12 | 28 | T(N)-1 | 1 | 6 | 2 | 9 | 18 | 4 |
| 13 | 46 | Abhaya | 5 | 16 | 8 | 13 | 42 | 12 |
| 14 | 43 | ASD-18 | 2 | 15 | 3 | 14 | 34 | 11 |
| 15 | 36 | Cul-8723 | 2 | 11 | 4 | 6 | 23 | 5 |
| 16 | 20 | IR-1552 | 12 | 13 | 4 | 15 | 44 | 13 |
| 17 | 100 | Cul-90-03 | 11 | 13 | 11 | 16 | 51 | 14 |

**Table 27. Ranking of genotypes based on LBI, normalized LBI, biomass *per se* and reduction of biomass (%) at 800ppm of Fe stress (Confirmation test-2)**

| Sl. No. | PGC No | Genotype | Ranking of genotypes | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | LBI (1) | Normalized LBI score (2) | Biomass (3) | Reduction of biomass (%) (4) | Total score (1 + 2+ 3+ 4) | Final ranking |
| 1 | 133 | AM-10-7 | 4 | 6 | 8 | 5 | 23 | 6 |
| 2 | 71 | Kargi | 7 | 5 | 8 | 7 | 27 | 7 |
| 3 | 34 | Cul-18716 | 1 | 4 | 1 | 2 | 8 | 2 |
| 4 | 104 | Cul-210-29 | 2 | 2 | 6 | 8 | 18 | 3 |
| 5 | 14 | Tulasi | 1 | 1 | 2 | 1 | 5 | 1 |
| 6 | 48 | ASD-16 | 5 | 8 | 6 | 4 | 23 | 6 |
| 7 | 17 | IR-36 | 3 | 10 | 5 | 12 | 30 | 9 |
| 8 | 31 | Cul-8709 | 7 | 15 | 6 | 16 | 44 | 14 |
| 9 | 115 | IVT-33 | 3 | 7 | 6 | 6 | 22 | 5 |
| 10 | 60 | PM-709 | 6 | 3 | 8 | 3 | 20 | 4 |
| 11 | 39 | Cul-3 | 6 | 9 | 3 | 10 | 28 | 8 |
| 12 | 28 | T(N)-1 | 5 | 12 | 4 | 11 | 32 | 10 |
| 13 | 46 | Abhaya | 6 | 16 | 7 | 9 | 38 | 11 |
| 14 | 43 | ASD-18 | 7 | 17 | 3 | 14 | 41 | 12 |
| 15 | 36 | Cul-8723 | 6 | 14 | 7 | 15 | 42 | 13 |
| 16 | 20 | IR-1552 | 6 | 11 | 7 | 17 | 41 | 12 |
| 17 | 100 | Cul-90-03 | 7 | 13 | 9 | 13 | 42 | 13 |

### 5.1.2. Hybridization

Results obtained in confirmation test 1 and 2 pointed out that genotypes Tulasi and Cul-18716 were more tolerant to iron stress while Cul-8709, Cul-90-03 and IR-1552 were the most susceptible of the lot.

Among these, genotype Tulasi (PGC 14) found to be most tolerant to iron stress (800ppm of Fe) was hybridized to the most susceptible genotype Cul-8709 (PGC 31) to obtain $F_1$'s.

## 5.2. Experiment 2: Study of parental polymorphism using molecular markers

Polymorphic microsatellites markers (SSR markers) are essential for bulk segregant analysis. Simple Sequence Repeat (SSR)/microsatellite markers have become the markers of choice for a wide spectrum of genetic, population, and evolutionary studies (Powell *et al.*, 1996).

Three hundred and thirty eight SSR markers distributed among the 12 chromosomes of rice (Fig 5) were used to deduce the molecular level polymorphism between the diverse parents (Tulasi and Cul-8709). Thirty seven markers were identified to differentiate the two parents. These 37 polymorphic rice microsatellites markers (SSR markers) were found to be distributed over all 12 linkage groups of rice varying between one in case on Chromosome 7 to five each on Chromosome 2, 9 and 10. The markers polymorphic between the parents have been highlighted in Fig 5.

The parental survey revealed 10.95 per cent polymorphism between the two parents (Tulasi and Cul-8709) used in the present study. Parental polymorphism per cent between any two parents depended on number of relevant primers selected for screening. Similar to the findings of the present study, Yadav *et al.* (2015) had identified 70 polymorphic markers out of the 500 markers used to survey the polymorphism at molecular level between the two parents (BPT-5204 and ARC-10531) explaining fourteen per cent polymorphism. Kanagaraj *et al.* (2010) screened 1206 rice microsatellite primer pairs between IR20/Nootripathu and identified 134 SSR polymorphic primers between these two parents. Govindaraj *et al.* (2005) had observed 63.95 per cent polymorphism using eighty- six SSR primers in parental polymorphism

survey involving two parents Basmati 370 and ASD16 of rice. Salunkhe *et al.* (2011) found 96 primers to be polymorphic between the parents IR20 and Nootripathu from among the 343 microsatellite markers indicating 27.99 per cent parental polymorphism; while Rani *et al.* (2012) could identify fifty-three polymorphic SSR primers distributed among different chromosomes out of 124 SSR markers screened between rice varieties IR 64 and Lalankada 4.

The thirty seven rice microsatellites markers (SSR markers) identified to be polymorphic between the two parents (Tulasi and Cul-8709) is a pointer to the existence of different alleles at each of the 37 marker locus. As the two parents differ from each other with respect to traits (eg. kernel colour) other than their reaction to iron stress, the 37 polymorphic markers may or may not be linked to leaf bronzing which is reliable indicator of iron toxicity tolerance. Onaga *et al.* (2013b) proposed that higher polymorphism information content (PIC) of markers is indicative of the utility of the markers for genetic diversity estimation of cultivars. Accordingly, markers RM10793, RM3412, RM333, RM562, RM13628, RM310, RM5749 and RM154 that recorded higher PIC values could be the best markers for delineating the differences among the rice genotypes at the genetic level. Out of these 37 polymorphic markers, only markers RM333, RM 263 and RM 107 were recognized by earlier studies as being associated with QTL for iron toxicity. Onaga *et al.* (2013b) had identified RM333 marker as being polymorphic marker between parents for iron toxicity tolerance while Dufey *et al.* (2009) had identified two markers (RM 263 and RM 107) as being polymorphic markers for iron toxicity.

### 5.3. Experiment 3: Raising of $F_1$'s

Main objective of raising $F_1$'s was production of $F_2$ population sufficient for bulk segregant analysis. The $F_1$ seeds obtained under hybridization programme (5.1.2) were raised in pots and selfed to obtain $F_2$ seeds (more than 1000 $F_2$ seeds).

### 5.4. Experiment 4: Bulk Segregant Analysis (BSA)

In order to detect the QTL associated with morphological traits of iron toxicity tolerance, a strategy of combining the DNA pooling from selected segregants and genotyping of DNA pooled samples along with parents was adopted. Three hundred $F_2$

seedlings of rice were used to for the bulk segregant analysis to identify putative markers linked to leaf bronzing as a reflection of tolerance to iron toxicity.

### 5.4.1. Phenotyping of $F_2$ plants for iron toxicity tolerance

Accurate phenotyping *i.e.*, screening of the plant material is required to identify QTLs for iron toxicity tolerance. According to Tanaka *et al.* (1966), rice growth can be reduced and bronzing appeared at $Fe^{2+}$ concentration of 10 to 500 mg $Fe^{2+}$ $L^{-1}$ in culture solutions and the degree of leaf bronzing is a straight forward indicator of $Fe^{2+}$ toxicity.

#### 5.4.1.1 Frequency distribution

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.

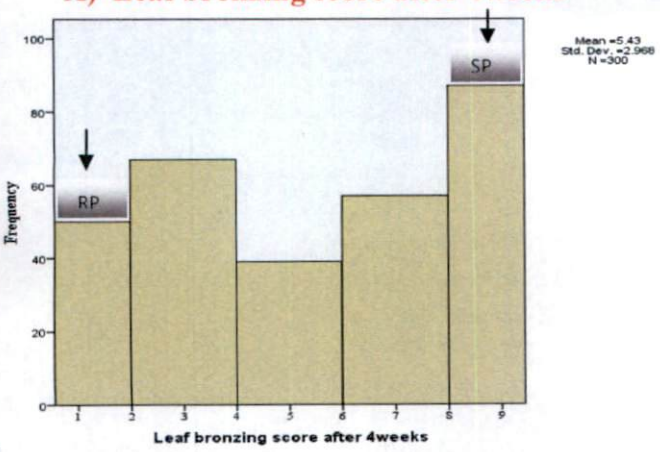
Results (Table 18, Plate 10 & 11 and Fig 1 & 2) indicated presence of wide variability for these traits among the $F_2$ plant population studied (Appendix II). Wu *et al.* (1997) had also observed wide variability among double haploid (DH) populations for leaf bronzing index and shoot weight in confirmation with the results of the present study.

Frequency distribution (Fig 1 and 2) of for the parameters studied indicated existence of clear difference b/w Tulasi and Cul 8709 with respect to the traits studied. Most $F_2$ individuals recorded phenotypic values between the susceptible and resistant parent under iron stress. The measures of skewness and kurtosis for various traits revealed existence of a large quantitative variability. However, none of the traits showed a perfect symmetrical data or skewness of zero. According to Fisher *et al.* (1932), the study of distribution using skewness provides information about nature of gene action while Robson (1956) opined that kurtosis is indicative of the number of genes controlling the traits.

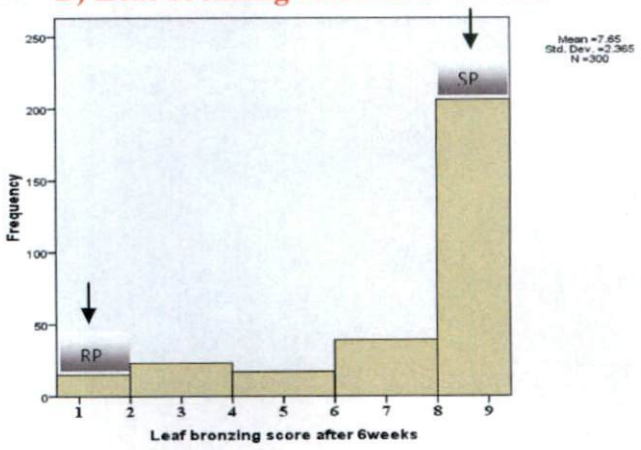
Distribution of root length, iron content in leaf and visual scoring for iron-toxicity symptoms of $F_2$ plants after 4 weeks was approximately symmetrical as

**Fig 1. Frequency distribution of $F_2$ plants for screening observations (I)**

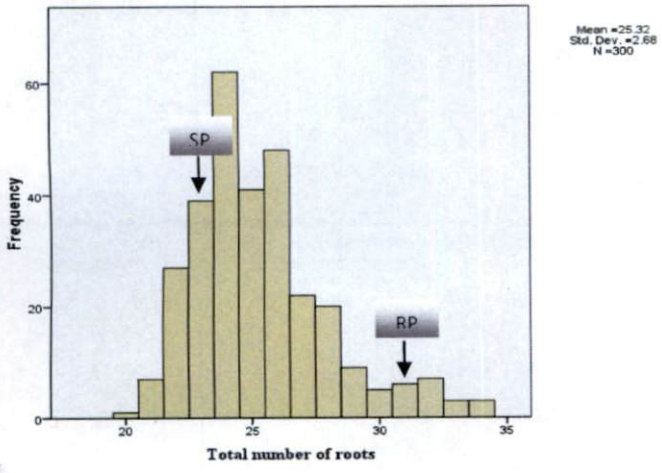
**A) Leaf bronzing score after 4weeks**



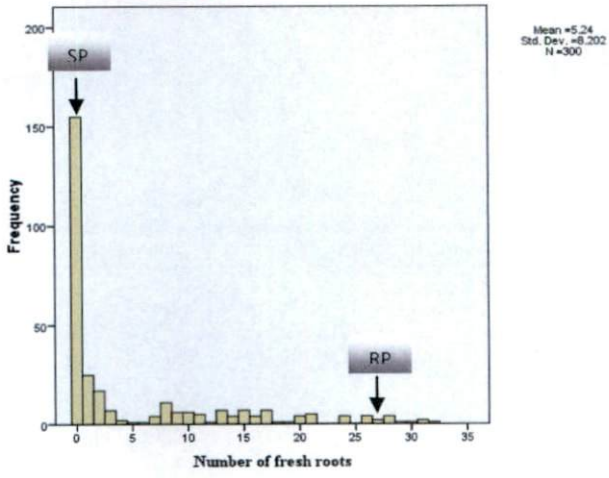

**B) Leaf bronzing score after 6weeks**



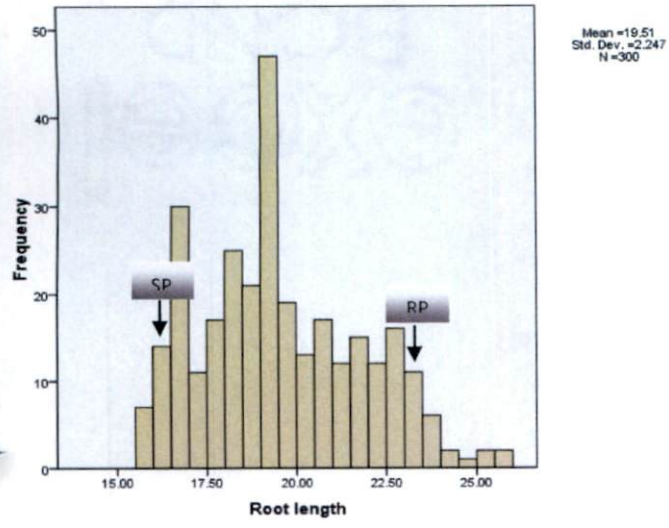

**C) Total number of roots**




**D) Number of Fresh roots**




**E) Root length**




**F) Shoot length**

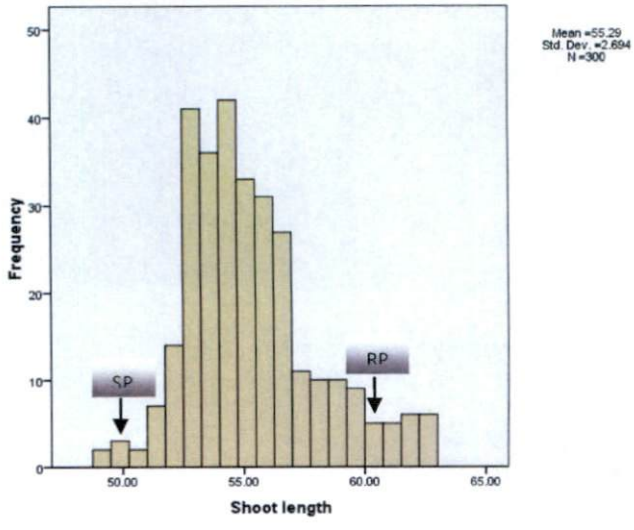
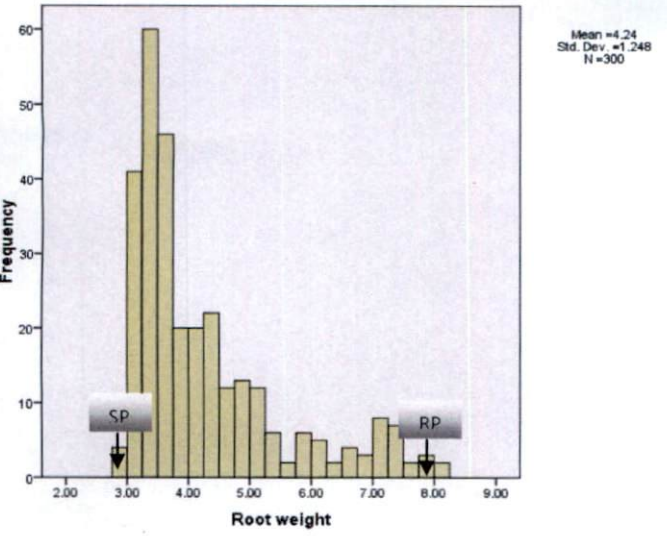
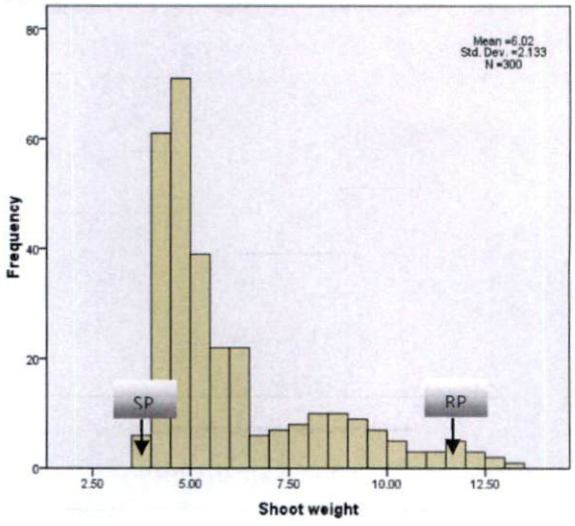


**Fig 2. Frequency distribution of $F_2$ plants for screening observations (II)**

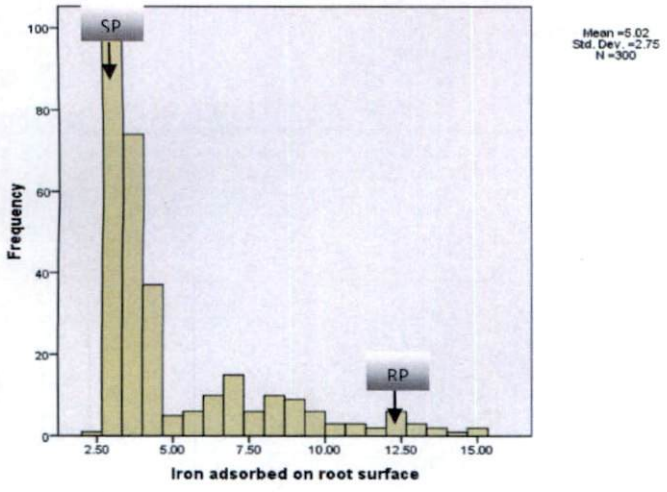
## G) Root weight



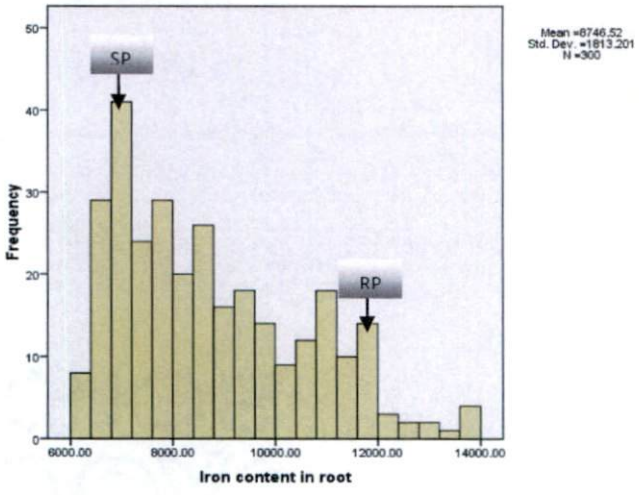

## H) Shoot weight



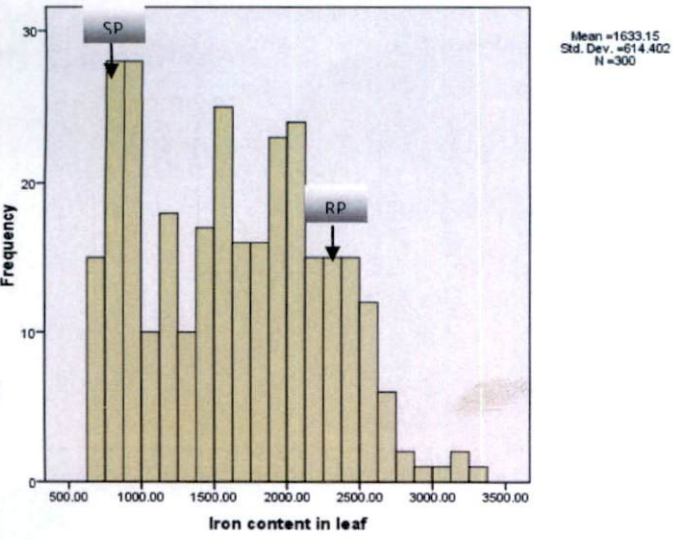

## I) Iron reversibly adsorbed on root surface




## J) Iron content in root




## K) Iron content in leaf



skewness of these characters ranged from -0.5 to 0.5 indicating a fairly normal frequency distribution under iron toxic conditions. All these traits exhibited a negative platykurtic distribution. A near zero skewness and negative value of kurtosis points to the absence of gene interaction (Ashwini *et al.*, 2011).

However, after 6 weeks of exposure to iron stress, the distribution of LBS was highly skewed with too many iron sensitive individuals. A negative skewness is indicative of duplicate (additive x additive) gene interactions while positive skewness is associated with complementary gene interactions (Ashwini *et al.*, 2011). The distribution was also platykurtic and positive. The traits with platykurtic distribution are considered to be controlled by a large number of genes (Kotch *et al.*, 1992). The results thus pointed out that the LBS after 6 weeks was controlled by multigenes that exhibit duplicate gene action. The efficiency of selection in a breeding programme depends on the amount of gene interaction. According to Choo and Reinberos (1982), improvement in population performance may be greater under complementary interaction rather than under duplicate gene interaction.

In case of total number of roots, shoot length, and iron content in root of $F_2$ plants, the distribution was moderately skewed (0.5 to 1.0) while a highly skewed (< -1 or > +1) distribution was observed for number of fresh roots, shoot weight, root weight, iron reversibly adsorbed on root surface and visual scoring for iron-toxicity symptoms of $F_2$ plants after 6 weeks. The genes controlling the trait with skewed distribution tend to be predominantly dominant irrespective of whether they have increasing or decreasing effect on the trait (Ashwini *et al.*, 2011). Maximizing the genetic gain in respect of traits with positively skewed distribution requires intense selection from the existing variability while genetic gain in respect of all the traits exhibiting negative skewed distribution will be rapid under mild selection from the existing variability (Roy, 2000).

All the above traits except iron content in root of $F_2$ lines exhibited positive platykurtic distribution. The platykurtic distribution for this trait was near zero (-0.37). Kurtosis is negative or close to zero in the absence of gene interaction and is positive in the presence of gene interactions (Choo and Reinbergs, 1982; Kotch *et al.*, 1992).

Measures of skewness and kurtosis also indicated that the performance of a few $F_2$ individuals were better than the resistant parent (Tulasi) while some were lower than that of susceptible parent (Cul-8709) for all observations except leaf bronzing score. This indicated occurrence of transgressive segregation in the $F_2$ population as observed in the variation in normal distribution of traits confirming the polygenic control of traits. In consonance with the study, Shimizu *et al.* (2005) and Dufey *et al.* (2015) had observed transgressive variation in segregating populations for leaf bronzing index (LBI) and all correlated parameters. According to Miles and Wayne (2008), the parental lines need not be phenotypically different for traits controlled by several genes; rather, they must simply contain different alleles at various loci, which are then reassorted by recombination in the derived population to produce a range of phenotypic values. Transgressive segregation indicated that the subset of $F_2$ population comprising of 300 individuals in the present study contained sufficient genetic variation for mapping QTLs for resistance to Fe toxicity.

#### 5.4.1.2 Association analysis

As resistance cannot be measured directly, several parameters were chosen as indicators of the degree of plant sensitivity to Fe toxicity. Iron toxicity tolerance is a complex character and is influenced by various other characters therefore it is essential to understand the association of other characters with iron toxicity tolerance in addition to the information on genetic variability (Dufey *et al.*, 2015). Hence, association analysis was undertaken to determine the direction of selection and number of characteristics to be considered in improving iron toxicity tolerance.

Among all parameters analyzed, the most indicative of the degree of plant sensitivity to Fe toxicity was the LBI (Tanaka *et al.*, 1966). This typical symptom of Fe toxicity, showed a strong negative correlation with shoot length, root length, total number of roots, number of fresh roots, shoot weight and root weight. The results indicated that leaf bronzing is associated with growth reduction due to $Fe^{2+}$ toxicity in this $F_2$ population. Wu *et al.* (1997) had reported that the leaf bronzing index is significantly negatively correlated with stem dry weight, tiller number and root dry weight. Previous studies have demonstrated that Fe toxicity in rice is characterized by bronzing spots on the lower leaves together with the formation of a red plaque on the

roots and decreased biomass production (Audebert, 2006; Becker and Asch, 2005; Dorlodot *et al.*, 2005; Green and Etherington, 1977; Howeler, 1973 and Sahrawat, 2004).

A study by Dufey *et al.* (2015) and Wan *et al.* (2003a) revealed negative correlation between leaf bronzing index (LBI) with the shoot dry weight (SDW) and root dry weight (RDW) (r = -0.41 and -0.39 respectively). Findings of the present study was in confirmation with the results of Olaleye *et al.* (2001) who reported a negative correlation between shoot length and shoot weight with leaf bronzing index. Fageria *et al.* (2008) and Dada and Aminu (2013) had also found a negative correlation between leaf bronzing index and shoot length.

In the present study, highly significant positive correlation between leaf bronzing index and iron content in leaf was observed. Similar findings were also reported by Asch *et al.* (2005) and Nyamangyoku and Bertin (2013). All the above correlations, confirms the usefulness of LBI as criterion for differentiating between genotypes susceptible and tolerance to iron toxicity. Several earlier workers (Dufey *et al.*, 2012; Wu *et al.*, 2014) had relied on LBI scoring to identify genotypes tolerant to Fe stress.

The expression of iron-toxicity symptom requires the excessive uptake of $Fe^{2+}$ by roots and its acropetal translocation via xylem flow into the leaves. Inside the leaf, excess amounts of $Fe^{2+}$ cause an elevated production of radicals, which can cause irreversible damage to cell structural components (Thompson and Legge, 1987) and lead to an accumulation of oxidized polyphenols (Yamauchi and Peng, 1995). At the cellular level, it is not only insolubility, but iron's high reactivity that can cause severe damage. Reactions involving iron in high concentrations in the interior of the cell may be highly damaging to the plant. These reactions can produce reactive species of oxygen, specifically the hydroxyl radical ($OH^-$), through the Fenton Reaction. The same physical properties that allow iron to act as an efficient cofactor and to catalyze controlled redox reactions also allow it to act as a powerful toxin when not protected from susceptible biomolecules. Numerous intracellular reactions use molecular oxygen as an electron acceptor producing superoxides ($O^{2-}$) or hydrogen peroxide ($H_2O_2$). These species are not harmful, but they contribute to the generation of reactive oxygen species,

hydroxyl radical ($OH^-$). Its formation is catalyzed by iron through the Fenton Reaction (Hell and Stephan, 2003). The typical visual symptom associated with those processes is the "bronzing" of the rice leaves (Howeler, 1973). Leaf Bronzing Symptom (LBS) was demonstrated to be highly correlated with yield formation under Fe-toxic field conditions (Audebert and Fofana, 2009).

A few $F_2$ plants (Plant no.20, Plant no.52, Plant no.110, Plant no.111, Plant no.156, Plant no.246, Plant no.248, Plant no.268, Plant no.287, Plant no.300, Plant no.308, Plant no.309, Plant no.319, Plant no.320 and Plant no.354) showed negligible leaf bronzing symptoms even at higher level of Fe content in their leaves. This indicated that tissue tolerance mechanism at leaf was also observed to some extent. On the subcellular level, the vacuole constitutes an important compartment for tissue tolerance at leaf through the storage of excess $Fe^{2+}$ ions (Moore *et al.,* 2014). Another mechanism of leaf tissue tolerance could be the scavenging of ROS through the plant's antioxidant network, thus avoiding the formation of oxidative stress. However, plants do not possess effective scavengers of the hydroxyl radical, the product of the Fenton reaction (Apel and Hirt, 2004). Therefore, antioxidants would have to remove the precursors of the hydroxyl radical such as hydrogen peroxide, which is reduced to water by antioxidant enzymes such as catalases and peroxidases (Blokhina *et al.,* 2003).

As in the present study, Dufey *et al.* (2015) had also identified a high and positive correlation of the leaf bronzing index (LBI) with the Fe concentration in the leaf ($r = 0.58$). Iron reversibly adsorbed on root surface and iron content in root characters were positively correlated with shoot length, root length, total number of roots, number of fresh roots, shoot weight and root weight. Ferritin is considered crucial for iron homeostasis. It is said to consist of a multimeric spherical protein called phytoferritin, which is able to store up to 4500 iron atoms inside its cavity in non-toxic form. A resistant variety may accumulate a larger amount of phytoferritin, which forms a complex that reduces iron toxicity (Rout and Sahoo, 2015). It has been reported that tolerant rice roots have Fe retaining, Fe oxidizing and Fe excluding powers that reduce the amount of Fe in shoot and leaf. According to Tadano (1975), these mechanisms invariably involved retention of Fe in the root preventing their transport to the shoot. Secondly ferrous ion is oxidized to the non active ferric oxide form.

Toxicity symptoms are usually correlated with iron deposition in the roots (Barbosa Filho *et al.,* 1994; Vahl, 1991). Kuraev (1966) reported that the initial toxic effect of high iron inhibits root development, and this was more pronounced at higher iron concentrations (200 mg $L^{-1}$), which may have been due to possible toxicity mechanisms such as the iron-induced production of superoxide ($O^{2-}$). Tanaka *et al.* (1966) reported that high iron concentrations may influence the growth and distribution of various wetland plant taxa. *Epilobium hirsutum* roots also have some capacity that is clearly inadequate in high iron environments.

Iron reversibly adsorbed on root surface was positively correlated with iron content in the root and observed in plants with lower leaf bronzing symptoms. It indicated that, physiological mechanisms like Fe exclusion from roots and root tissue tolerance at higher Fe content in roots are predominant in Fe toxicity tolerance. Snowden and Wheeler (1995) found evidence of a clear relationship between the iron tolerance of a species and the nature of the root precipitate. Becker and Asch (2005) identified exclusion of Fe at the root surface by oxidation of $Fe^{2+}$ into insoluble $Fe^{3+}$ which leads to the formation of a root plaque i.e. precipitation of Fe at the root surface. Root architectural traits favoring this process include the formation of an aerenchyma and a large number of lateral fine roots, which facilitate the diffusion of oxygen into the rhizosphere, thereby increasing the redox potential above the threshold for Fe oxidation (Wu *et al.,* 2014). Higher iron content in the root due to regulating mechanisms for the transport of iron from roots to aerial parts are involved in those plants that show iron tolerance (Curie and Briat, 2003).

Negative correlation of iron content in leaf with iron content in root was supported by Majerus *et al.* (2007). Iron content in leaf was negatively correlated with root length, shoot length, root weight, shoot weight, total number of roots, number of fresh roots and iron reversibly adsorbed on root. Similarly, Onaga *et al.* (2013a) observed a significant negative correlation of iron content in leaf with root weight, shoot weight and tiller number under iron toxic conditions. Nyamangyoku and Bertin (2013) also observed highly significant negative correlation of leaf iron concentration with leaf dry weight. The yield reduction by Fe toxicity was associated with the growth inhibition, especially at the later stages of growth. During this period, the Fe content of

roots of tolerant lines increased more slowly than those of susceptible lines. Also, the Fe content in soil solution sampled from plots of tolerant lines was higher than in those of susceptible lines. These findings suggest that a Fe exclusion mechanism is operating in the roots of tolerant lines (Nozoe *et al.*, 2008).

Traits like root length, shoot length, root weight, shoot weight, total number of roots, number of fresh roots, iron reversibly adsorbed on root and iron content in root were positively correlated each other. Similarly, Wang *et al.* (2013) observed positive correlation among shoot length, root length, root weight and shoot weight characters. Highly significant positive correlation between root weight and shoot weight characters was also observed by Wan *et al.* (2003a), Onaga *et al.* (2013a) and Dufey *et al.* (2015). Olaleye *et al.* (2001) observed positive correlation between shoot length and shoot weight and similar results were observed in the present study.

Based on the LBI from among the 300 $F_2$ phenotyped plants, fifteen $F_2$ individuals with an LBI score of 1 at Fe stress (800ppm) were identified to be tolerant iron stress while 71 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 28 and 29) following the procedure enumerated under section 4.4.3.

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 from that of ten most tolerant $F_2$ plants and DNA of susceptible bulk was prepared from DNA of 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

**Table 28. Ranking of $F_2$ genotypes that exhibited high tolerance to iron stress**

| Sl. 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 ($\Sigma$ 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 29. Ranking of $F_2$ genotypes that exhibited high susceptibility to iron stress**

| Sl. 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 ($\Sigma$ 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 |

Table 29. Continued.....

| Sl. 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 ($\Sigma$ 1 to 11) | Final ranking |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 |
| 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 |

**Table 29. Continued.....**

| Sl. 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 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 |
| 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 |

**Table 29. Continued.....**

| Sl. 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 ($\Sigma$ 1 to 11) | Final ranking |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 |
| 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 |

**Table 29. Continued.....**

| Sl. 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 ($\Sigma$ 1 to 11) | Final ranking |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 |

selected region and are seemingly heterozygous and monomorphic for all other regions (Michelmore *et al.,* 1991).

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 root surface, iron content in root and leaf of sensitive bulk and resistant bulk differed from each other (Table 30 & Fig 3).

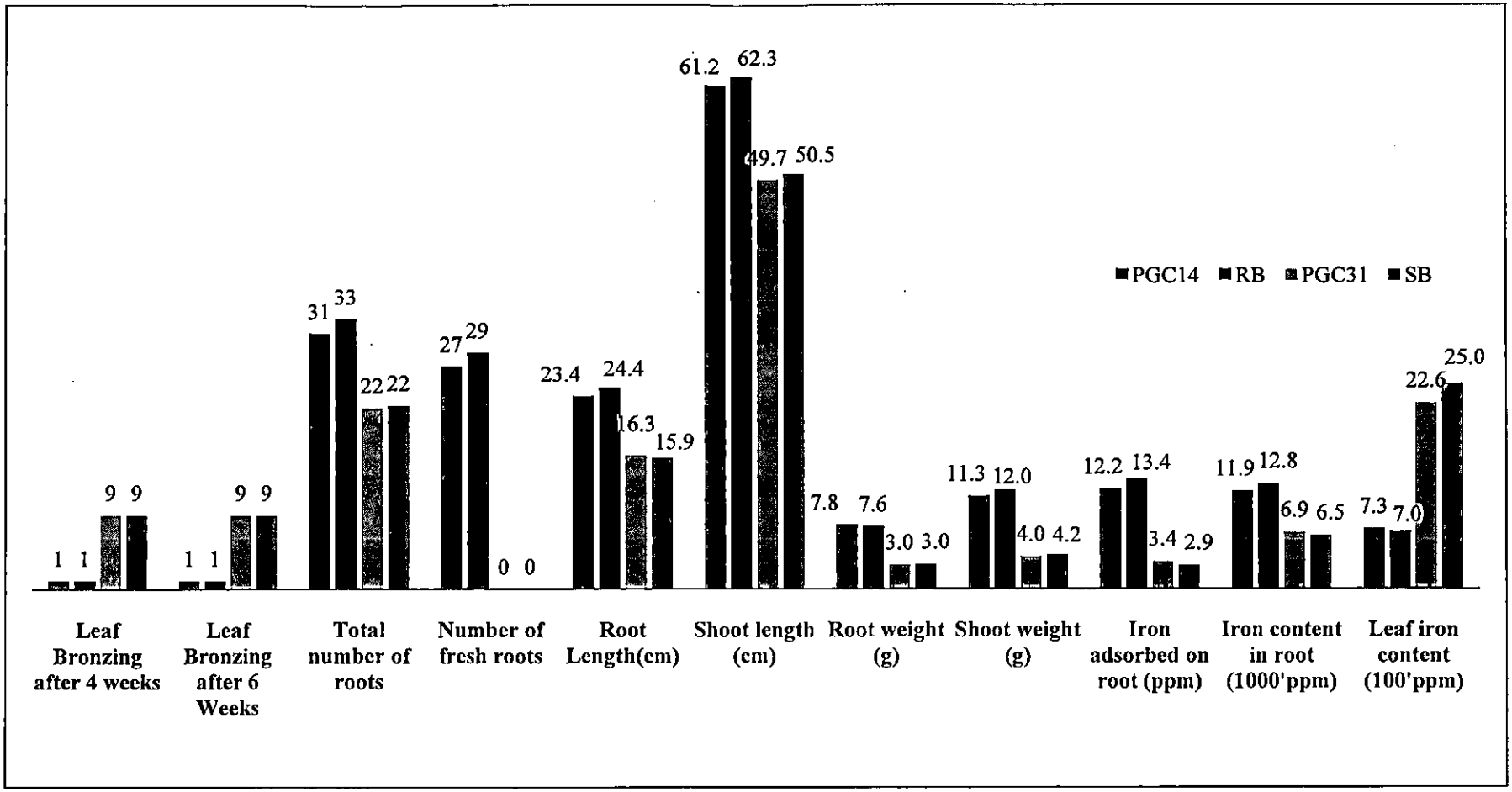
Higher values of shoot length, root length, total number of roots, number of fresh roots, shoot weight, root weight, iron reversibly adsorbed on root surface, iron content in root and lower values of leaf bronzing score and iron content in leaf were observed in resistant parent Tulasi and individuals of resistant bulk (Appendix III, Table 21), while the inverse relationship among the above traits was observed in susceptible parent Cul-8709 and individuals of susceptible bulk (Appendix IV, Table 21).

**Table 30. Performance of Resistant parent (PGC 14 - Tulasi), Resistant Bulk (RB), Susceptible parent (PGC 31 - Cul 8709) and Susceptible Bulk (SB)**

| Sl. 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 |

These results confirmed that the resistant parent (Tulasi) and individuals constituting the resistant bulk differed from susceptible parent (Cul-8709) and

**Fig 3. Performance of resistant parent (PGC 14 - Tulasi), resistant Bulk (RB), susceptible parent (PGC 31 - Cul 8709) and susceptible Bulk (SB)**



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.

### 5.4.2. Genotyping of parents, susceptible and resistant bulks

Results of parental polymorphism survey with 338 microsatellite markers under section of 4.2. (Parental polymorphism study using molecular markers) revealed that thirty seven were polymorphic between the parents (Tulasi and Cul-8709). The procedure of associating putative markers based on DNA pooling from selected segregants was established by QTL mapping method (Michelmore *et al.*, 1991).

Genotyping the resistant parent, resistant bulk, susceptible parent and susceptible bulk with the thirty seven polymorphic markers indicated that seven markers showed complete co-segregation among resistant parent and resistant bulk, susceptible parent and susceptible bulk (Plate 12 and 13). The difference between the bulked extremes is very clear when the bulk size was with ten $F_2$ individuals of phenotypic extremes. Of the seven markers that co-segregated, two markers RM 12292 and RM 12255 markers were present on chromosome number 1 while, markers RM 13619 and RM 263 were present on chromosome 2. Markers RM 107, RM 24616 and RM 24664 were presented on chromosome 9. The co-segregation of the seven markers between the resistant parent and resistant bulk, the susceptible parent and the susceptible bulk thus indicated these may be putatively linked to Leaf bronzing score which is a strong indicator of tolerance to Fe stress. Similar to the findings of the study, Dufey *et al.* (2009) had also identified markers RM 263 and RM 107 located on chromosome no 2 and 9 respectively to be a putative marker for leaf bronzing index.

The genetic architecture of tolerance to Fe toxicity in rice appears to be complex. Although quite a few studies reported quantitative trait loci (QTL) for different phenotypes related to Fe toxicity (Dufey *et al.*, 2015; Wu *et al.*, 2014), no major locus has been identified, fine-mapped, or cloned so far.

### 5.4.3. Confirmation of putative markers

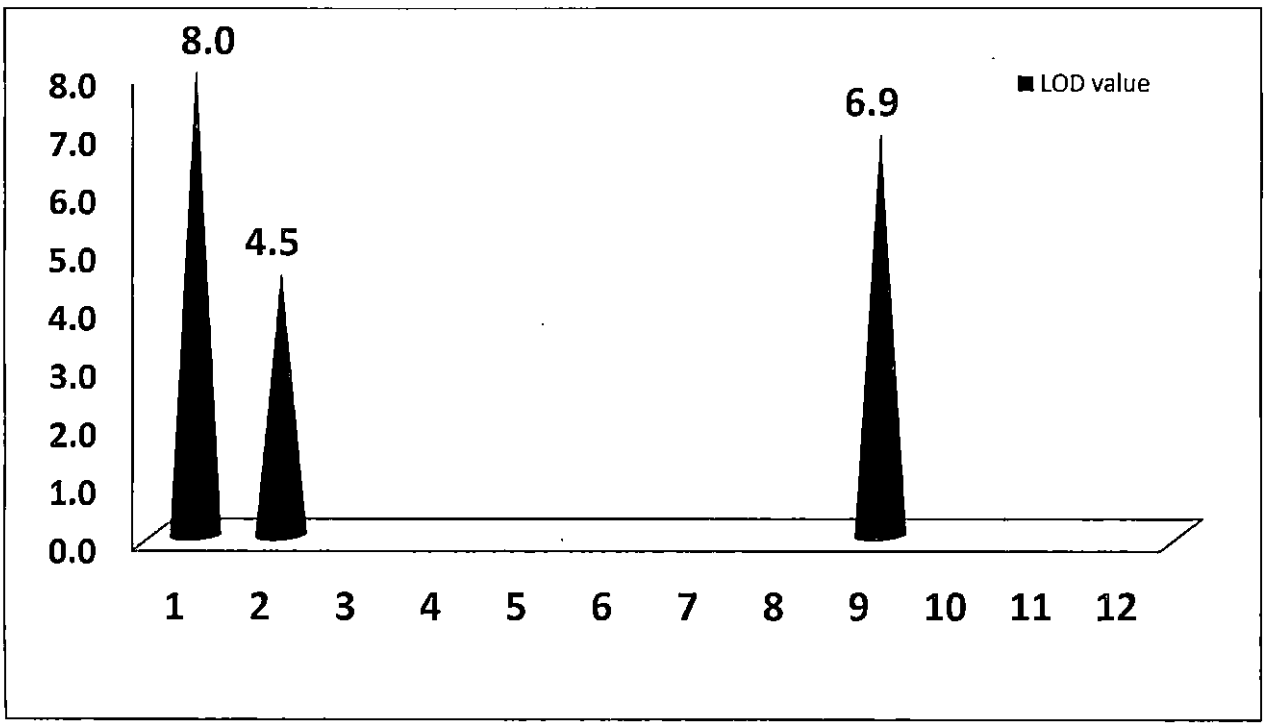
Putative markers identified from genotyping of parents, susceptible bulk and resistant bulks were used to genotype the $F_2$ individuals. In our study, seven markers RM 263, RM 107, RM 12292, RM 24616, RM 24664, RM 13619 and RM 12255 were identified as putatively linked to visual scoring for iron-toxicity symptoms (leaf bronzing index) based on the analysis of bulked extremes (Plate 14 to 16).

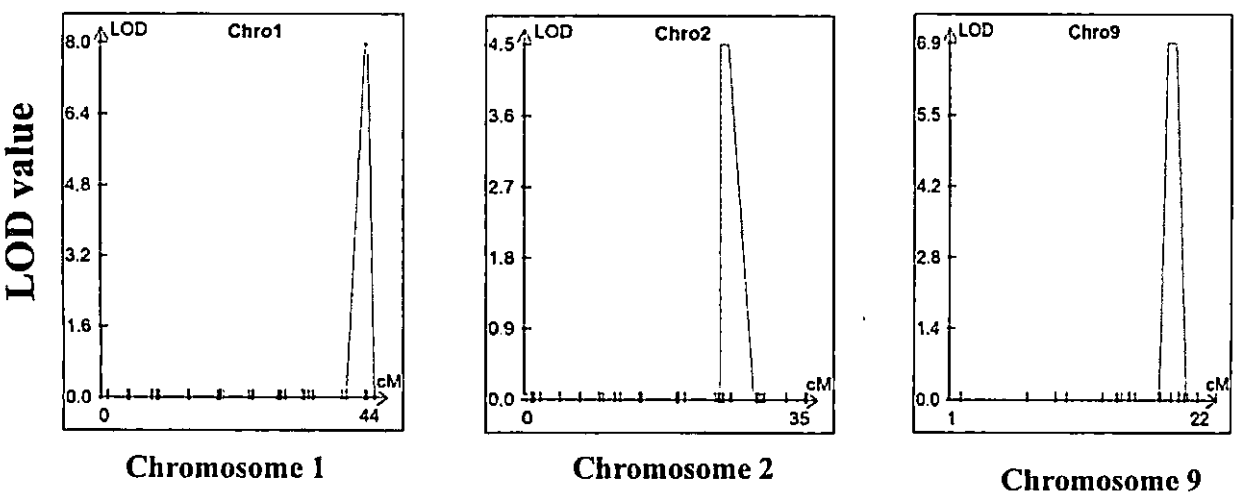
Probability of association all seven putative markers with variation of visual scoring for iron-toxicity symptoms (leaf bronzing index) was highly significant ($p<0.001$). Results indicated strong association of these putative markers of genomic region with visual scoring for iron-toxicity symptoms (leaf bronzing index). Positions of RM 12255, RM 12292, RM 13619, RM 263, RM 24616, RM 107 and RM 24664 were determined to be at 42.8 Mb (chromosome no: 1), 43.2 Mb (chromosome no: 1), 24.9 Mb (chromosome no: 2), 25.9 Mb (chromosome no: 2), 19.3 Mb (chromosome no: 9), 19.8 Mb (chromosome no: 9) and 20.1 Mb (chromosome no: 9). As these markers were linked to the quantitative trait loci for LBI which is considered as a reliable indicator of tolerance to iron toxicity, the markers can be considered putatively linked the genomic region governing tolerance to iron toxicity .

Totally three probable quantitative trait loci (QTL's) were identified based on probability of association all seven putative markers with variations of leaf bronzing index through single marker loci analysis (Fig 4, Fig 5 and Fig 6) associated with variations of leaf bronzing index through single marker loci analysis (Table 31). Significant QTLs were detected for trait leaf bronzing index after 8weeks of stress exposure. A LOD value of three indicates chance of presence of QTL is more ($10^3$ times) than absence of QTL for this character. A total of three QTLs with LOD values of 8.0, 4.5 and 6.9 respectively were mapped on chromosome 1, 2, and 9 respectively. LOD value of QTL present on chromosome 1 was higher than LOD values of remaining two QTL and indicated that stronge linkage of this QTL with leaf bronzing index of iron toxicity tolerance.

According to Wu *et al.,* (2014), major loci and some minor loci responsible for iron toxicity tolerance or susceptibility can be identified by bulk segregant analysis, if

**Fig 4. Distributions of quantitative trait loci (QTL's) associated with leaf bronzing index of Fe toxicity**




**Distributions of quantitative trait loci (QTL's) on chromosome 1, 2 and 9**

**Fig 5. Positions of three quantitative trait loci (QTL's) associated with leaf bronzing index of Fe toxicity**

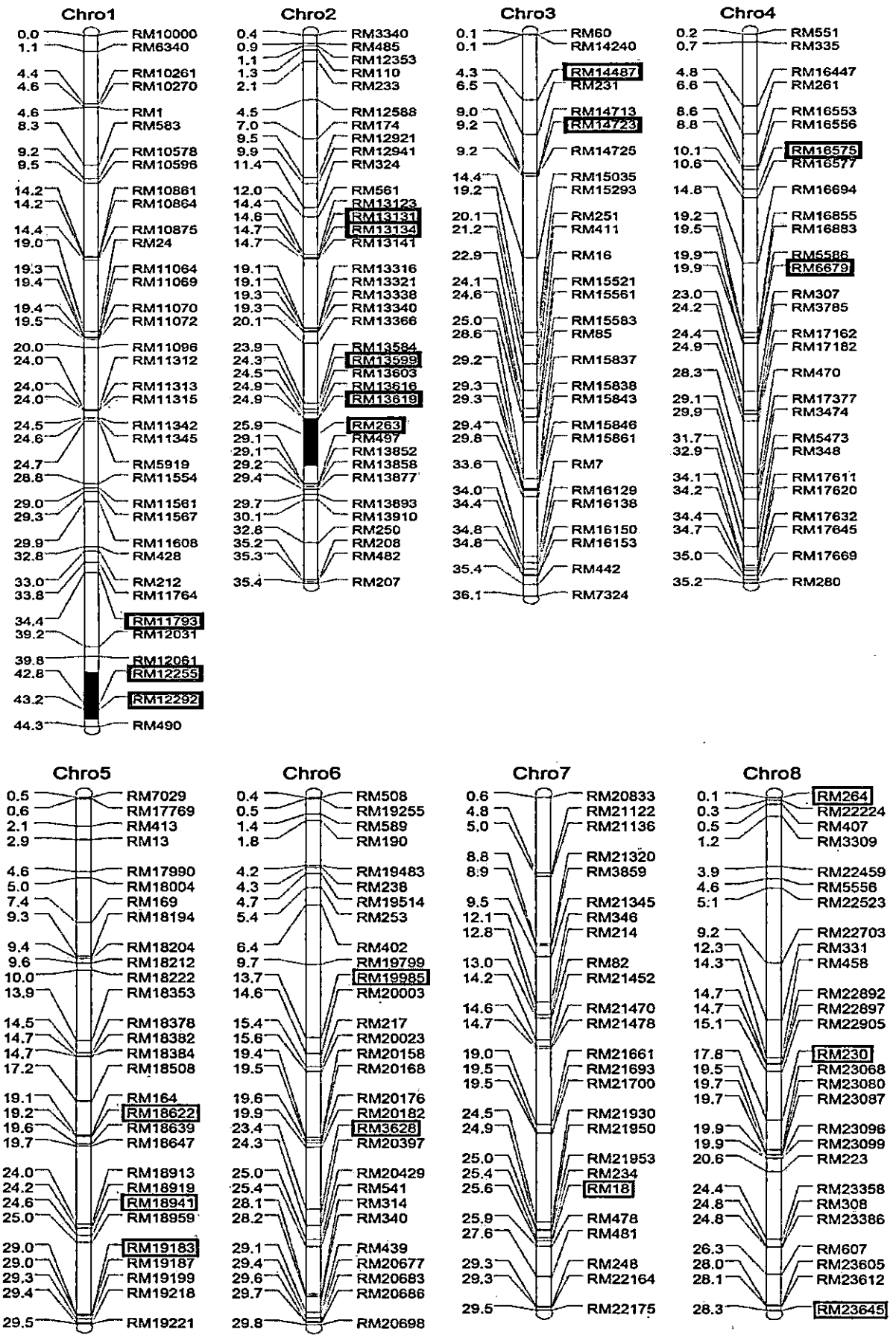
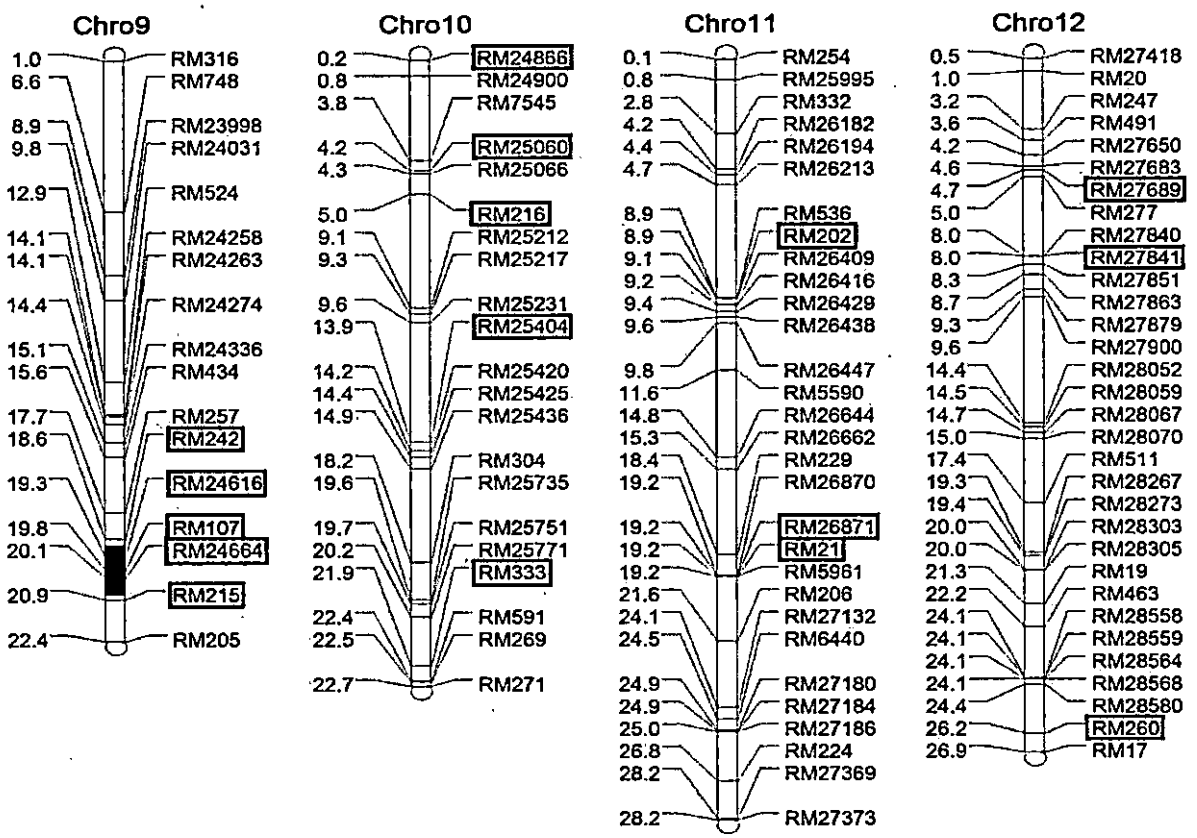


**Fig 6. Positions of three quantitative trait loci (QTL's) associated with leaf bronzing index of Fe toxicity (II)**



they are present in either of most resistant plants of resistant bulk or most susceptible plants of susceptible bulk. This method may not identify some other minor loci responsible for iron toxicity tolerance or susceptibility in moderately resistant and moderately susceptible plants of segregating population. This can be corrected by RIL's population mapping which identify QTL's which have less or negligible effects on iron toxicity tolerance or susceptibility.

**Table 31. Characteristics of QTL linked to LBI candidate markers as indicator for tolerance to iron toxicity in $F_2$ population of Tulasi x Cul-8709**

| QTL number | Marker interval | Chromosome number | QTL Position (Mb) | LOD value |
|---|---|---|---|---|
| 1 | RM 12255- RM 12292 | 1 | 42.8 - 43.2 | 8.0 |
| 2 | RM 13619 - RM 263 | 2 | 24.9 – 25.9 | 4.5 |
| 3 | RM 24616- RM 24664 | 9 | 19.3 – 20.1 | 6.9 |

Association of markers RM 12255 and RM 12292 located on chromosome number 1 with the variation of leaf bronzing index was high and significant ($P < 0.001$). Similarly, Wu *et al.* (1997) had observed that the tolerance may be largely controlled by a major gene located on chromosome one which makes the population a suitable for genetic investigation of $Fe^{2+}$ tolerance mechanism in rice. QTLs associated with leaf bronzing score were reported to be located on chromosome 1 of rice by several earlier workers. A QTL for leaf bronzing index was reported at the region of RG345-RG381 on chromosome 1 (Wu *et al.*, 1998). Wan *et al.* (2003b) had identified one QTL controlling Leaf bronzing index (LBI) located at the region of C955-C885 on chromosome 1. Wan *et al.* (2005) had identified a QTL on chromosome number 1 for leaf bronzing through mapping of $F_2$ and $F_3$ population of Longza 8503 x IR64 cross. Shimizu (2009) reported one QTL on chromosome number 1 representing leaf bronzing index of iron toxicity tolerance. Wu *et al.* (2014) identified one QTL for leaf bronzing on chromosome number 1 in RIL's population in IR29/ Pokkali. Dufey *et al.* (2015) had identified two QTL's responsible for iron toxicity tolerance on chromosome number 1.

In the present study, markers RM 263 and RM 13619 on chromosome number 2 also exhibited high significant ($P < 0.001$) association with the variation of leaf bronzing index. Similar to the findings of the study, Dufey *et al.* (2015) identified one QTL on chromosome number 2 responsible for leaf bronzing index and supported to

results of current study. However, Fukuda *et al.* (2012) had identified QTL for iron toxicity tolerance through root weight on chromosome number 2 in 39 CSSLs carrying Kasalath chromosomal segments in a background of Koshihikari. Presence of two QTL's for iron toxicity tolerance on chromosome number 2 through shoot weight and Fe concentration in leaf was reported by Shimizu *et al.* (2005).

RM 107, RM 24616 and RM 24664 markers on chromosome number 9 are highly significantly ($P < 0.001$) associated with the variation of leaf bronzing index. Similarly, Wan *et al.* (2005) had identified a QTL on chromosome number 9 for leaf bronzing character through mapping of $F_2$ and $F_3$ population of Longza 8503 x IR64 cross. Shimizu *et al.* (2005) identified two QTL's on chromosome number 9 and representing shoot weight and Fe concentration for leaf of iron toxicity tolerance.

Bulk segregating analysis is rapid screening method of identification of molecular markers linked to trait of interest and locate genomic region responsible for this trait of interest. In our study, seven markers were identified and contributed to three major loci associated with iron toxicity tolerance through leaf bronzing index. These markers therefore can be used to identify loci linked to LBI as an indicator for iron toxicity tolerance and incorporated in marker-assisted selection programmes aiming to develop varieties tolerant to iron toxicity.

# Summary

# VI. SUMMARY

The present investigation on 'Identification of molecular markers linked to iron toxicity tolerance through bulk segregant analysis (BSA) in rice (*Oryza sativa* L.)' was conducted at the Department of Plant Breeding and Genetics, College of Horticulture, Kerala Agricultural University, Vellanikkara, Thrissur during 2013-2015. The study involved screening of rice genotypes at seedling phase under the stress induced by excessive iron in growth medium, identification of the most susceptible as well as the most resistant genotypes under such situation followed by hybridization between them followed by raising of $F_1$s and production of $F_2$ generation. Phenotyping of $F_2$ population under iron stress through hydroponics was accompanied by extensive parental polymorphism survey using microsatellite markers sourced from Gramene database. Segregation of the markers found to be polymorphic between the parents were assessed through bulk segregant analysis and the probable markers linked to QTL conferring tolerance to iron at toxic levels were delineated. The salient findings of the study are summarized below.

**Parental selection and hybridization**

**Confirmation test 1:**

1) Screening the thirty rice genotypes (Confirmation test 1) selected from the KSCSTE project: 'Donor identification for tolerance to iron toxicity in rice (*Oryza sativa* L.)', under varying concentrations of iron (0ppm, 600ppm and 800 ppm) revealed that there existed high significant differences among the genotypes with respect to leaf bronzing and biomass production under all the three concentrations of iron.
2) At a higher concentration of Fe (800 ppm), the genotypes Cul-8723, Cul-18716, Tulasi, IVT-33 and Kargi exhibited lower Leaf bronzing index (<6.0). Cul-8723 had exhibited the least leaf bronzing score (4.7) at 800 ppm and was significantly different from all the other genotypes. The genotypes Tulasi, Cul-18716, IVT 33 and Kargi were on par and found to be next best to Cul-8723.
3) Among the 30 genotypes, the biomass *per se* genotypes and the per cent reduction in biomass over control at both 600 and 800ppm of Fe stress, were the highest in

genotype Tulasi (0.75g) followed by Cul-18716 (0.73g), Kargi and Cul-8723 (0.72g each).

4) Considering lower leaf bronzing and reduction in biomass at higher concentrations of Fe as a valid criterion for identifying genotypes tolerant to Fe stress, Cul-8723, Tulasi, Cul-18716, Kargi and IVT-33 were identified as the most tolerant genotypes to iron toxicity. A similar ranking of genotypes on the basis of leaf bronzing index and biomass production at 600ppm of iron revealed that the twelve genotypes *viz.*, Cul-8709, Cul-210-29, AM-10-7, Cul-90-03, PM-709, ASD-16, ASD-18, Abhaya, IR-1552, T(N)-1, IR-36 and Cul-3 were highly susceptible to iron stress. The above mentioned 17 genotypes were selected for further screening for tolerance to iron stress under Confirmation test 2.

**Confirmation test 2:**

5) Variance due to genotypes was found to be highly significant for visual bronzing scores (toxicity) and biomass under confirmation test-2 indicating that the genotypes differed significantly at all three levels of iron (control, 600ppm Fe and 800ppm of Fe).

6) At 800 ppm of Fe, almost all the genotypes except Tulasi and Cul-18716 had registered a leaf bronzing score of above 7.4. Among the two, Tulasi exhibited the lowest leaf bronzing score (5.4) at 800 ppm and was significantly different from all other genotypes. Cul-18716 (5.7) was found next best to Tulasi.

7) Among the 17 genotypes screened at 600 ppm of Fe, Cul-8709, IR-1552 and Cul-90-03 recorded the highest leaf bronzing score of 9.0. These genotypes had also exhibited a high reduction in biomass over control. Considering the above, the three genotypes were identified as susceptible genotypes for further studies. Of the three, Cul 8709 (PGC 31) that had registered the highest per cent reduction in biomass at 600ppm of Fe was adjudged to be the most susceptible to iron stress.

**Hybridisation and production of $F_2$ population**

8) Genotype Tulasi (PGC 14) found to be the most tolerant one to iron stress (at 800ppm of Fe) was hybridized to the susceptible genotype Cul-8709 (PGC 31) to obtain $F_1$s. The $F_1$ plants were selfed to obtain $F_2$ population to be used for Bulk Segregant Analysis.

**Bulk Segregant Analysis (BSA)**

**Phenotyping of $F_2$ plants for iron toxicity tolerance**

9) Results indicated wide variability in various parameters among the $F_2$ plant population *viz.,* shoot length, root length, total number of roots, number of fresh roots, shoot weight, root weight, amount of iron adsorbed on root surface, iron content in root and leaf and visual scoring for iron-toxicity symptoms of $F_2$ plants.
10) Measures of skewness and kurtosis confirmed the existence of high quantitative variability among $F_2$ individuals. It explicitly indicated that the performance of a few $F_2$ individuals were better than that of the resistant parent (Tulasi) while some were lower than that of susceptible parent (Cul-8709) for all observations except leaf bronzing score. This indicated the occurrence of transgressive segregation in the $F_2$ population.
11) Distribution of root length, iron content in leaf and visual scoring for iron-toxicity symptoms of $F_2$ plants after 4 weeks was approximately symmetrical as skewness of these characters ranged from -0.5 to 0.5 indicating a fairly normal frequency distribution under iron toxic conditions. All these traits exhibited a negative platykurtic distribution indicating absence of gene interaction.
12) However, after 6 weeks of exposure to iron stress, the distribution of LBS was highly skewed with too many iron sensitive individuals. A negative skewness is indicative of duplicate (additive x additive) gene interactions. All the traits mentioned above except iron content in root of $F_2$ lines exhibited positive platykurtic distribution indicative of presence of gene interactions in trait expression.
13) Correlation between leaf bronzing score and iron content in the leaf was highly significant and positive while that between leaf bronzing score and traits root length, shoot length, total number of roots, number of fresh roots, root weight, shoot weight, iron adsorbed on root surface and iron content in the root was significant and negative.

**Parental polymorphism study using molecular markers**

14) Parental polymorphism survey using 338 Rice Microsatellites (RM) markers revealed that 37 RM markers were polymorphic between the two parental lines

(Tulasi and CUL-8709). These polymorphic rice microsatellites markers (SSR markers) captured 10.95 per cent of polymorphism between the two parents (Tulasi and Cul-8709).

15) The thirty-seven polymorphic microsatellites markers were found to be distributed over all 12 linkage groups of rice varying between one in case on Chromosome 7 to five each on Chromosome 2, 9 and 10.

**Genotyping of parents, susceptible and resistant bulks**

16) Molecular assay of resistant parent, susceptible parent, resistant bulk and susceptible bulk with the 37 polymorphic RM markers indicated that seven markers *viz.*, RM 263, RM 107, RM 12292, RM 24616, RM 24664, RM 13619 and RM 12255 exhibited clear co-segregation between resistant parent and resistant bulk, susceptible parent and susceptible bulk. Hence, these markers were considered as putatively linked to Leaf bronzing index (LBI).

**Confirmation of putative markers**

17) Probability of all the seven putative markers was highly significant ($P < 0.001$) pointing to strong association of these markers with the genomic region governing Leaf Bronzing Index which is the valid indicator of iron toxicity tolerance.
18) Of the seven markers, RM 12292 and RM 12255 were found to be present on chromosome number 1 while, RM 13619 and RM 263 on chromosome 2. Markers RM 107, RM 24616 and RM 24664 were observed to be present on chromosome 9.
19) Single marker loci analysis associated with variations of leaf bronzing index pointed to presence of three quantitative trait loci (QTL) one each on chromosome 1, chromosome 2 and chromosome 9.

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# Appendices

## Appendix I. List of 338 rice microsatellites used for the study of parental polymorphism

| Sl. No | Name of rice microsatellite | Chromosome number | Sequence of Farward primer | Sequence of Reverse primer |
|---|---|---|---|---|
| 1 | RM 1 | 1 | GCGAAAACACAATGCAAAAA | GCGTTGGTTGGACCTGAC |
| 2 | RM 10000 | 1 | AATCCAGATGTGCGCCAACC | GAGTGAAGCCAGCTCCTCAACC |
| 3 | RM 10261 | 1 | ACGCTACACTACCAAGAAATCC | TGAGAAAGGAGGGAGTAGTTAGC |
| 4 | RM 10270 | 1 | ATGAGTCGGTGAGTGAGTACG | GCTCCGTCAAATACTTTCTAGC |
| 5 | RM 10578 | 1 | GCGAGTAATTATGGTGGTGTTTGG | CCCTCGACCATTCGTATAGTTACTCC |
| 6 | RM 10596 | 1 | CAACAACCTGGCAGAGAATTTCG | CTGCACGTGATGTCAGAGTTCG |
| 7 | RM 10861 | 1 | GATCGAGGAATATGATCCAACTCG | ATTTCTGGTGTGAGCACTGATCG |
| 8 | RM 10864 | 1 | GAGGTGAGTGAGACTTGACAGTGC | GCTCATCATCCAACCACAGTCC |
| 9 | RM 10875 | 1 | TCCGCAATAAACAGCCACACG | CGTCCGGTACTTCTCCTTGAGC |
| 10 | RM 11064 | 1 | TCGCGTGTGTCTTGTGTGTTTCC | CACATCAACGGTGCAGATTGTAGG |
| 11 | RM 11069 | 1 | GGTACAATGAAGCTTGGCAACG | CGGTGGAGTAGAACCACGAAGC |
| 12 | RM 11070 | 1 | TCCCTACTCACTCTTTCTTCTGC | TGTACGGAGTGTGTAAGAGAAGC |
| 13 | RM 11072 | 1 | TGGAGAACAATCAAGAGGCTTCC | GGGCATCCTATAAATGCTGATTGG |
| 14 | RM 11096 | 1 | AGGGAAGGGAAGTATGTATGTACACG | TATGAGTGATGCCAGCTCAATGG |
| 15 | RM 11312 | 1 | CAGGCTAACAGCGGTAGAACACC | GCAAACAAGGCTCGAAAGAACC |
| 16 | RM 11313 | 1 | TGAGGCTGATAGAAAGCAGAATGC | CCCGTTTCTTCCATATCATGTCG |
| 17 | RM 11315 | 1 | CATGAGGTTCTGAGTGGATCACC | TCGATCGATCACGTACCAGTCC |
| 18 | RM 11342 | 1 | CCATCCATGCACATTTAGGAGTAGG | TGTACAATCACGTCGCTCTACACG |
| 19 | RM 11345 | 1 | CAGAGAGTGTAGTCTTCCAACG | ATGCTTGGAGTTGAGATTGC |
| 20 | RM 11554 | 1 | AGGACTTAGGGTACGTTTGAATCTCC | GACGATGATTGTCTCCTAAGTCTGC |
| 21 | RM 11561 | 1 | AGTAGCATGAGTCACATCCAATCC | GCTCGAACTTGAGTTTATCGAACC |
| 22 | RM 11567 | 1 | CAAACCTCACCTACTGCGTTCG | CTCACAATCTGCTGGACAACTGG |
| 23 | RM 11608 | 1 | GTATAATGGTGTGTGTCAGTGTGTGC | ACTGTGAGCCTGTGAGCCTACC |
| 24 | RM 11764 | 1 | CACTGTCATCGTCGCCAAACG | GTTAATGAGCTACTCCCTCCGTCTCC |
| 25 | RM 11793 | 1 | GAGACTACCAAACTCCTAACTACCG | GATTCATAGGCCGAGACTGC |
| 26 | RM 12031 | 1 | TCCCTAGCTAGCTCTCCATCTCC | AGTACTACCGCTACATGTCTTCTTGG |
| 27 | RM 12061 | 1 | GTCGGTTTGGGAATTTGACTAGTAGG | TAATTGTGGACTGCTCGTTTCTGG |
| 28 | RM 12255 | 1 | CCTCCCGTAAATAGTCCATACAGC | CTTCACGCATCCACTGATTATTCC |
| 29 | RM 12292 | 1 | ATGAGACGATGAAAGCCTCAAGC | GTGGGACAAGCAAATTGAAACG |
| 30 | RM 212 | 1 | AAGGTCAAGGAAACAGGGACTGG | AGCCACGAATTCCACTTTCAGC |
| 31 | RM 24 | 1 | CTAAATTTCTGGCCGTAGGATCTTGG | GGGTAGTGGACGGCGAATGC |
| 32 | RM 428 | 1 | AACAGATGGCATCGTCTTCC | CGCTGCATCCACTACTGTTG |
| 33 | RM 490 | 1 | ATCTGCACACTGCAAACACC | AGCAAGCAGTGCTTTCAGAG |
| 34 | RM 583 | 1 | GTTGCGGTTTGTTCGTTCTTGC | TAGATCCCAGCAGACGGATCAGC |
| 35 | RM 5919 | 1 | AGCGGCTTTGTCACTGTATTTCC | GACGAGTATAGTGATAGCGTTTGACG |

| Sl. No | Name of rice microsatellite | Chromosome number | Sequence of Forward primer | Sequence of Reverse primer |
|---|---|---|---|---|
| 36 | RM 6340 | 1 | GCATGATGCAACGGAGATCG | CTTCCTCATCTCCCTCACCTTCC |
| 37 | RM 110 | 2 | AAATTCGAAGCCATCCACCAACG | GCCGACGAGGTCGAGTAGAAGG |
| 38 | RM 12353 | 2 | TACTTCTCCCACTTGGACTTTGC | GATCAGTTCTTGAGATGGGATGG |
| 39 | RM 12588 | 2 | TGTCCAGATTCGTGGTATAAGC | AAAGGCTGGTCGTCTTTCTAGG |
| 40 | RM 12921 | 2 | TCGTATTTCCCGGTGTCTCAGG | ACTAGTACTCGGTGCAGGGAATCG |
| 41 | RM 12941 | 2 | TTATGCCATGTGGTCCAATCAGC | ATTTGAACCATTTGGGCCTTGG |
| 42 | RM 13123 | 2 | AGTAGGTGGATGAGCAGATGTGG | CATCACTCCTCTCCAAGAAAGAGC |
| 43 | RM 13131 | 2 | TGGAGTGAGGGTAGGTGGGTTGG | CTCGAGAGTTGTGCCCATCATCC |
| 44 | RM 13134 | 2 | CCGATCTTTAACTGAGGTGAGAGG | TGAGACGAGAGATGAGAAGAGTGG |
| 45 | RM 13141 | 2 | CGGTTTGGAAGAGATTGTGTTTGG | CGGAAGGGAGTTGCTGATGG |
| 46 | RM 13316 | 2 | TTATCAGGAGGCGTTTGATCTGG | GTGCTTGGTAGTTGGGCTAATGG |
| 47 | RM 13321 | 2 | ATCTTTCCTTACGGCTTTCACG | TTAGTTATAGCACACCCTGGATGC |
| 48 | RM 13338 | 2 | TTGATAGCCCGACCTCTTCTCG | ATGGGATAGCGGGATAGTCAATCG |
| 49 | RM 13340 | 2 | CACTCGCGGTTCAAATGCTTACC | TGAACGGCTCCAACGTGAAAGG |
| 50 | RM 13366 | 2 | GAATGGACGACATGTACGACACC | GGATGACGGACGAAAGCTAAGG |
| 51 | RM 13584 | 2 | TCCATCTTCACAATCAGCAACC | CCCTGTAACAATGTTGAGAACACC |
| 52 | RM 13599 | 2 | GTTCATGGCACTCCTCTCCTAGC | GAGGAATGAACAGTGCCTACACG |
| 53 | RM 13603 | 2 | GTACATATACGGACCACTTCTGC | GTTACGACCTAAATCTGCAACC |
| 54 | RM 13616 | 2 | GATCTAAACCCTCTTTCCACAAGC | CGGCCAATATATAATGCACTCC |
| 55 | RM 13619 | 2 | TGGCTCCGTCGAGTCAAACTGG | TGATGCTTTGGCCTTCAAGTAGCC |
| 56 | RM 13852 | 2 | TACTGTATGGGAAAGGAGAGG | CCACTTGTACTACGATCACTTAGC |
| 57 | RM 13858 | 2 | AATATTGGTTTGGCCCTTACCC | TTATTCTCCGGTGTCCACTTCC |
| 58 | RM 13877 | 2 | ACTAGTCAGGGCCAACAGACAGG | CAAGCATGCACCATCATCTCC |
| 59 | RM 13893 | 2 | CAGTCTCATTTGATGCAGTGTACG | CCTCTACCTATATGATGCACAACACG |
| 60 | RM 13910 | 2 | GAGCGAGCTATACCACCGTGACC | ATCGCGTCCAAGAAAGGTGTCG |
| 61 | RM 174 | 2 | ATAAGCGACGCCAAGACAAGTCG | GGAAGCAAGAAGGAAAGAGAGATGG |
| 62 | RM 207 | 2 | ATCCTAGTGGATAAGGCACAGACTGG | CCCTTGCTCTTCCACCTCATCC |
| 63 | RM 208 | 2 | AGTACCACCACCATTCTCTGCAAGC | TCGATTGGCCATGAGTTCTCG |
| 64 | RM 233 | 2 | TATTGCTAGAGCCTACCTTTCC | CCAACCAGTCTTACAATCAACC |
| 65 | RM 250 | 2 | GTTCAAACCAAGCTGATCACAAGC | GGCGTCAGAGTCAGAGATGAAGG |
| 66 | RM 263 | 2 | AATCTATGGACCTGGGAGGAACC | TGACGAGAGTGCTACGTTTGAGC |
| 67 | RM 324 | 2 | GATTCCACGTCAGGATCTTCTGG | GCTCACCAGTTGAGATTGAAAGG |
| 68 | RM 3340 | 2 | GAGAGAGACACCAAATGATCCATCC | ACTGATTTGGCCCTTGTTCTTGG |
| 69 | RM 482 | 2 | TCTGAAAGCCTGACTCATCG | GTCAATTGCAGTGCCCTTTC |
| 70 | RM 485 | 2 | CATCCACCGTCCGATTCTACC | TTCCAGTCCTCTCCTCTCTTTGG |

| Sl. No | Name of rice microsatellite | Chromosome number | Sequence of Forward primer | Sequence of Reverse primer |
|---|---|---|---|---|
| 71 | RM 497 | 2 | GCTGCTTGTGTTGTTGTTGTCG | CACAGGCTCCTCTTCACCTATGG |
| 72 | RM 561 | 2 | GAGCTGTTTTGGACTACGGC | GAGTAGCTTTCTCCCACCCC |
| 73 | RM 14240 | 3 | GCTCAAAGAATGACACCGATGC | CATGGTGAGTTTGGAGTGATTGG |
| 74 | RM 14487 | 3 | TGCACACTCTGCCTAAATTTGC | CGAGAGTGTCTGTCTAGATTTCAGG |
| 75 | RM 14713 | 3 | CTCGAGGCGTTCATGGTTACTTCG | TGCGTTGCACTGGGTGATTACG |
| 76 | RM 14723 | 3 | GCAAAGTCCTTTGGACAGGTAGC | CGTCCCAGATCAAAGTACACTCTTCC |
| 77 | RM 14725 | 3 | CCACATAAGTATTGGAGTGCATCG | AGATGTTAACCCACGAGGAATGG |
| 78 | RM 15035 | 3 | TTCGTGATCGGTCATCCATCC | AGATTCATATGCTGCGCTGTTACC |
| 79 | RM 15293 | 3 | ATCGGCAAGCAATGTCATCAAGC | GTGCATGTGCAAGACACGTTCC |
| 80 | RM 15521 | 3 | GGTGAAGAACCTTCCTCCTTTATACC | TCATACTGTCCTGGACCACATCC |
| 81 | RM 15561 | 3 | ATTAGCTTGGGCGTCTTCCTCTGG | TGCAAACAATGGCTTCACATCG |
| 82 | RM 15583 | 3 | CCCAAATAGTCACCAGCATTATCG | TTGCCTGTGCAACCTTATGAACC |
| 83 | RM 15837 | 3 | GACCATAGTTGACTCCCATTGAGC | ACGTCACGTATTTGTGGCTAAGG |
| 84 | RM 15838 | 3 | CGATGTCATTCGGTAGAAACAAGC | CCTAGTCAAGGCATGGTCAATCC |
| 85 | RM 15843 | 3 | TGTGGAGTCAACTTATGCTCATGC | CTAGGACACTGAGGCCCAACC |
| 86 | RM 15846 | 3 | CAACTCGGGTCAATAACCACTGC | AGGAATCGAAGTCGTCCTGTGC |
| 87 | RM 15861 | 3 | GGTGTGTGAGAAGAGGAGATTGG | GGCCCTTTGAAGACAACACTAGG |
| 88 | RM 16 | 3 | GTGCGCCAGGAGTAGTTGTCTCC | GACGTGTACACATAGCCAAATCATCC |
| 89 | RM 16129 | 3 | TGAGCACGGAATTTATGGTTGC | ACATTTAGGCACCGGATAGTTGG |
| 90 | RM 16138 | 3 | CTGCACTAATGAACAAGCGAAACC | TTCTGTAAACGCTCCTTCAGTGG |
| 91 | RM 16150 | 3 | TCCATGGGCTTCTTAGTAATGC | GTTGAGAAAGAGAACGGGAAGG |
| 92 | RM 16153 | 3 | TGGTTGTGGTATAGCACGGTAAGC | TGACCCAAGGAGATACTAGGTTGC |
| 93 | RM 231 | 3 | CCAGATTATTTCCTGAGGTC | CACTTGCATAGTTCTGCATTG |
| 94 | RM 251 | 3 | GAATGGCAATGGCGCTAG | ATGCGGTTCAAGATTCGATC |
| 95 | RM 411 | 3 | GTAGGAAATTCTTCGCCAGATGC | CCGAGACTTGGAACAATCTTAGGC |
| 96 | RM 442 | 3 | CTTAAGCCGATGCATGAAGG | ATCCTATCGACGAATGCACC |
| 97 | RM 60 | 3 | CAAGTTCACCCGCCTTCTCG | TTTCCATCATTAGCAGGCAGTAGC |
| 98 | RM 7 | 3 | TTCGCCATGAAGTCTCTCG | CCTCCCATCATTTCGTTGTT |
| 99 | RM 7324 | 3 | GAGAGAGAGAGAGGAGAGGCG | GATGCACATCTCGACAGCTC |
| 100 | RM 85 | 3 | CCAAAGATGAAACCTGGATTG | GCACAAGGTGAGCAGTCC |
| 101 | RM 16447 | 4 | CGGGTCTGTCTTTCAGTTTGC | AGTGGCGTACTTGCTCTACTGC |
| 102 | RM 16553 | 4 | CATAGCCACTTATCGTTGTTACGC | TGTCCATCTATGACTGTCCACTACG |
| 103 | RM 16556 | 4 | TTGGACCAGGAGATCAATGAAGG | GTGCGCACACTCTTCTATGTGC |
| 104 | RM 16575 | 4 | CACCAACTACACTCCTACACTCC | CTAGATCATAGGCGGTCACG |
| 105 | RM 16577 | 4 | GGTGAATTCTACTAAGACGGATCG | AGCCTTATTAGTCTCACCTCGTAACC |

| Sl. No | Name of rice microsatellite | Chromosome number | Sequence of Forward primer | Sequence of Reverse primer |
|---|---|---|---|---|
| 106 | RM 16694 | 4 | GCGTGATAGATGGATCTGTTGG | CATCCGATAGTACTACCTCCATCC |
| 107 | RM 16855 | 4 | GCGAGCCTAGTTCTGATTCTAGC | TGGAGTACGTATGTTGGGTATGC |
| 108 | RM 16883 | 4 | TGCCATGATATGATTCCTGTGG | GGTCCTATTACAAGCATGCAGTCC |
| 109 | RM 17162 | 4 | GATGTACCAGTCCAGTTACAAAGACC | CCTTCAGAGTCTGCACACAGG |
| 110 | RM 17182 | 4 | TGCAGCGTCTCATCATAAAGTCG | GCTTAGTGCTGTGAACTGTGAAGACC |
| 111 | RM 17377 | 4 | ATATTACTTCGACGCTGGATCAGG | GTCAGTTCGTCAGGCACAACG |
| 112 | RM 17611 | 4 | GAGCAAATCCAGACCAGAAGTGC | ACACCTGGCAGCCAAGATATGG |
| 113 | RM 17620 | 4 | ACCATCTCGTATTTGGCTCATCC | AACATGCACTGGATGATCTCTCG |
| 114 | RM 17632 | 4 | ACAGCATGCGCACCACATAAAGG | CGTGGTTCACACACTTACATTGTTGG |
| 115 | RM 17645 | 4 | GCTTTGTTGGGTGATCGTCTAGG | GGCGATCTACTGTTCTTGTCACC |
| 116 | RM 17669 | 4 | AGAGCCAAATCCAACGGTATGTAGC | CCAACATTCAGGCGACAGAGG |
| 117 | RM 261 | 4 | CTACTTCTCCCCTTGTGTCG | TGTACCATCGCCAAATCTCC |
| 118 | RM 280 | 4 | GTGCTCTCCATGTCGGATTATGC | CAAGGCAACAAGATTGGTTAGTGG |
| 119 | RM 307 | 4 | GTACTACCGACCTACCGTTCAC | CTGCTATGCATGAACTGCTC |
| 120 | RM 335 | 4 | GTACACACCCACATCGAGAAGC | TCCATGGATATACGAGGAGATGC |
| 121 | RM 3474 | 4 | ACCTCACCTTTCCCTCGATTGG | GTTGGTTGCTTCCTCCCATACG |
| 122 | RM 348 | 4 | CATGAAGCTGTGTTGCTGTTGC | CGCTACTAATAGCAGAGAGACCATCG |
| 123 | RM 3785 | 4 | GCAAGCAGCAAGAGCGAAGAGG | CTCAAGGCCGCCTCTCAAATCC |
| 124 | RM 470 | 4 | CCCTCCCGTAGACCTTGTACCC | CCACAGCTAACCAATCCTTCTCC |
| 125 | RM 5473 | 4 | GGAGATAAGACACGAGGGAATTATGC | AGATTAACTACGCGCGCTCATCC |
| 126 | RM 551 | 4 | CTTACTCCATTGGGCTGGAACC | TGTAGGGTGGTAAGAGATCCACTCC |
| 127 | RM 5586 | 4 | AGATGGCTGGCCAACAGACTGG | ACAATGCCCATCCACTGCTTCC |
| 128 | RM 6679 | 4 | TTTAGGCCGTAAGAGCGAACATGG | ATATGCCGATGCAGAACAAGATCG |
| 129 | RM 13 | 5 | TCCAACATGGCAAGAGAGAG | GGTGGCATTCGATTCCAG |
| 130 | RM 164 | 5 | TCTTGCCCGTCACTGCAGATATCC | GCAGCCCTAATGCTACAATTCTTC |
| 131 | RM 169 | 5 | CACCTCCTCCAAGATCCTTATGC | CTCTCTGTCTCGCTGTCTGTTGC |
| 132 | RM 17769 | 5 | CATGCATGCAGTGAATTTCAGG | GCTAAGCTAAGTTGGTCTCAGTTGC |
| 133 | RM 17990 | 5 | TCTCCACACAATACAAGTCACG | GAGAGTTGGAGAGAAAGGAAGG |
| 134 | RM 18004 | 5 | CTCGAAGCTATTAGCCGGGATCG | ATCTTCTTCCTCGCCGTCTTCC |
| 135 | RM 18194 | 5 | CCTCTTCCAATGTTCTCAAGATCG | TATTTCCACGGACAAGAGTAGGG |
| 136 | RM 18204 | 5 | GAAACTAGAGATGCACACATCC | ATGGTAAGTACTCCCTCCATCC |
| 137 | RM 18212 | 5 | TAGATGTCAGTGGTCAGTACAGG | TGAACTAGTACTCCATCCAACC |
| 138 | RM 18222 | 5 | TGATTCCTCTATATGCAGCCTTGG | TATCGTGGTTTCATCGTGTGTGC |
| 139 | RM 18353 | 5 | AGATCTCACTATTGAGTAGCCCATGC | CACCTTGCCCTTAAATACCAACC |
| 140 | RM 18378 | 5 | GCTATCCTAGTGCTTTGTGTCC | TTCTCATGGTGGAGTTATAGGC |

| Sl. No | Name of rice microsatellite | Chromosome number | Sequence of Forward primer | Sequence of Reverse primer |
|---|---|---|---|---|
| 141 | RM 18382 | 5 | GGAATTAAATGTGCGGGAATGC | TGTAAGTACAAATCCGGCACCTATGG |
| 142 | RM 18384 | 5 | GCAGCAGAAAGGGAGAGAGTATGG | CAGCAACGTACGTACCAACAGG |
| 143 | RM 18508 | 5 | CATCGACTCTGCCTTCAAAGACC | GGGCTTGTCCTGGTAGATCAGC |
| 144 | RM 18622 | 5 | GGCATGCATGTGTCTAACATTCG | AAGCAGAATTTGGCCGTGTTAGC |
| 145 | RM 18639 | 5 | CATCATGTGGTAAGTGTGCAACG | GGTTGCGATGAGATTACGAGACC |
| 146 | RM 18647 | 5 | ATTTCTAGCCCTCACGGTAAATGTGG | GGGTGAAACGGTGTCTGACTGG |
| 147 | RM 18913 | 5 | CAAGATCTTTGCAACTGAGGAAGG | TTGCTACTTCGTGTTGTCACTCTACG |
| 148 | RM 18919 | 5 | AGGAGTTCAGTTTCTGCAAGTCAGG | CAGCATGCCGTAGTTCACACC |
| 149 | RM 18941 | 5 | GTGAAGTGCAGCCGAAGAGC | ATCGATCTCTCATCACGATCAACC |
| 150 | RM 18959 | 5 | GAGCTATTCCATTACAGGTAGTGAGG | GGGTGAGATATGGGATAAGAACG |
| 151 | RM 19183 | 5 | CATAAGCTAAGCACACCCACTCG | GCTTCATCGACGTCAACTACACG |
| 152 | RM 19187 | 5 | ACCACCGATGACAATGAAATGC | TGCAGGATGGCACATGTAGTACC |
| 153 | RM 19199 | 5 | GCTCTACCAGGTATTATAGCCGATCC | AACTCCTCCAAGGTTCCATAGCC |
| 154 | RM 19218 | 5 | CGGAGGGAGTAGGTACGTAGGG | CCCATTCCATTCTACACTGACG |
| 155 | RM 19221 | 5 | CCGATAATCACCTCCATTCCTAGC | AATGGAGTAGACGGAGCACTAATCG |
| 156 | RM 413 | 5 | CCAATCTTGTCTTCCGGATCTTGC | AGATAGCCATGGGCGATTCTTGG |
| 157 | RM 7029 | 5 | CTTGATCAGTTAAAGAGCCAGTCTGC | TTCAAGGCAGACAACAACATGG |
| 158 | RM 190 | 6 | GCTACAAATAGCCACCCACACC | CAACACAAGCAGAGAAGTGAAGC |
| 159 | RM 19255 | 6 | TTAAGCTAGGGAATCAGCGGTTAGC | GGAGTTGCAGTGTGGTGTGTGG |
| 160 | RM 19483 | 6 | CCAACTAAACAAGCCCTGACTATGG | GGTTGTCCCGTCAATAAAGTACCC |
| 161 | RM 19514 | 6 | TTTATGGGATGGAGGAGTATCG | CAGAAAGTAAGCGTGTTCAAGACC |
| 162 | RM 19799 | 6 | GAGAGACATGTTGTTGTGTTCTCC | CTGGTTGATGTTACCAATCACG |
| 163 | RM 19985 | 6 | AGCAGATATCACACACAGCATTGG | GGAGCTTCATTTGTGATGAACCTAGC |
| 164 | RM 20003 | 6 | TGCACTATTGGCAGTAACATCG | GATGTGGATGGTATGAGAGTTGG |
| 165 | RM 20023 | 6 | CTGACCTGACGGCTGACATGACC | CAAGCAACCTTTCGGGATTTGC |
| 166 | RM 20158 | 6 | ACTCACCGTACGAACTCGATGC | ATCTGTCCTGAACCCGATACTGC |
| 167 | RM 20168 | 6 | GAATATCCTTGGCTCTCTAGACTTGG | TGGGACTTGACTTGGACTATTTGC |
| 168 | RM 20176 | 6 | CCCTCTGTAACATCTGCATTCC | CTACCAACACATGCACAATTCG |
| 169 | RM 20182 | 6 | CCTTATTGGGCCAGAGATAGTTGG | CAGTGTTGTCGACGGTACAATGC |
| 170 | RM 20397 | 6 | CCATTTAAACTCAGGACCGATGC | CTGAACACGAAAGGGCATGTAGC |
| 171 | RM 20429 | 6 | AGTTTCCTAGCGCTTCAGCATCC | TGTGCGTATGAGAACCATCATGC |
| 172 | RM 20677 | 6 | TTCCTTCCAGATTTGCACGTACC | CGAGTAATGGATGGATGGATGG |
| 173 | RM 20683 | 6 | ATGATGATCCCTTCAGCCTTTCG | TGTCAGTGCCTCCTCTTCATTCC |
| 174 | RM 20686 | 6 | ATGCACACATAGTCAACAGCTTCC | GTGATCACCACACAGACTGAAACC |
| 175 | RM 20698 | 6 | ACGGTCGTAGCAATAACTAGC | GGTCATAGGTCATAACTAGTCTGC |

| Sl. No | Name of rice microsatellite | Chromosome number | Sequence of Forward primer | Sequence of Reverse primer |
|---|---|---|---|---|
| 176 | RM 217 | 6 | ATCGCAGCAATGCCTCGT | GGGTGTGAACAAAGACAC |
| 177 | RM 238 | 6 | GATGGAAAGCACGTGCACTA | ACAGGCAATCCGTAGACTCG |
| 178 | RM 253 | 6 | CCATCTCTGCCTCTGACTCACC | TCCTTCAATGGTCGTATCTTCTCC |
| 179 | RM 314 | 6 | CTAGCAGGAACTCCTTTCAGG | AACATTCCACACACACACGC |
| 180 | RM 340 | 6 | GGGTAAATGGACAATCCTATGG | ACCCTATTCTGGAGTTCATCTGG |
| 181 | RM 3628 | 6 | GCCCTAGACACACCCGTACC | TGCCAGATCAGAAATCATGC |
| 182 | RM 402 | 6 | CATCTCTGCTAGGTGGTGAATGG | CTCAGCTGGCCTATGACAATGG |
| 183 | RM 439 | 6 | CTGGGTCTAATCTCGTCCTAAATTGC | CGCCTCTCATAACAGTCCACTCC |
| 184 | RM 508 | 6 | AGAAGCCGGTTCATAGTTCATGC | ACCCGTGAACCACAAAGAACG |
| 185 | RM 541 | 6 | TATAACCGACCTCAGTGCCC | CCTTACTCCCATGCCATGAG |
| 186 | RM 589 | 6 | GTGGCTTAACCACATGAGAAACTACC | TCACATCATTAGGTGGCAATCG |
| 187 | RM 18 | 7 | TTCCCTCTCATGAGCTCCAT | GAGTGCCTGGCGCTGTAC |
| 188 | RM 20833 | 7 | GATATGGTTCCACTTCACCATGC | TTAGAAACTCGCCTTCAGAACTGC |
| 189 | RM 21122 | 7 | ATGTGCAAAGCTGAATCCATCG | TGATCACATACCCTCCGAATTGC |
| 190 | RM 21136 | 7 | GAAGCCAAACGCAACCAAGG | TCGGTGAATTGTCCTGTATCAGC |
| 191 | RM 21320 | 7 | CGTGCAACCCTATATGTAGATTGTGG | GGAGCCCGGAGTAATTTCTAAAGC |
| 192 | RM 21345 | 7 | GCATGCTAAGCTGTAGAAGTTAGTGG | GCTACATGTCACCGATCAGACC |
| 193 | RM 214 | 7 | CTGATGATAGAAACCTCTTCTC | AAGAACAGCTGACTTCACAA |
| 194 | RM 21452 | 7 | GGTTATCCAACCGGGACTACC | CATACACCTGAGTGTACGAAAGAGC |
| 195 | RM 21470 | 7 | TCTTGCCATCACATAGCAACAGG | ACTCGGTGAGCATCCAATGTCC |
| 196 | RM 21478 | 7 | TAACACAGTTCTTCTCGCAACG | AAGTTCCCTTGTGTGATTGACC |
| 197 | RM 21661 | 7 | CTCCGCAGGGTCTGTTTAGTTTCC | GACGATATTGTTGCAAGCGTGAGG |
| 198 | RM 21693 | 7 | GCACAGACCAGAACTTTCTTCG | TGGCGAGTGTAGATGTAATTGG |
| 199 | RM 21700 | 7 | GCGGGTGCCTACACATTTAAGG | GGCCGAATATTTAGCTCAACAGC |
| 200 | RM 21930 | 7 | TAGCTGTTGTGCATGATGTTCG | GCTGGACTCCTCTTGATCTCTCC |
| 201 | RM 21950 | 7 | AACCTTGCACCATTCTCTTCTGG | GGAATGGTTTACATCTCCGATCC |
| 202 | RM 21953 | 7 | TTGGCAAAGAAGCTCACAACAGG | GAAGAGATGGTGGACGATGATGAGG |
| 203 | RM 22164 | 7 | TGGATCTTCGATCTCTCACTCACC | GCATATGCATGTTCCATGATCG |
| 204 | RM 22175 | 7 | CCTTCCCAAATCAGTTCACAACC | TGTTGTTGGCTTGATGATGAGC |
| 205 | RM 234 | 7 | TTCAGCCAAGAACAGAACAGTGG | CTTCTCTTCATCCTCCTCCTTGG |
| 206 | RM 248 | 7 | AGAGAGCAAGTTTGAAGCGAAGC | ACCAAGAGGGTAGCCTAGCATGG |
| 207 | RM 346 | 7 | CGAGAGAGCCCATAACTACG | ACAAGACGACGAGGAGGGAC |
| 208 | RM 3859 | 7 | CTCATGCTTTCAGTCATTCAGTGC | TCCTGGATTCATGGTGTCTTTAGC |
| 209 | RM 478 | 7 | GGGTGGAGTGTAATAATAGCAAGC | AACACGTCCAAAGTCACAGAGC |
| 210 | RM 481 | 7 | TAGCTAGCCGATTGAATGGC | CTCCACCTCCTATGTTGTTG |

| Sl. No | Name of rice microsatellite | Chromosome number | Sequence of Forward primer | Sequence of Reverse primer |
|---|---|---|---|---|
| 211 | RM 82 | 7 | TGCTTCTTGTCAATTCGCC | CGACTCGTGGAGGTACGG |
| 212 | RM 22224 | 8 | CAAATTGTCCATGTGGATCACC | AAGGGCAACATATGAGGAATGC |
| 213 | RM 223 | 8 | GAGTGAGCTTGGGCTGAAAC | GAAGGCAAGTCTTGGCACTG |
| 214 | RM 22459 | 8 | ACCACCGCGACTTCAGTTCTCC | CGGAGGTGTTGGTGGAAGAGG |
| 215 | RM 22523 | 8 | CGTGCTTAATTACCTGGCTTCG | GCACGTATTCACAGCTAGCAACC |
| 216 | RM 22703 | 8 | GGTCAATTAGGAGCGCGTATGG | CTCTTCCTTCGCTGCCCTATCC |
| 217 | RM 22892 | 8 | GAACATGTCTTGGGTGTGATACAGG | TATGTTTAAACGGGCTCCAACC |
| 218 | RM 22897 | 8 | AGAGAGGAGCGACAGGGAGGAAGG | TGGCCCAAGTCGGCTTAGATGC |
| 219 | RM 22905 | 8 | CACTGCTCACTGCTGCCTTGC | CACGGGAGCTTCTGTCAGTGG |
| 220 | RM 230 | 8 | GCCAGACCGTGGATGTTC | CACCGCAGTCACTTTTCAAG |
| 221 | RM 23068 | 8 | AGAAGCGTCCTCCTCCTCCTACTCC | GCGAGCTTCTTGGATCTGTGACC |
| 222 | RM 23080 | 8 | CAACCTCCCGCCCTAACTACC | ATCAACAGAAGAAACCGGCTACC |
| 223 | RM 23087 | 8 | GATATTAGCTAGACATGGCACTCTGC | GTACATCCGCATGAATAGAGTGG |
| 224 | RM 23096 | 8 | GTGCAATCATGTTCACATCAGC | AAATAGACTACTGGGTGCGTTCG |
| 225 | RM 23099 | 8 | GACACGCCTGGAGACAATAGTAGG | TTTATTCGGGATGCGTGATGC |
| 226 | RM 23358 | 8 | CAGAGAGGTGAGATTGTGACG | ACAGTAACAGAGTGCTGAAACG |
| 227 | RM 23386 | 8 | AGGTTGACCTGTGTGAGTAGCAAGG | ACATCGCCAACCATCTCAAGG |
| 228 | RM 23605 | 8 | TTCTCTAGATAGAACTCAGGCTCTGC | TGGATTAGCCGACTAGGTTAGAGC |
| 229 | RM 23612 | 8 | CGATCGACCACAAGTAGAGTACG | CAATCACGTACAGGCAAGATCG |
| 230 | RM 23645 | 8 | CATACAGCATGCTCACAGTTGATCG | CATCAGCATCTGGGACCTCTCC |
| 231 | RM 264 | 8 | GTTGCGTCCTACTGCTACTTC | GATCCGTGTCGATGATTAGC |
| 232 | RM 308 | 8 | GGCTGCACACGCACACTATA | TTACGCATATGGTGAGTAGGC |
| 233 | RM 3309 | 8 | GCCTACTCAGCTTCCTCTCCTTCG | CGCCATTTACGGCAGCAACC |
| 234 | RM 331 | 8 | ATGTTGCACTCCTTCAATGTCC | CATGAGACAATGCCAGAAAGC |
| 235 | RM 407 | 8 | GACTACGAGACGAGTGATTTGAACC | GCGTGGGAAATGACTAGGAGTAGG |
| 236 | RM 458 | 8 | GGTGATCTGCATTGTCAACG | TGCAATGGATCTAGCGACTG |
| 237 | RM 5556 | 8 | GTAAGCCATTTGCACGGACAAGG | GAGCTCAGGATCATCCCTACATGC |
| 238 | RM 607 | 8 | TTGCTAGTGCTTACCACCCC | TCCCAGTCACCCTGCTACTC |
| 239 | RM 107 | 9 | TCTTACTGCGTCCTCTGGGTTCC | ATTCTTGCGGCGATTCATCTTCC |
| 240 | RM 205 | 9 | CCTAAGAGGAGCCATCTAACAACTGG | CTTGGATATACTGGCCCTTCACG |
| 241 | RM 215 | 9 | GAGCAGCAAGAGCAGCAGAGG | CATGCTCGACTTCAGAAGCTTGG |
| 242 | RM 23998 | 9 | CTGCACGTACGGTCAAGTCTACC | GCATTGCAAGGGTTGAAGTGG |
| 243 | RM 24031 | 9 | AAGGTGGTAGCTGCTTCATTCG | GGAGATCATATGGAGGGAAAGG |
| 244 | RM 242 | 9 | AAACACATGCTGCTGACACTTGC | TTACTAGATTTACCACGGCCAACG |
| 245 | RM 24258 | 9 | GTTTGGAACAGAGGAATTCAGG | TTCCTTGCGGTATGTAACTCTCC |

| Sl. No | Name of rice microsatellite | Chromosome number | Sequence of Forward primer | Sequence of Reverse primer |
|---|---|---|---|---|
| 246 | RM 24263 | 9 | CTCATCGGCGACATATCACAGC | ATGGGATGTACACAGCCAAACG |
| 247 | RM 24274 | 9 | CCTTTGTCCCACTTTGGACTACC | CCATGATTCCCTACAGGATACTTCC |
| 248 | RM 24336 | 9 | GATTAGCTAACCGGAGGCAACC | AACCGCCAGTACAACTCTACAACG |
| 249 | RM 24616 | 9 | CACCTTGGCCAACTAACTAATCG | GGGCAAGAGGAATTCACAACC |
| 250 | RM 24664 | 9 | ATCTAAACACAGCCCTAGTTGC | GAGCCATAACTTTACCATGTGC |
| 251 | RM 257 | 9 | CCGTGCAACTTAAATCCAAACAGG | GGAATCCTATATGAGCCAGTGATGG |
| 252 | RM 316 | 9 | CTAGTTGGGCATACGATGGC | ACGCTTATATGTTACGTCAAC |
| 253 | RM 434 | 9 | TCTCTAGTTGCCTCATCCCTCTAACC | GGCTCAACCTCTATATTTGCTGATCG |
| 254 | RM 524 | 9 | ATCATAGCCCAGACCAAGAATGC | AGATGAAGAGCAGGAACCGTAGG |
| 255 | RM 748 | 9 | CGACCCAATATCTTTCTGCC | CATTGGTCGTGCTCAACAAG |
| 256 | RM 216 | 10 | GATGGTAAAGGAAGAACGTGTGC | CACTCATAGACGCATCACATAGCC |
| 257 | RM 24866 | 10 | CCCTTTCATTTGCGCTTTATGG | GGGTTATTTCAGTCCGTGATTGC |
| 258 | RM 24900 | 10 | CACAAGTTCTGGGTCTCAGTGG | AGAGGATCAACATCGCAATACC |
| 259 | RM 25060 | 10 | AGCTTTGACTGAGGTGTGGAGTGG | TGGAGCGTTGTATCGATGTGG |
| 260 | RM 25066 | 10 | GTTGTTAGGTGTAGCCGTGTAGG | GTACACCAATAACTGTGGAAGAGC |
| 261 | RM 25212 | 10 | ACGTACGGTGTTCCAATTGTGG | GATTTGTATAGGGTGGGAAACACTCG |
| 262 | RM 25217 | 10 | TGGCAGCCTCTATGTTAGACC | GATGCATATCGGTGATTTGG |
| 263 | RM 25231 | 10 | CCAGCCTGAAGGCAAGGGTAGC | CCGGCCCAAAGTTTAGGGATGC |
| 264 | RM 25404 | 10 | GCAACGGTTCTCCTTCCACTACC | CCATGATAGCGTTAGCCATAAACG |
| 265 | RM 25420 | 10 | CAAGGGTGAGGCCATGATTTCC | CGTGTGATGTGGTCATGAAATGG |
| 266 | RM 25425 | 10 | CCAGCCCAAACAGCTCTTGC | GGGCACTGTTTGTCTTTCTGTGC |
| 267 | RM 25436 | 10 | CGATCACTCACTCATCCACATCG | GGTTAACCAAACAGAGACAACTGAGC |
| 268 | RM 25735 | 10 | AGGCAGGCAAGCAGTAGTTTCG | ATCAAGATCAGGAGCCGCAAGG |
| 269 | RM 25751 | 10 | TGACGTCAGCAGAAACCATTCC | CTTGCCTTGCTTCTTCATTTGG |
| 270 | RM 25771 | 10 | CCTCTGTGGCTCTGTGACGTACC | GCATTTGGCTCTTATTCGTTGTCC |
| 271 | RM 269 | 10 | GAAAGCGATCGAACCAGC | GCAAATGCGCCTCGTGTC |
| 272 | RM 271 | 10 | TCAGATCTACAATTCCATCC | TCGGTGAGACCTAGAGAGCC |
| 273 | RM 304 | 10 | TCAAACCGGCACATATAAGACC | CGTTGTAGTGTCAGCAAGATAGGG |
| 274 | RM 333 | 10 | GATGTACTTGCCAACATGCTCTCC | AGCACACGCGAGTGATGTAACG |
| 275 | RM 591 | 10 | CTCATAGGTGGGTTAGTTTCTTGG | GCTGGTTTACAACTTGCTACTCTACC |
| 276 | RM 7545 | 10 | GTTTCCATATCCGTGCTATTCG | CACGATTCCTACAATACGAGAGC |
| 277 | RM 202 | 11 | TGGAACACCCATAGACAAACAGC | TGGCAAGTGGTATTCTTCCTTCC |
| 278 | RM 206 | 11 | ATCGATCCGTATGGGTTCTAGC | GTCCATGTAGCCAATCTTATGTGG |
| 279 | RM 21 | 11 | ACAGTATTCCGTAGGCACGG | GCTCCATGAGGGTGGTAGAG |
| 280 | RM 224 | 11 | ATCGATCGATCTTCACGAGG | TGCTATAAAAGGCATTCGGG |

| Sl. No | Name of rice microsatellite | Chromosome number | Sequence of Forward primer | Sequence of Reverse primer |
|---|---|---|---|---|
| 281 | RM 229 | 11 | CACTCACACGAACGACTGAC | CGCAGGTTCTTGTGAAATGT |
| 282 | RM 254 | 11 | AGCCCCGAATAAATCCACCT | CTGGAGGAGCATTTGGTAGC |
| 283 | RM 25995 | 11 | TTGGGAAGGAGGGAGTAGATTTGAGC | GGCATGAACGACAACATGAACG |
| 284 | RM 26182 | 11 | TGGGTCCTCCGATCCTTGTACTCG | GCTGGTGTTGACGGTCATTAAGTGC |
| 285 | RM 26194 | 11 | GTGGTGGTCAAACACTCCACACC | ATTCCAGGATTGGACAAACAGAGC |
| 286 | RM 26213 | 11 | GCCACAGGAGACAGCAAGAACC | CGATCCAATTCCAGCCTAGATAGC |
| 287 | RM 26409 | 11 | TCCCTTTCCTATATATCGGCTCACC | ACATAATGTGCGAGCCTGTGC |
| 288 | RM 26416 | 11 | CGTGCCTGGAGAAAGCATATAACC | AGAATCCCTCAATCCGAACAACC |
| 289 | RM 26429 | 11 | ATTCCCTTGTTATGGCCAGTTTGC | CACGACTGACCATCGTGAGAAACC |
| 290 | RM 26438 | 11 | GCTCCTGCATTTAGGCGACTCC | TTGTCTCAACCACCCAAGAAAGC |
| 291 | RM 26447 | 11 | TGCAGGTTGTACAAGCAACTCC | TTGCTTCGAGTATGATCGTCAGG |
| 292 | RM 26644 | 11 | GGGCAGTGACCTAGAGTTTCTCG | CGTGATTTGAGAAGTGGTTCAAGG |
| 293 | RM 26662 | 11 | AACTCCACCTTTACCCACACTGC | CGCCAGTAGGAAGGAGATGATAAGG |
| 294 | RM 26870 | 11 | GTCTGCCTGGTTAGACATGGTACG | GGACCATTTGGACCCACATACG |
| 295 | RM 26871 | 11 | CTCCACAGCTCTCTTCTTCTTTCACG | GAGATTTCTGTCTCCGCTGATGG |
| 296 | RM 27132 | 11 | GAATCAAGGATAGACACGAAGG | TCTAAGTTCTACCACCACAACC |
| 297 | RM 27180 | 11 | AAGAAGAGAAGGGATGGGATCTGG | TCTAAACAGGGCCTCAAACTGTATCC |
| 298 | RM 27184 | 11 | ATGTGACCTCGTCGATCTTGTTCC | CCGAGTACAGCAGCACACAGC |
| 299 | RM 27186 | 11 | GGTTCCGACCTCCGAACACAGC | CTCCCAACCCTTCTCCTCCTTCC |
| 300 | RM 27369 | 11 | ACATATCGACGGTGGATGAGAGC | TCCGTGTGCATACATTCTTGAGC |
| 301 | RM 27373 | 11 | ATGTCCACATGGGTAGCTGATCG | CAGCAAACAGAAGAGCACACAGG |
| 302 | RM 332 | 11 | GAAGGCGAAGGTGAAGAAGAAGC | CCTCCCTTGCATGATACCTTGG |
| 303 | RM 536 | 11 | TACCAGGATCATGTTTCTCTCC | ACTGTGAGATTGACTGACAGTGG |
| 304 | RM 5590 | 11 | GAGGGAGGGAGTACATATCTGATCG | AGCAACTGGATAAGCGATTGAGG |
| 305 | RM 5961 | 11 | GATCAGCAGTGGACGATTCACC | TCTCCTGTATGCTCCTCCTCACC |
| 306 | RM 6440 | 11 | GATAGTGATTGCTGGTCTATCG | ATATTGTCTCAGTTGGGTCTGC |
| 307 | RM 17 | 12 | TGCCCTGTTATTTTCTTCTCTC | GGTGATCCTTTCCCATTTCA |
| 308 | RM 19 | 12 | CAAAAACAGAGCAGATGAC | CTCAAGATGGACGCCAAGA |
| 309 | RM 20 | 12 | ATCTTGTCCCTGCAGGTCAT | GAAACAGAGGCACATTTCATTG |
| 310 | RM 247 | 12 | AAGGCGAACTGTCCTAGTGAAGC | CAGGATGTTCTTGCCAAGTTGC |
| 311 | RM 260 | 12 | ACTCCACTATGACCCAGAG | GAACAATCCCTTCTACGATCG |
| 312 | RM 27418 | 12 | CAAACTGCATGTACGTCTTCTTGC | GGTTTGGATTGTGATGGTTTGG |
| 313 | RM 27650 | 12 | AGCTTGACGTCATTGCTCTCAGC | GAGAGTACTGCTACCTCCGTTTCAGG |
| 314 | RM 27683 | 12 | TGAGTCGAGATTTCACATCAGG | TTTCCTTCCAAAGGTAGAGGTAGG |
| 315 | RM 27689 | 12 | AACCTGCAATTACCATCCAAGC | AATACACACCCACAGTTCCACACC |

| Sl. No | Name of rice microsatellite | Chromosome number | Sequence of Forward primer | Sequence of Reverse primer |
|---|---|---|---|---|
| 316 | RM 277 | 12 | CGGTCAAATCATCACCTGAC | CAAGGCTTGCAAGGGAAG |
| 317 | RM 27840 | 12 | TTTGCGTGCTAGGGAGATTAGC | CATTATGTACTTACTCCCTCCCTTCC |
| 318 | RM 27841 | 12 | TAAATACCCGACAATGCCCTAGC | GGAAATCCCATCAATCACAAGAGC |
| 319 | RM 27851 | 12 | CCCATCTCAACTTCCATGGTACGG | CGCGTGGAAGGCAAAGATACG |
| 320 | RM 27863 | 12 | AAACATGTGCAGACTTTCCCAGTCC | GGTGGGATTGAGATTGGTCATCG |
| 321 | RM 27879 | 12 | GCTGTAATTGTACGGCTCCAACC | ATAACGAGCTGATCGCAATGTGG |
| 322 | RM 27900 | 12 | CAAATATAACCGCATGGAGACACG | AGCAGTACTCCCTCCCTCCTTCC |
| 323 | RM 28052 | 12 | ACTAAAGATCTTCGAGCTGACC | GCTACATGGAGTATGGGTTCC |
| 324 | RM 28059 | 12 | TGGCCGGTTAGATTTGATAGAGC | GATGTAATCAACCAAGGGACACG |
| 325 | RM 28067 | 12 | GAGGGAGTACTAAACTTCCTAACG | ATGTGGATGTGATCTCCATAGG |
| 326 | RM 28070 | 12 | AAGGCACCAGGAATATGACAAGC | GGGATGTGGGATTTGGAGAGG |
| 327 | RM 28267 | 12 | GCATAGCCCTGTTTGTTGCATGG | CGGTCCTTCCTCTTCTGTCATAACG |
| 328 | RM 28273 | 12 | TTGAAGGTTGGTTGTTCTACGG | GGATACATGCAACTATCAGCAAGG |
| 329 | RM 28303 | 12 | AGGACTTAAGGCGTCGAAAGATAGC | CTAGCTGGGTTGGTGTTCTCTAGG |
| 330 | RM 28305 | 12 | GTCATCTTCGCAAATGGTGATGG | GGTCGTCGTGGTGTTATTCTTGG |
| 331 | RM 28558 | 12 | TATATATACGTGCGCGTGTGC | TAGTAGCTTGAGTTGTACCGCTTACC |
| 332 | RM 28559 | 12 | TTGTGCGTACTTGCTTGTCATGG | CTGTTGTTGTTTGCCGCTAATCC |
| 333 | RM 28564 | 12 | ATACAATTGTGTGGGCACTCC | GCTCTCCAACTGGAAGATTAGC |
| 334 | RM 28568 | 12 | GCAGTACATGTATCCAGTATCGTACC | TTGTATGTGTGCGCGTATTCC |
| 335 | RM 28580 | 12 | GCACAATGGGATGATACGAGACC | TTGGGAACTAGTAGCAGGCAAGG |
| 336 | RM 463 | 12 | GAGGATTAATTAGCGTGTGACC | GTCGTGACATCTACTCAAATGG |
| 337 | RM 491 | 12 | CACATGATGCGTAGCGAGTTGC | TTATGCCTCTCCCTTCCCAATTCC |
| 338 | RM 511 | 12 | AACGAAAGCGAAGCTGTCTCC | ATTTGTTCCCTTCCTTCGATCC |

**Appendix II. Phenotypic data of $F_2$ population screened for response to stress at 800 ppm of iron in BSA**

| $F_2$ plant no | Leaf bronzing ($4^{th}$ week) | Leaf bronzing ($6^{th}$ week) | Total no of roots | No of fresh roots | Root length (cm) | Shoot length (cm) | root weight (g) | shoot weight (g) | Fe adsorbed on root (mg $l^{-1}$) | Iron content in root (mg $kg^{-1}$) | Iron content in leaf (mg $kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 9 | 9 | 24 | 0 | 19.3 | 55.1 | 3.05 | 4.25 | 2.90 | 6567.86 | 2265.00 |
| 2 | 3 | 9 | 26 | 4 | 19.2 | 52.6 | 4.60 | 6.20 | 4.25 | 8155.00 | 1382.50 |
| 3 | 7 | 9 | 22 | 0 | 16.6 | 52.9 | 3.40 | 3.60 | 3.13 | 10428.85 | 1918.75 |
| 5 | 7 | 9 | 24 | 0 | 20.4 | 53.9 | 3.25 | 3.50 | 3.49 | 9503.52 | 2036.25 |
| 7 | 5 | 9 | 25 | 2 | 23.1 | 55.4 | 5.20 | 5.65 | 4.03 | 6900.24 | 1552.50 |
| 8 | 9 | 9 | 24 | 0 | 16.1 | 55.7 | 4.80 | 8.05 | 3.00 | 8068.18 | 2145.00 |
| 9 | 7 | 9 | 23 | 0 | 19.8 | 53.7 | 3.75 | 4.55 | 3.81 | 6957.77 | 2060.00 |
| 10 | 3 | 9 | 24 | 3 | 18.7 | 59.8 | 3.05 | 5.55 | 3.89 | 7762.50 | 3068.66 |
| 11 | 9 | 9 | 23 | 0 | 18.8 | 53.9 | 3.35 | 6.65 | 3.17 | 6990.00 | 2477.50 |
| 12 | 9 | 9 | 22 | 0 | 15.8 | 50.1 | 2.85 | 4.00 | 2.80 | 6391.67 | 2560.00 |
| 14 | 5 | 9 | 24 | 1 | 16.8 | 55.5 | 3.75 | 7.75 | 3.88 | 11964.29 | 1586.25 |
| 15 | 9 | 9 | 22 | 0 | 16.6 | 54.4 | 3.35 | 4.55 | 3.28 | 6504.08 | 2232.50 |
| 17 | 9 | 9 | 23 | 0 | 21.6 | 53.5 | 4.75 | 4.95 | 3.13 | 6953.13 | 2350.00 |
| 18 | 9 | 9 | 21 | 0 | 15.6 | 49.4 | 2.85 | 3.90 | 2.81 | 6547.15 | 2255.00 |
| 19 | 9 | 9 | 21 | 0 | 16.8 | 53.6 | 4.35 | 4.65 | 3.19 | 6935.55 | 2230.00 |
| 20 | 1 | 1 | 34 | 31 | 24.4 | 61.8 | 7.85 | 12.10 | 12.60 | 11763.89 | 736.84 |
| 21 | 5 | 9 | 25 | 1 | 16.5 | 52.5 | 3.35 | 4.60 | 4.36 | 7874.33 | 1566.25 |
| 22 | 3 | 5 | 28 | 15 | 22.7 | 56.3 | 5.45 | 8.05 | 7.69 | 11655.74 | 912.50 |
| 24 | 9 | 9 | 22 | 0 | 16.7 | 52.9 | 3.20 | 4.80 | 3.52 | 6357.14 | 2307.50 |
| 26 | 9 | 9 | 24 | 0 | 19.4 | 55.0 | 3.15 | 5.65 | 3.15 | 8857.14 | 2170.00 |
| 27 | 5 | 9 | 24 | 0 | 16.8 | 53.4 | 3.40 | 4.75 | 3.90 | 8482.14 | 1521.25 |
| 28 | 5 | 5 | 25 | 10 | 22.5 | 56.1 | 3.60 | 6.40 | 5.89 | 11564.16 | 1502.50 |
| 30 | 3 | 7 | 25 | 9 | 18.8 | 56.7 | 3.75 | 9.25 | 5.81 | 7917.81 | 1242.50 |
| 31 | 3 | 7 | 24 | 8 | 19.1 | 54.1 | 3.20 | 5.00 | 5.72 | 11373.42 | 1151.25 |
| 32 | 3 | 5 | 28 | 15 | 22.0 | 57.3 | 7.95 | 8.85 | 8.54 | 8502.44 | 921.25 |
| 35 | 9 | 9 | 25 | 0 | 20.1 | 54.0 | 3.70 | 4.50 | 3.13 | 6383.93 | 2238.75 |
| 36 | 9 | 9 | 24 | 0 | 19.4 | 52.3 | 3.15 | 4.45 | 3.35 | 7823.68 | 2355.00 |
| 37 | 3 | 7 | 27 | 10 | 19.9 | 55.7 | 4.40 | 8.85 | 4.91 | 7845.39 | 1221.25 |
| 38 | 3 | 9 | 26 | 3 | 19.2 | 53.6 | 3.30 | 6.60 | 5.33 | 9820.36 | 1286.25 |
| 39 | 9 | 9 | 22 | 0 | 17.9 | 53.0 | 3.85 | 5.10 | 3.00 | 8254.90 | 3324.22 |
| 40 | 9 | 9 | 23 | 0 | 22.6 | 54.5 | 3.65 | 4.90 | 3.31 | 6785.42 | 2457.50 |
| 41 | 7 | 9 | 21 | 0 | 16.1 | 54.9 | 4.30 | 6.10 | 3.70 | 10339.80 | 1858.75 |
| 43 | 7 | 9 | 24 | 0 | 23.1 | 54.7 | 5.05 | 5.90 | 3.28 | 6940.48 | 1741.25 |
| 44 | 7 | 9 | 24 | 0 | 18.1 | 52.5 | 4.20 | 4.35 | 3.76 | 7539.37 | 1955.00 |
| 45 | 5 | 9 | 25 | 1 | 22.8 | 53.1 | 3.60 | 6.15 | 3.84 | 6914.89 | 1540.00 |

| $F_2$ plant no | Leaf bronzing ($4^{th}$ week) | Leaf bronzing ($6^{th}$ week) | Total no of roots | No of fresh roots | Root length (cm) | Shoot length (cm) | root weight (g) | shoot weight (g) | Fe adsorbed on root (mg $l^{-1}$) | Iron content in root (mg $kg^{-1}$) | Iron content in leaf (mg $kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 46 | 9 | 9 | 25 | 0 | 22.3 | 54.7 | 3.65 | 4.35 | 3.13 | 6966.39 | 2506.25 |
| 47 | 9 | 9 | 23 | 0 | 16.6 | 55.5 | 3.35 | 5.00 | 3.16 | 8500.00 | 2175.00 |
| 48 | 9 | 9 | 22 | 0 | 16.9 | 51.8 | 4.25 | 4.15 | 3.17 | 7185.42 | 2230.00 |
| 49 | 7 | 9 | 24 | 0 | 19.3 | 53.1 | 3.50 | 4.55 | 3.36 | 8061.76 | 1961.25 |
| 50 | 9 | 9 | 23 | 0 | 19.5 | 52.9 | 3.15 | 4.65 | 2.95 | 8951.92 | 2691.25 |
| 51 | 5 | 7 | 24 | 8 | 16.6 | 57.0 | 4.00 | 9.20 | 4.56 | 8644.56 | 1558.75 |
| 52 | 1 | 1 | 33 | 28 | 23.5 | 62.5 | 7.20 | 12.00 | 14.40 | 11318.52 | 732.50 |
| 53 | 9 | 9 | 26 | 0 | 22.6 | 55.9 | 5.90 | 7.30 | 3.06 | 6924.07 | 2515.00 |
| 56 | 7 | 9 | 24 | 0 | 21.9 | 54.1 | 3.25 | 4.45 | 3.61 | 8808.08 | 1975.00 |
| 58 | 3 | 7 | 27 | 10 | 20.8 | 56.3 | 4.45 | 8.80 | 6.44 | 11237.62 | 1192.50 |
| 59 | 7 | 9 | 25 | 1 | 19.9 | 55.1 | 3.55 | 4.90 | 3.28 | 9686.87 | 2090.00 |
| 61 | 7 | 9 | 26 | 2 | 23.0 | 58.1 | 6.85 | 9.55 | 3.78 | 7600.00 | 2045.00 |
| 63 | 9 | 9 | 23 | 0 | 19.1 | 54.2 | 3.65 | 5.20 | 3.31 | 6932.04 | 2471.25 |
| 64 | 9 | 9 | 24 | 0 | 18.7 | 53.5 | 3.20 | 4.85 | 2.89 | 6891.79 | 2247.50 |
| 65 | 9 | 9 | 24 | 0 | 17.9 | 52.9 | 3.35 | 4.70 | 3.11 | 6982.95 | 2816.58 |
| 66 | 9 | 9 | 23 | 0 | 16.1 | 50.6 | 3.00 | 4.20 | 2.71 | 6746.53 | 2533.75 |
| 67 | 9 | 9 | 22 | 0 | 18.7 | 52.7 | 3.85 | 5.00 | 2.85 | 7584.91 | 2308.75 |
| 68 | 9 | 9 | 27 | 0 | 18.2 | 53.1 | 3.70 | 5.35 | 3.10 | 8259.76 | 2390.00 |
| 69 | 5 | 9 | 24 | 0 | 20.6 | 55.9 | 3.35 | 4.50 | 3.86 | 11098.88 | 1608.75 |
| 70 | 5 | 7 | 26 | 9 | 21.9 | 53.8 | 3.40 | 4.35 | 3.91 | 9635.59 | 1587.50 |
| 71 | 9 | 9 | 26 | 0 | 18.1 | 54.6 | 3.30 | 4.05 | 2.85 | 7366.94 | 2198.75 |
| 73 | 9 | 9 | 25 | 0 | 19.9 | 53.9 | 3.95 | 5.95 | 2.78 | 6861.70 | 2016.25 |
| 74 | 3 | 9 | 24 | 2 | 19.4 | 55.1 | 3.60 | 5.05 | 3.91 | 8504.72 | 1173.75 |
| 75 | 9 | 9 | 26 | 0 | 16.9 | 56.2 | 3.35 | 4.90 | 2.68 | 7954.27 | 2653.75 |
| 76 | 5 | 9 | 28 | 2 | 22.6 | 56.5 | 4.00 | 5.10 | 3.69 | 7702.90 | 1577.50 |
| 77 | 9 | 9 | 22 | 0 | 16.8 | 52.7 | 3.15 | 4.40 | 2.90 | 7461.65 | 2417.50 |
| 78 | 9 | 9 | 22 | 0 | 16.9 | 51.8 | 3.20 | 4.35 | 2.77 | 8685.90 | 2310.00 |
| 79 | 5 | 7 | 24 | 8 | 18.1 | 52.6 | 4.20 | 5.85 | 3.83 | 8810.27 | 1362.50 |
| 80 | 3 | 7 | 26 | 10 | 17.6 | 53.6 | 3.85 | 5.30 | 5.39 | 9962.64 | 1128.75 |
| 81 | 1 | 3 | 31 | 21 | 22.7 | 58.2 | 6.70 | 9.40 | 9.98 | 10878.01 | 827.50 |
| 82 | 1 | 3 | 32 | 24 | 23.1 | 59.9 | 6.55 | 9.15 | 10.00 | 10660.85 | 800.00 |
| 84 | 9 | 9 | 22 | 0 | 16.8 | 52.6 | 3.15 | 4.25 | 2.99 | 7361.84 | 2477.50 |
| 85 | 9 | 9 | 25 | 0 | 18.6 | 53.7 | 3.25 | 4.60 | 2.95 | 6781.91 | 2200.00 |
| 86 | 9 | 9 | 22 | 0 | 16.5 | 52.9 | 3.15 | 4.40 | 3.49 | 8100.00 | 2256.25 |
| 87 | 9 | 9 | 23 | 0 | 19.4 | 52.7 | 3.50 | 4.30 | 2.88 | 8889.53 | 2682.50 |

| $F_2$ plant no | Leaf bronzing ($4^{th}$ week) | Leaf bronzing ($6^{th}$ week) | Total no of roots | No of fresh roots | Root length (cm) | Shoot length (cm) | root weight (g) | shoot weight (g) | Fe adsorbed on root ($mg\ l^{-1}$) | Iron content in root ($mg\ kg^{-1}$) | Iron content in leaf ($mg\ kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 88 | 7 | 7 | 23 | 5 | 17.0 | 54.6 | 4.20 | 6.20 | 3.93 | 9037.88 | 1698.75 |
| 89 | 1 | 3 | 27 | 19 | 19.2 | 56.8 | 7.10 | 9.00 | 9.44 | 10919.58 | 767.50 |
| 91 | 3 | 7 | 24 | 8 | 18.3 | 53.7 | 4.00 | 4.95 | 4.58 | 8681.99 | 1121.25 |
| 92 | 7 | 9 | 25 | 0 | 19.7 | 55.7 | 3.95 | 5.40 | 3.14 | 9652.27 | 1900.00 |
| 93 | 9 | 9 | 26 | 0 | 18.9 | 53.8 | 3.20 | 4.80 | 2.83 | 7533.02 | 2092.50 |
| 94 | 9 | 9 | 24 | 0 | 20.8 | 55.1 | 3.90 | 5.15 | 3.14 | 7856.56 | 1975.00 |
| 96 | 9 | 9 | 22 | 0 | 18.9 | 53.5 | 3.50 | 4.30 | 3.16 | 6753.68 | 2468.75 |
| 97 | 7 | 9 | 26 | 1 | 18.0 | 53.7 | 3.60 | 4.55 | 3.11 | 7386.67 | 2023.75 |
| 99 | 9 | 9 | 26 | 0 | 21.2 | 56.5 | 3.45 | 4.60 | 2.89 | 6819.03 | 2287.50 |
| 100 | 7 | 9 | 25 | 0 | 19.1 | 52.7 | 3.50 | 4.95 | 3.20 | 11142.00 | 1831.25 |
| 101 | 1 | 9 | 24 | 2 | 18.9 | 53.1 | 3.80 | 4.35 | 6.19 | 11731.25 | 976.25 |
| 104 | 1 | 9 | 26 | 4 | 19.4 | 54.3 | 4.50 | 5.90 | 6.55 | 11549.50 | 985.00 |
| 105 | 5 | 9 | 26 | 2 | 20.1 | 58.5 | 6.25 | 8.20 | 4.25 | 6337.06 | 1596.25 |
| 106 | 7 | 9 | 25 | 1 | 19.9 | 56.0 | 4.45 | 6.10 | 3.35 | 7555.08 | 2057.50 |
| 107 | 1 | 7 | 26 | 12 | 20.3 | 56.1 | 3.45 | 5.40 | 7.26 | 10068.75 | 966.25 |
| 108 | 9 | 9 | 22 | 0 | 18.0 | 53.6 | 3.25 | 4.95 | 2.65 | 7424.42 | 2276.25 |
| 109 | 9 | 9 | 22 | 0 | 15.7 | 49.0 | 3.00 | 3.95 | 3.07 | 6583.33 | 2483.75 |
| 110 | 1 | 1 | 32 | 28 | 23.9 | 61.1 | 7.15 | 11.60 | 11.90 | 12691.29 | 763.75 |
| 111 | 1 | 1 | 34 | 31 | 25.6 | 62.3 | 8.20 | 12.30 | 13.40 | 11514.12 | 692.50 |
| 113 | 9 | 9 | 22 | 0 | 16.0 | 51.2 | 3.05 | 4.35 | 2.82 | 6558.14 | 2468.75 |
| 115 | 9 | 9 | 21 | 0 | 16.6 | 55.3 | 3.15 | 5.00 | 2.95 | 6943.63 | 2246.25 |
| 117 | 5 | 9 | 25 | 1 | 20.6 | 56.1 | 3.55 | 6.20 | 3.82 | 11068.18 | 1688.75 |
| 118 | 3 | 5 | 28 | 14 | 22.6 | 56.5 | 4.65 | 5.60 | 6.51 | 11559.52 | 1062.50 |
| 119 | 1 | 5 | 28 | 17 | 21.9 | 57.0 | 4.40 | 9.25 | 8.80 | 10729.38 | 842.50 |
| 120 | 3 | 5 | 26 | 13 | 22.2 | 56.7 | 4.95 | 9.50 | 7.30 | 8626.09 | 1013.75 |
| 122 | 9 | 9 | 22 | 0 | 16.2 | 50.3 | 3.20 | 4.70 | 2.84 | 6160.38 | 2382.50 |
| 124 | 9 | 9 | 21 | 0 | 16.7 | 51.5 | 3.15 | 4.45 | 3.37 | 7495.28 | 2438.75 |
| 126 | 1 | 7 | 24 | 11 | 20.6 | 54.8 | 4.20 | 6.50 | 6.85 | 9584.46 | 937.50 |
| 127 | 3 | 9 | 25 | 3 | 21.0 | 54.1 | 4.35 | 6.20 | 3.57 | 7625.00 | 1475.00 |
| 130 | 3 | 7 | 24 | 7 | 20.6 | 55.0 | 3.50 | 5.85 | 7.08 | 8509.80 | 1271.25 |
| 131 | 9 | 9 | 22 | 0 | 16.6 | 51.6 | 3.20 | 4.50 | 3.19 | 6722.22 | 2448.75 |
| 132 | 7 | 9 | 25 | 0 | 20.8 | 53.6 | 3.45 | 5.15 | 3.31 | 7942.50 | 2033.75 |
| 133 | 3 | 7 | 25 | 8 | 19.4 | 55.4 | 5.65 | 8.70 | 4.52 | 9814.52 | 1168.75 |
| 134 | 9 | 9 | 23 | 0 | 19.1 | 53.1 | 3.55 | 5.10 | 3.19 | 7063.73 | 2292.50 |
| 135 | 3 | 5 | 27 | 14 | 20.6 | 55.1 | 3.85 | 4.95 | 6.17 | 8933.21 | 1108.75 |

| $F_2$ plant no | Leaf bronzing ($4^{th}$ week) | Leaf bronzing ($6^{th}$ week) | Total no of roots | No of fresh roots | Root length (cm) | Shoot length (cm) | root weight (g) | shoot weight (g) | Fe adsorbed on root (mg $l^{-1}$) | Iron content in root (mg $kg^{-1}$) | Iron content in leaf (mg $kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 137 | 3 | 9 | 23 | 1 | 17.1 | 53.0 | 3.60 | 4.50 | 4.67 | 10911.76 | 1407.50 |
| 138 | 3 | 9 | 24 | 2 | 18.1 | 56.3 | 3.20 | 4.65 | 4.51 | 10823.01 | 1505.00 |
| 140 | 7 | 9 | 26 | 1 | 19.0 | 55.2 | 3.65 | 7.90 | 3.17 | 10857.14 | 2005.00 |
| 141 | 3 | 3 | 27 | 16 | 23.0 | 59.1 | 4.50 | 8.95 | 8.87 | 9506.00 | 878.75 |
| 142 | 3 | 9 | 24 | 2 | 21.9 | 58.5 | 4.20 | 6.55 | 4.29 | 8383.72 | 1286.25 |
| 143 | 3 | 3 | 29 | 17 | 22.6 | 59.5 | 7.25 | 10.00 | 8.34 | 8932.35 | 867.50 |
| 145 | 1 | 7 | 27 | 13 | 23.1 | 57.2 | 4.85 | 6.20 | 6.79 | 9992.91 | 866.25 |
| 147 | 5 | 9 | 23 | 0 | 17.2 | 52.6 | 3.40 | 4.40 | 3.87 | 8496.91 | 1635.00 |
| 148 | 9 | 9 | 24 | 0 | 18.5 | 53.0 | 3.55 | 5.20 | 3.20 | 7668.18 | 2821.25 |
| 150 | 5 | 9 | 24 | 0 | 17.9 | 53.6 | 3.25 | 4.95 | 3.75 | 10979.17 | 1642.50 |
| 151 | 7 | 9 | 26 | 0 | 22.8 | 59.0 | 4.65 | 5.95 | 3.26 | 7965.22 | 2065.00 |
| 152 | 9 | 9 | 27 | 0 | 21.6 | 58.2 | 3.25 | 5.35 | 2.85 | 7097.50 | 2157.50 |
| 156 | 1 | 1 | 32 | 27 | 24.9 | 62.6 | 8.20 | 11.50 | 15.10 | 13723.96 | 726.25 |
| 157 | 3 | 9 | 26 | 2 | 19.7 | 55.0 | 4.20 | 9.05 | 3.58 | 8970.83 | 1353.75 |
| 158 | 5 | 9 | 23 | 0 | 17.2 | 53.1 | 3.30 | 4.35 | 3.69 | 6656.72 | 1617.50 |
| 159 | 3 | 7 | 23 | 6 | 16.8 | 52.1 | 3.20 | 5.90 | 4.47 | 8454.35 | 1177.50 |
| 161 | 1 | 3 | 30 | 24 | 22.7 | 60.6 | 6.30 | 9.75 | 10.30 | 9987.36 | 852.50 |
| 163 | 1 | 3 | 28 | 20 | 21.9 | 60.2 | 5.30 | 7.50 | 9.86 | 11845.24 | 817.50 |
| 164 | 9 | 9 | 24 | 0 | 18.1 | 54.6 | 3.35 | 4.30 | 3.09 | 8089.55 | 3153.74 |
| 165 | 9 | 9 | 25 | 0 | 19.4 | 55.0 | 3.50 | 4.60 | 2.99 | 7268.18 | 2127.50 |
| 166 | 9 | 9 | 24 | 0 | 18.1 | 54.6 | 3.20 | 4.85 | 3.01 | 8768.94 | 2730.00 |
| 167 | 5 | 9 | 23 | 0 | 18.7 | 54.1 | 3.25 | 4.50 | 4.28 | 8657.35 | 1471.25 |
| 168 | 9 | 9 | 22 | 0 | 16.9 | 52.3 | 3.25 | 4.30 | 2.75 | 7911.11 | 2511.25 |
| 169 | 7 | 9 | 25 | 1 | 19.9 | 55.7 | 3.60 | 6.20 | 3.11 | 10563.29 | 1942.50 |
| 170 | 3 | 7 | 25 | 7 | 19.1 | 55.7 | 3.20 | 5.65 | 4.39 | 11845.59 | 1202.50 |
| 172 | 7 | 9 | 21 | 0 | 16.5 | 53.0 | 3.30 | 4.55 | 3.17 | 12156.78 | 1946.25 |
| 173 | 9 | 9 | 20 | 0 | 17.0 | 53.9 | 3.35 | 4.70 | 2.88 | 8976.42 | 3177.78 |
| 174 | 9 | 9 | 25 | 0 | 17.6 | 52.3 | 3.35 | 4.45 | 2.91 | 8365.74 | 2585.00 |
| 175 | 3 | 9 | 26 | 3 | 18.2 | 54.2 | 3.25 | 4.80 | 3.97 | 8433.96 | 1386.25 |
| 176 | 1 | 7 | 28 | 13 | 22.8 | 59.8 | 7.40 | 8.15 | 7.92 | 9479.76 | 858.75 |
| 177 | 1 | 7 | 29 | 14 | 21.8 | 57.5 | 5.85 | 6.15 | 6.93 | 9487.44 | 868.75 |
| 178 | 3 | 7 | 25 | 8 | 19.9 | 54.4 | 4.35 | 7.20 | 4.84 | 11660.71 | 1111.25 |
| 179 | 1 | 9 | 24 | 3 | 17.9 | 54.0 | 3.20 | 4.60 | 7.44 | 11926.14 | 936.25 |
| 181 | 5 | 9 | 27 | 2 | 22.2 | 56.5 | 6.00 | 7.50 | 4.28 | 7825.44 | 1497.50 |
| 182 | 9 | 9 | 26 | 0 | 19.0 | 55.5 | 3.55 | 5.20 | 2.77 | 6625.00 | 2168.75 |

| $F_2$ plant no | Leaf bronzing ($4^{th}$ week) | Leaf bronzing ($6^{th}$ week) | Total no of roots | No of fresh roots | Root length (cm) | Shoot length (cm) | root weight (g) | shoot weight (g) | Fe adsorbed on root (mg $l^{-1}$) | Iron content in root (mg $kg^{-1}$) | Iron content in leaf (mg $kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 183 | 9 | 9 | 23 | 0 | 16.6 | 51.2 | 3.25 | 4.30 | 3.24 | 6688.30 | 1961.25 |
| 184 | 7 | 9 | 24 | 0 | 16.2 | 55.6 | 3.20 | 4.55 | 3.89 | 11941.82 | 1981.25 |
| 185 | 9 | 9 | 28 | 0 | 18.8 | 54.9 | 3.20 | 4.10 | 3.11 | 7346.59 | 2006.25 |
| 186 | 5 | 9 | 24 | 0 | 19.2 | 54.3 | 4.95 | 7.00 | 4.18 | 7027.78 | 1611.25 |
| 188 | 7 | 9 | 24 | 0 | 19.8 | 55.1 | 3.60 | 5.05 | 3.71 | 7290.11 | 1791.25 |
| 189 | 5 | 9 | 23 | 0 | 19.2 | 53.5 | 4.45 | 5.30 | 3.99 | 8335.94 | 1557.50 |
| 191 | 7 | 9 | 24 | 0 | 18.4 | 53.2 | 4.20 | 4.35 | 3.63 | 7887.99 | 1813.75 |
| 192 | 9 | 9 | 23 | 0 | 17.9 | 53.9 | 3.50 | 4.25 | 3.23 | 6954.55 | 2148.75 |
| 194 | 9 | 9 | 21 | 0 | 15.9 | 49.9 | 3.25 | 4.75 | 2.85 | 6775.59 | 2417.50 |
| 195 | 9 | 9 | 24 | 0 | 17.6 | 53.1 | 4.25 | 4.30 | 3.34 | 7750.00 | 2510.00 |
| 196 | 7 | 9 | 22 | 0 | 17.2 | 52.6 | 3.65 | 4.35 | 3.54 | 7886.36 | 2018.75 |
| 197 | 3 | 9 | 25 | 2 | 19.3 | 54.2 | 4.45 | 5.05 | 4.47 | 8093.75 | 1180.00 |
| 198 | 1 | 7 | 26 | 11 | 19.2 | 54.5 | 4.55 | 5.60 | 7.49 | 9619.13 | 965.00 |
| 199 | 1 | 5 | 26 | 16 | 18.9 | 55.0 | 4.70 | 5.95 | 8.69 | 10791.67 | 862.50 |
| 200 | 3 | 9 | 22 | 1 | 16.8 | 52.8 | 3.55 | 4.30 | 4.47 | 8263.06 | 1436.25 |
| 202 | 9 | 9 | 22 | 0 | 15.8 | 49.5 | 2.90 | 3.80 | 2.86 | 6666.67 | 2646.25 |
| 203 | 9 | 9 | 23 | 0 | 19.4 | 53.4 | 3.15 | 4.50 | 2.83 | 6906.25 | 2046.25 |
| 204 | 7 | 9 | 22 | 0 | 16.4 | 53.0 | 3.30 | 4.45 | 3.16 | 10259.80 | 2076.25 |
| 205 | 9 | 9 | 22 | 0 | 16.5 | 52.0 | 3.20 | 4.35 | 2.80 | 7465.75 | 1922.50 |
| 207 | 7 | 9 | 23 | 0 | 17.7 | 53.5 | 3.60 | 4.50 | 3.25 | 9295.00 | 1901.25 |
| 208 | 7 | 9 | 25 | 0 | 19.5 | 54.2 | 5.00 | 5.25 | 3.25 | 7646.20 | 1800.00 |
| 209 | 3 | 7 | 25 | 8 | 18.8 | 55.9 | 3.40 | 4.75 | 4.48 | 9274.34 | 1096.25 |
| 211 | 5 | 9 | 24 | 0 | 19.4 | 54.5 | 3.25 | 4.60 | 3.80 | 9571.43 | 1408.75 |
| 212 | 7 | 9 | 24 | 0 | 16.4 | 54.8 | 3.45 | 4.55 | 3.19 | 6510.42 | 1800.00 |
| 213 | 9 | 9 | 23 | 0 | 16.4 | 52.1 | 3.05 | 4.30 | 3.03 | 6724.45 | 2548.75 |
| 214 | 5 | 9 | 25 | 1 | 18.4 | 56.1 | 4.35 | 5.30 | 3.94 | 11852.27 | 1510.00 |
| 215 | 9 | 9 | 23 | 0 | 16.2 | 51.3 | 3.25 | 4.25 | 2.98 | 7454.08 | 2108.75 |
| 216 | 9 | 9 | 23 | 0 | 17.0 | 54.5 | 3.30 | 4.40 | 2.96 | 7489.58 | 2097.50 |
| 217 | 9 | 9 | 22 | 0 | 16.5 | 53.2 | 3.20 | 4.30 | 3.15 | 6921.88 | 2012.50 |
| 219 | 1 | 3 | 26 | 18 | 17.8 | 57.5 | 3.35 | 7.85 | 9.22 | 10430.77 | 795.00 |
| 221 | 7 | 9 | 26 | 0 | 19.4 | 58.0 | 5.90 | 8.00 | 3.67 | 7362.86 | 1920.00 |
| 223 | 7 | 9 | 24 | 0 | 18.2 | 55.1 | 4.65 | 5.60 | 3.40 | 7162.00 | 1690.00 |
| 224 | 9 | 9 | 23 | 0 | 17.7 | 54.2 | 3.20 | 4.50 | 2.94 | 6998.03 | 2375.00 |
| 225 | 5 | 9 | 25 | 0 | 19.2 | 56.5 | 5.15 | 8.65 | 3.80 | 7752.33 | 1583.75 |
| 226 | 7 | 9 | 23 | 0 | 16.9 | 53.8 | 4.30 | 4.90 | 3.35 | 8254.17 | 1696.25 |

| $F_2$ plant no | Leaf bronzing ($4^{th}$ week) | Leaf bronzing ($6^{th}$ week) | Total no of roots | No of fresh roots | Root length (cm) | Shoot length (cm) | root weight (g) | shoot weight (g) | Fe adsorbed on root (mg $l^{-1}$) | Iron content in root (mg $kg^{-1}$) | Iron content in leaf (mg $kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 227 | 1 | 9 | 26 | 1 | 17.5 | 55.2 | 4.70 | 6.00 | 6.21 | 8951.30 | 1008.75 |
| 230 | 1 | 7 | 24 | 9 | 17.1 | 53.9 | 4.15 | 4.60 | 8.54 | 9532.00 | 928.75 |
| 231 | 9 | 9 | 23 | 0 | 15.9 | 51.3 | 3.45 | 4.85 | 2.95 | 6443.07 | 2585.00 |
| 232 | 7 | 9 | 23 | 0 | 18.1 | 53.0 | 3.95 | 4.35 | 3.31 | 7036.26 | 1943.75 |
| 235 | 5 | 9 | 23 | 0 | 18.3 | 54.2 | 3.25 | 4.50 | 3.75 | 6647.35 | 1547.50 |
| 236 | 5 | 9 | 24 | 0 | 18.2 | 53.8 | 4.30 | 4.45 | 4.01 | 9492.27 | 1391.25 |
| 237 | 3 | 9 | 26 | 3 | 17.8 | 55.0 | 3.40 | 6.00 | 4.40 | 10850.39 | 1330.00 |
| 238 | 3 | 9 | 25 | 2 | 16.7 | 56.2 | 3.35 | 6.15 | 3.78 | 8489.58 | 1235.00 |
| 239 | 5 | 9 | 22 | 0 | 16.9 | 54.5 | 3.50 | 4.90 | 3.78 | 10559.90 | 1623.75 |
| 240 | 3 | 9 | 24 | 1 | 17.9 | 55.8 | 3.45 | 5.70 | 4.52 | 10788.64 | 1256.25 |
| 241 | 7 | 9 | 26 | 1 | 19.5 | 56.2 | 3.95 | 4.60 | 3.38 | 6820.18 | 1770.00 |
| 242 | 9 | 9 | 22 | 0 | 16.3 | 51.8 | 3.15 | 4.20 | 2.82 | 7573.01 | 2313.75 |
| 243 | 3 | 9 | 27 | 2 | 21.6 | 56.7 | 4.85 | 6.15 | 4.42 | 8178.99 | 1220.00 |
| 244 | 1 | 5 | 28 | 17 | 21.7 | 59.5 | 5.05 | 7.65 | 8.49 | 9880.53 | 853.75 |
| 245 | 3 | 9 | 26 | 0 | 18.2 | 58.8 | 3.20 | 5.35 | 4.08 | 7907.08 | 1457.50 |
| 246 | 1 | 1 | 32 | 30 | 25.3 | 61.6 | 7.40 | 11.20 | 12.20 | 11965.36 | 716.25 |
| 247 | 3 | 5 | 28 | 15 | 21.6 | 57.7 | 5.00 | 8.65 | 6.75 | 8446.28 | 940.00 |
| 248 | 1 | 1 | 33 | 32 | 25.9 | 62.8 | 7.55 | 11.60 | 12.80 | 13932.69 | 688.75 |
| 249 | 9 | 9 | 24 | 0 | 19.8 | 55.1 | 2.85 | 3.90 | 3.03 | 7398.76 | 2333.75 |
| 250 | 1 | 3 | 30 | 20 | 22.8 | 59.3 | 5.45 | 9.55 | 10.70 | 10028.37 | 788.75 |
| 251 | 7 | 9 | 24 | 0 | 18.8 | 53.6 | 3.20 | 4.35 | 3.34 | 12944.21 | 2101.25 |
| 252 | 1 | 3 | 30 | 21 | 22.2 | 60.9 | 5.80 | 10.50 | 9.03 | 10642.44 | 812.50 |
| 253 | 1 | 3 | 31 | 24 | 22.3 | 60.2 | 5.35 | 9.25 | 9.59 | 10961.36 | 807.50 |
| 256 | 9 | 9 | 26 | 0 | 21.2 | 55.2 | 5.20 | 6.35 | 3.07 | 6980.00 | 2436.25 |
| 257 | 1 | 3 | 29 | 20 | 21.1 | 58.1 | 4.10 | 6.95 | 9.19 | 10987.90 | 812.50 |
| 258 | 3 | 3 | 27 | 16 | 20.9 | 58.6 | 6.95 | 10.40 | 8.36 | 8818.86 | 875.00 |
| 259 | 1 | 3 | 30 | 21 | 22.0 | 60.7 | 4.70 | 10.30 | 10.20 | 10259.38 | 767.50 |
| 260 | 5 | 9 | 23 | 0 | 19.9 | 55.0 | 3.60 | 4.30 | 4.59 | 11426.34 | 1592.50 |
| 261 | 5 | 9 | 24 | 0 | 18.8 | 53.1 | 3.30 | 4.45 | 4.08 | 7427.93 | 1627.50 |
| 264 | 9 | 9 | 25 | 0 | 19.3 | 54.2 | 3.55 | 4.30 | 3.16 | 6841.73 | 2283.75 |
| 265 | 7 | 9 | 24 | 0 | 20.0 | 53.2 | 3.25 | 4.40 | 3.71 | 7795.45 | 1993.75 |
| 266 | 7 | 9 | 26 | 1 | 19.2 | 54.1 | 3.60 | 4.65 | 3.62 | 7047.50 | 1821.25 |
| 267 | 7 | 9 | 24 | 0 | 19.0 | 54.2 | 3.30 | 5.35 | 3.63 | 6637.32 | 1677.50 |
| 268 | 1 | 1 | 32 | 27 | 25.1 | 61.8 | 7.45 | 11.30 | 12.60 | 12189.29 | 678.75 |
| 269 | 9 | 9 | 26 | 0 | 19.4 | 55.3 | 3.25 | 4.35 | 3.67 | 6323.25 | 2283.75 |

| $F_2$ plant no | Leaf bronzing ($4^{th}$ week) | Leaf bronzing ($6^{th}$ week) | Total no of roots | No of fresh roots | Root length (cm) | Shoot length (cm) | root weight (g) | shoot weight (g) | Fe adsorbed on root (mg $l^{-1}$) | Iron content in root (mg $kg^{-1}$) | Iron content in leaf (mg $kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 270 | 9 | 9 | 23 | 0 | 16.3 | 52.3 | 3.10 | 4.35 | 3.21 | 6335.99 | 1890.00 |
| 272 | 5 | 9 | 23 | 0 | 17.7 | 52.8 | 3.20 | 5.20 | 3.72 | 8231.06 | 1385.00 |
| 273 | 7 | 9 | 26 | 1 | 21.2 | 56.0 | 4.10 | 5.05 | 3.69 | 7427.50 | 1663.75 |
| 274 | 9 | 9 | 24 | 0 | 19.1 | 55.3 | 3.25 | 4.80 | 3.18 | 6766.39 | 1933.75 |
| 275 | 9 | 9 | 26 | 0 | 21.1 | 56.3 | 5.10 | 5.35 | 3.27 | 6691.06 | 2521.25 |
| 276 | 1 | 3 | 30 | 21 | 22.2 | 60.8 | 4.20 | 9.50 | 10.90 | 10913.46 | 728.75 |
| 277 | 1 | 3 | 31 | 24 | 23.0 | 60.1 | 5.50 | 9.05 | 11.10 | 10764.81 | 738.75 |
| 278 | 3 | 7 | 28 | 11 | 20.2 | 56.8 | 4.20 | 5.85 | 6.89 | 8441.56 | 902.50 |
| 280 | 3 | 7 | 27 | 10 | 21.0 | 57.2 | 4.35 | 8.05 | 6.93 | 7768.57 | 912.50 |
| 281 | 9 | 9 | 26 | 0 | 22.2 | 56.5 | 4.10 | 5.10 | 3.58 | 6845.07 | 1873.75 |
| 282 | 9 | 9 | 24 | 0 | 19.3 | 55.0 | 3.70 | 4.50 | 3.48 | 6412.83 | 2110.00 |
| 283 | 5 | 9 | 25 | 1 | 19.1 | 55.5 | 4.15 | 5.80 | 4.29 | 9897.06 | 1428.75 |
| 284 | 3 | 7 | 27 | 9 | 18.8 | 56.8 | 5.20 | 6.35 | 6.19 | 9779.41 | 930.00 |
| 285 | 3 | 7 | 26 | 8 | 20.2 | 58.1 | 5.20 | 6.25 | 7.14 | 8435.64 | 1158.75 |
| 287 | 1 | 1 | 32 | 28 | 23.6 | 62.1 | 7.25 | 12.30 | 13.50 | 13360.82 | 748.75 |
| 288 | 1 | 7 | 26 | 11 | 19.6 | 57.3 | 3.65 | 4.60 | 7.14 | 9524.73 | 873.75 |
| 289 | 3 | 7 | 27 | 9 | 21.1 | 56.2 | 3.95 | 5.80 | 6.21 | 9508.13 | 1030.00 |
| 291 | 9 | 9 | 25 | 0 | 20.6 | 55.8 | 3.50 | 4.30 | 2.96 | 7354.35 | 2952.50 |
| 293 | 5 | 9 | 26 | 1 | 19.1 | 54.6 | 4.05 | 5.05 | 3.91 | 8580.88 | 1473.75 |
| 294 | 1 | 7 | 25 | 11 | 21.4 | 53.8 | 4.45 | 5.05 | 8.32 | 9304.51 | 886.25 |
| 297 | 9 | 9 | 26 | 0 | 18.9 | 54.1 | 4.80 | 5.30 | 3.25 | 6901.32 | 2012.50 |
| 298 | 7 | 9 | 27 | 1 | 19.3 | 54.8 | 5.20 | 5.85 | 3.32 | 8407.83 | 1842.50 |
| 299 | 3 | 7 | 28 | 10 | 20.6 | 56.4 | 4.05 | 6.20 | 5.64 | 8977.68 | 996.25 |
| 300 | 1 | 1 | 31 | 26 | 23.5 | 61.6 | 7.20 | 11.40 | 12.60 | 13762.20 | 691.25 |
| 301 | 9 | 9 | 26 | 0 | 20.1 | 56.3 | 5.10 | 7.20 | 3.26 | 6386.65 | 2023.75 |
| 302 | 7 | 9 | 23 | 0 | 18.2 | 52.6 | 3.25 | 4.30 | 3.51 | 7746.75 | 1842.50 |
| 303 | 9 | 9 | 23 | 0 | 16.2 | 52.4 | 3.30 | 4.40 | 2.74 | 7041.26 | 2653.75 |
| 304 | 3 | 9 | 25 | 0 | 19.4 | 54.6 | 3.20 | 4.45 | 4.21 | 7935.19 | 1355.00 |
| 305 | 3 | 9 | 23 | 0 | 17.9 | 52.3 | 3.20 | 4.30 | 3.86 | 8704.79 | 1171.25 |
| 307 | 3 | 5 | 27 | 14 | 19.9 | 56.8 | 3.65 | 4.70 | 6.20 | 9350.81 | 908.75 |
| 308 | 1 | 1 | 32 | 26 | 23.6 | 61.6 | 7.30 | 13.00 | 12.40 | 11719.95 | 760.00 |
| 309 | 1 | 1 | 34 | 29 | 24.3 | 62.8 | 7.80 | 13.50 | 12.10 | 12979.04 | 658.75 |
| 311 | 7 | 9 | 24 | 0 | 18.1 | 52.6 | 3.60 | 4.65 | 3.76 | 6700.73 | 1751.25 |
| 312 | 9 | 9 | 26 | 0 | 19.2 | 53.5 | 3.35 | 4.30 | 3.41 | 6902.88 | 2061.25 |
| 313 | 1 | 3 | 29 | 21 | 22.6 | 59.8 | 4.45 | 10.10 | 9.67 | 10136.94 | 765.00 |

| $F_2$ plant no | Leaf bronzing ($4^{th}$ week) | Leaf bronzing ($6^{th}$ week) | Total no of roots | No of fresh roots | Root length (cm) | Shoot length (cm) | root weight (g) | shoot weight (g) | Fe adsorbed on root (mg $l^{-1}$) | Iron content in root (mg $kg^{-1}$) | Iron content in leaf (mg $kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 314 | 7 | 9 | 24 | 0 | 21.2 | 55.6 | 3.35 | 4.65 | 3.58 | 8363.37 | 1916.25 |
| 315 | 7 | 9 | 26 | 1 | 19.9 | 56.0 | 5.25 | 5.35 | 3.74 | 7167.13 | 1860.00 |
| 316 | 1 | 7 | 29 | 13 | 22.2 | 56.7 | 6.05 | 9.55 | 6.99 | 9344.53 | 850.00 |
| 317 | 3 | 3 | 28 | 17 | 21.9 | 56.5 | 5.75 | 8.30 | 8.53 | 8345.12 | 953.75 |
| 318 | 3 | 5 | 27 | 13 | 22.6 | 55.3 | 4.45 | 8.50 | 7.55 | 9259.06 | 886.25 |
| 319 | 1 | 1 | 31 | 26 | 23.1 | 61.4 | 7.10 | 11.90 | 13.00 | 12468.93 | 665.00 |
| 320 | 1 | 1 | 33 | 28 | 23.6 | 62.3 | 7.35 | 12.60 | 14.90 | 13685.57 | 656.25 |
| 321 | 7 | 9 | 26 | 0 | 21.6 | 58.5 | 6.50 | 8.20 | 3.73 | 8158.91 | 1628.75 |
| 323 | 7 | 9 | 25 | 0 | 19.4 | 53.1 | 4.85 | 5.00 | 3.66 | 6808.82 | 1787.50 |
| 324 | 5 | 9 | 27 | 1 | 20.2 | 53.8 | 3.65 | 5.10 | 4.13 | 7116.88 | 1457.50 |
| 325 | 3 | 9 | 24 | 2 | 17.2 | 52.5 | 3.50 | 4.35 | 4.50 | 10837.91 | 1213.75 |
| 327 | 3 | 3 | 29 | 17 | 21.6 | 57.3 | 6.05 | 7.65 | 9.26 | 10039.31 | 840.00 |
| 328 | 1 | 7 | 28 | 13 | 20.9 | 56.8 | 6.20 | 7.90 | 8.39 | 9391.18 | 907.50 |
| 329 | 3 | 5 | 28 | 15 | 23.3 | 58.2 | 7.05 | 10.30 | 6.43 | 7904.64 | 937.50 |
| 332 | 7 | 9 | 24 | 0 | 19.4 | 53.5 | 3.90 | 4.75 | 3.16 | 6791.38 | 1662.50 |
| 333 | 7 | 9 | 25 | 1 | 18.4 | 52.9 | 4.25 | 5.40 | 3.23 | 7190.56 | 1641.25 |
| 334 | 9 | 9 | 23 | 0 | 15.9 | 51.1 | 3.05 | 4.15 | 3.17 | 6546.51 | 2541.25 |
| 335 | 7 | 9 | 25 | 0 | 17.8 | 54.5 | 4.80 | 5.10 | 3.35 | 6865.77 | 1713.75 |
| 336 | 7 | 9 | 24 | 0 | 20.2 | 55.9 | 4.35 | 5.65 | 3.45 | 6436.96 | 2027.50 |
| 337 | 3 | 9 | 24 | 2 | 18.2 | 52.1 | 3.40 | 4.30 | 4.58 | 8500.00 | 1240.00 |
| 338 | 3 | 7 | 26 | 8 | 19.1 | 56.0 | 3.20 | 4.90 | 4.58 | 7533.02 | 975.00 |
| 339 | 1 | 5 | 29 | 17 | 23.0 | 59.0 | 7.10 | 10.80 | 8.82 | 10375.00 | 835.00 |
| 340 | 9 | 9 | 24 | 0 | 16.3 | 52.1 | 3.30 | 4.45 | 3.11 | 7055.28 | 1991.25 |
| 341 | 7 | 9 | 23 | 0 | 20.1 | 54.6 | 3.95 | 5.30 | 3.60 | 8360.50 | 1795.00 |
| 343 | 5 | 7 | 24 | 7 | 18.3 | 54.6 | 3.45 | 4.50 | 4.09 | 7956.77 | 1518.75 |
| 344 | 7 | 9 | 22 | 0 | 16.8 | 53.3 | 3.45 | 4.30 | 3.22 | 10855.26 | 1893.75 |
| 346 | 7 | 9 | 23 | 0 | 18.7 | 54.7 | 3.55 | 4.35 | 3.44 | 6550.88 | 1727.50 |
| 347 | 5 | 9 | 24 | 0 | 19.1 | 54.6 | 4.65 | 6.30 | 3.81 | 7663.31 | 1666.25 |
| 349 | 3 | 7 | 25 | 9 | 20.3 | 56.5 | 3.90 | 4.65 | 5.95 | 11183.47 | 1021.25 |
| 350 | 3 | 7 | 26 | 8 | 20.8 | 56.9 | 4.05 | 4.65 | 7.01 | 8863.64 | 1148.75 |
| 351 | 5 | 9 | 24 | 0 | 19.4 | 54.6 | 3.65 | 4.50 | 3.91 | 6790.91 | 1561.25 |
| 352 | 3 | 3 | 27 | 15 | 21.9 | 58.2 | 4.50 | 8.05 | 8.22 | 11569.67 | 855.00 |
| 353 | 3 | 9 | 26 | 0 | 19.1 | 55.1 | 3.70 | 4.50 | 4.44 | 8533.85 | 1187.50 |
| 354 | 1 | 1 | 31 | 26 | 23.3 | 60.9 | 7.15 | 10.90 | 12.90 | 11687.85 | 706.25 |
| 355 | 3 | 5 | 28 | 15 | 20.9 | 58.8 | 4.85 | 7.30 | 6.80 | 10825.20 | 968.75 |

| $F_2$ plant no/parent | Leaf bronzing ($4^{th}$ week) | Leaf bronzing ($6^{th}$ week) | Total no of roots | No of fresh roots | Root length (cm) | Shoot length (cm) | root weight (g) | shoot weight (g) | Fe adsorbed on root (mg $l^{-1}$) | Iron content in root (mg $kg^{-1}$) | Iron content in leaf (mg $kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 356 | 5 | 9 | 26 | 0 | 18.8 | 54.9 | 4.30 | 5.20 | 3.98 | 11810.48 | 1601.25 |
| 357 | 5 | 9 | 25 | 1 | 19.7 | 57.8 | 3.85 | 4.70 | 4.11 | 6875.78 | 1488.75 |
| 359 | 5 | 9 | 24 | 0 | 20.9 | 55.6 | 3.25 | 4.30 | 4.41 | 7786.67 | 1451.25 |
| 360 | 3 | 9 | 27 | 3 | 17.1 | 53.5 | 5.80 | 7.25 | 4.47 | 8615.08 | 1267.50 |
| 361 | 3 | 5 | 29 | 15 | 20.8 | 56.5 | 6.80 | 7.45 | 8.37 | 8109.85 | 887.50 |
| 362 | 3 | 9 | 24 | 1 | 18.1 | 54.0 | 3.50 | 4.55 | 4.38 | 10988.89 | 1496.25 |
| 363 | 1 | 3 | 28 | 20 | 21.3 | 57.5 | 4.80 | 8.75 | 11.40 | 10589.02 | 791.25 |
| 364 | 7 | 9 | 25 | 0 | 19.2 | 56.3 | 3.70 | 4.65 | 3.76 | 12343.25 | 1882.50 |
| 365 | 3 | 5 | 26 | 13 | 20.1 | 54.5 | 3.85 | 5.60 | 7.93 | 9129.92 | 882.50 |
| 366 | 9 | 9 | 27 | 0 | 20.8 | 55.9 | 6.50 | 6.75 | 3.07 | 6972.92 | 2621.25 |
| 367 | 7 | 9 | 24 | 0 | 18.9 | 53.5 | 3.75 | 4.35 | 3.31 | 10387.76 | 1932.50 |
| 368 | 1 | 9 | 28 | 2 | 22.3 | 59.7 | 4.80 | 6.35 | 6.88 | 9842.98 | 937.50 |
| 371 | 5 | 9 | 23 | 0 | 17.0 | 55.2 | 3.30 | 4.65 | 3.86 | 10470.00 | 1507.50 |
| 372 | 7 | 9 | 25 | 0 | 19.2 | 53.2 | 3.60 | 4.50 | 3.65 | 9701.27 | 1867.50 |
| 373 | 7 | 9 | 26 | 0 | 18.4 | 56.3 | 4.75 | 6.30 | 3.27 | 7565.89 | 1875.00 |
| 374 | 3 | 7 | 27 | 8 | 19.2 | 54.6 | 3.45 | 4.85 | 4.89 | 11543.03 | 1072.50 |
| 375 | 7 | 7 | 25 | 7 | 16.8 | 53.9 | 3.50 | 4.60 | 3.56 | 8743.30 | 1610.00 |
| 376 | 3 | 9 | 28 | 2 | 22.4 | 58.9 | 5.25 | 8.20 | 4.58 | 8586.96 | 1310.00 |
| 377 | 3 | 3 | 28 | 16 | 22.6 | 59.4 | 6.20 | 8.75 | 8.81 | 9342.98 | 896.25 |
| 378 | 3 | 3 | 29 | 17 | 21.3 | 57.9 | 7.50 | 9.90 | 9.35 | 9175.48 | 841.25 |
| *PGC14 | 1 | 1 | 31 | 27 | 23.4 | 61.2 | 7.80 | 11.30 | 12.20 | 11918.52 | 731.25 |
| **PGC31 | 9 | 9 | 22 | 0 | 16.3 | 49.7 | 2.95 | 3.95 | 3.36 | 6889.42 | 2258.75 |

* PGC 14 (Tulasi) – Resistant parent **PGC 31 (Cul-8709) – Susceptible parent

**Appendix III. Phenotypic data of individual $F_2$ plants resistant to iron toxicity at 800ppm of iron**

| $F_2$ plant no / Parent | Leaf bronzing score ($4^{th}$ week) | Leaf bronzing score ($6^{th}$ 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)

## Appendix IV. Phenotypic data of individual $F_2$ plants susceptible to iron toxicity at 800ppm of iron

| $F_2$ plant no | Leaf bronzing score ($4^{th}$ week) | Leaf bronzing score ($6^{th}$ 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 |

| $F_2$ plant no | Leaf bronzing score ($4^{th}$ week) | Leaf bronzing score ($6^{th}$ 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 $kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 |
| 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 |

| $F_2$ plant no / parent | Leaf bronzing score ($4^{th}$ week) | Leaf bronzing score ($6^{th}$ 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 $kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 |
| 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)

# IDENTIFICATION OF MOLECULAR MARKERS LINKED TO IRON TOXICITY TOLERANCE THROUGH BULK SEGREGANT ANALYSIS (BSA) IN RICE (*Oryza sativa* L.)

By

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## ABSTRACT OF THE THESIS

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## ABSTRACT

Globally rice is the most important food crop, serving as staple food for more than half of the world's population. As in other parts of the country, rice is the major food crop grown in Kerala too. The total annual production of rice is however insufficient to meet the total demand in the state. Iron toxicity prevalent in the rice growing tracts of the state, further compounds the problem of low rice production. Although, several attempts to ameliorate the iron toxic soil conditions are being made, the best way to combat this stress and increase rice production in the affected soils is to develop varieties tolerant to iron toxicity.

The present investigation on 'Identification of molecular markers linked to iron toxicity tolerance through bulk segregant analysis (BSA) in rice (*Oryza sativa* L.)' was conducted at College of Horticulture, Kerala Agricultural University (KAU), Vellanikkara, Thrissur during 2013 to 2015 year. The study involved screening of thirty rice genotypes for response to iron at toxic levels, hybridization between the most tolerant and susceptible genotype, production of $F_2$ generation of this cross, parental polymorphism study using molecular markers and Bulk Segregant Analysis (BSA). The thirty rice genotypes were selected on the basis of their response to iron stress under KSCSTE project: 'Donor identification for tolerance to iron toxicity in rice (*Oryza sativa* L.)'.

Further screening of the thirty genotypes (Confirmation test 1 and 2) as per the method advocated by Shimizu *et al.* (2005) to confirm their tolerance or susceptibility to iron toxicity revealed existence of high significant differences among the genotypes with respect to leaf bronzing and biomass produced under varying concentrations of iron (0 ppm, 600ppm and 800ppm of Fe). Considering that at higher concentrations of Fe, a lower leaf bronzing and reduction in biomass, is a valid criterion for identifying genotypes tolerant to Fe stress, twelve genotypes *viz.*, Cul-8709, Cul-210-29, AM-10-7, Cul-90-03, PM-709, ASD-16, ASD-18, Abhaya, IR-1552, T(N)-1, IR-36 and Cul-3

were found to be highly susceptible to iron stress while genotypes Cul-8723, Tulasi, Cul-18716, Kargi and IVT-33 were identified as the most tolerant ones.

Selfing of $F_1$s obtained on hybridizing the genotype (Tulasi) and genotype (CUL-8709) which were found respectively to be most tolerant and most susceptible to iron stress was done, to produce $F_2$ population for the conduct of bulk sergegant analysis (BSA).

Phenotyping of $F_2$ plants under iron at toxic levels indicated presence of wide variability for shoot length, root length, total number of roots, number of fresh roots, shoot weight, root weight and visual scoring for iron-toxicity symptoms. The measures of skewness and kurtosis for various traits revealed a large quantitative variability. All the above traits except iron content in root of $F_2$ lines exhibited a positive platykurtic distribution pointing to presence of gene interaction in trait expression. Measures of skewness and kurtosis also indicated occurrence of transgressive segregation in the $F_2$ population. Leaf bronzing the typical symptom of Fe toxicity, showed a strong negative correlation with shoot length, root length, total number of roots, number of fresh roots, shoot weight and root weight. The results indicated that leaf bronzing is associated with growth reduction due to $Fe^{2+}$ toxicity in this $F_2$ population.

Parental polymorphism (Tulasi and CUL-8709) survey using 338 Rice Microsatellites (RM) markers revealed 37 RM markers polymorphic between the two. These 37 polymorphic rice microsatellites markers (SSR markers) were found to be distributed over all 12 linkage groups of rice varying between one in case on Chromosome 7 to five each on Chromosome 2, 9 and 10.

Bulk segregant analysis indicated that out of the 37 microsatellite markers that were polymorphic between parents seven viz., RM 263, RM 107, RM 12292, RM 24616, RM 24664, RM 13619 showed clear co-segregation with the susceptible parent and susceptible bulk, and resistant parent and resistant bulk. Probability of all seven putative markers was highly significant ($P < 0.001$) indicating strong association of these markers to the genomic region governing Leaf Bronzing Index which is a valid indicator of tolerance to iron toxicity. Through single marker analysis, three probable quantitative trait loci (QTL's) of Leaf Bronzing Index were identified, each on

chromosome 1, 2 and 9. The QTL on chromosome 1 was located between 42.8 Mb and 43.2 Mb and associated with markers RM 12255 and RM 12292. The QTL for LBI was found to be associated with RM 13619 and RM 263 markers and placed between 24.9 Mb and 25.9 Mb on chromosome 2 while on chromosome 9, it was a located between 19.3 Mb and 20.1 Mb and linked to marker RM 107, RM 24616 and RM 24664.