Assessment of useful genetic variation for nutrient use efficiency in *aus* rice (*Oryza sativa* L.) using genome wide association studies

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By

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It is further certified that the assistance and help availed by him from various sources during the course of investigation has been duly acknowledged.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

	population.	
4.9	Significant association for root length on chromosome 4 at 26861688bp a) Local Manhattan plot (top) and LD heat map (bottom) of QTL on Chromosome 4 (243 kbp upstream and downstream of the peak SNP) and blue dash line represents the candidate gene *OsSAUR19* (LOC_Os04g45370); b) Boxplot and significance test representation of haplotypes for flag leaf width (c) Nonsynonymous SNPs in the candidate gene *OsSAUR19* significantly associated with root length, and amino acid variations and haplotype mean	80
4.10	Significant association for shoot length on chromosome 6 at 25259001bp a) Local Manhattan plot (top) and LD heat map (bottom) of QTL on chromosome 6 (243 kbp upstream and downstream of the peak SNP) and blue dash line represents the candidate gene *CCR7* (LOC_Os06g41840); b) Boxplot and significance test representation of haplotypes for shoot length (c) Nonsynonymous SNPs in the candidate gene *CCR7* significantly associated with shoot length, and amino acid variations and haplotype mean	80
4.11	Significant association for soot dry weight on chromosome 3 at 14212541bp a) Local Manhattan plot (top) and LD heat map (bottom) of QTL on chromosome 3 (243 kbp upstream and downstream of the peak SNP) and blue dash line represents the candidate gene *SCL28* (LOC_Os03g25040); b) Boxplot and significance test representation of haplotypes for shoot length (c) Nonsynonymous SNPs in the candidate gene *SCL28* significantly associated with shoot dry weight, and amino acid variations and haplotype mean	81
4.12	Manhattan plots from GWA mapping of total root volume, average root diameter, shoot length and number of leaves under iron deficient condition of BAAP population.	89
4.13	Significant association for root volume, average root diameter on chromosome 4 at 32141074bp a) Local Manhattan plot (top) and LD heat map (bottom) of QTL on chromosome 3 (243 kbp upstream and downstream of the peak SNP) and blue dash line represents the candidate gene *OsBIDK1* (LOC_Os04g54200); b) Boxplot and significance test representation of haplotypes for shoot length (c) Nonsynonymous SNPs in the candidate gene *OsBIDK1* significantly associated with root volume, average root diameter and amino acid variations and haplotype mean.	91
4.14	Significant association for shoot length, number of leaves on chromosome 11 at 16362848bp a) Local Manhattan plot (top) and LD heat map (bottom) of QTL on chromosome 11 (243 kbp upstream and downstream of the peak SNP) and blue dash line represents the candidate gene *OsFLA* (LOC_Os11g28360); b) Boxplot and significance test representation of haplotypes for shoot length (c) Nonsynonymous SNPs in the candidate gene *OsFLA* significantly associated with shoot length, number of leaves and amino acid variations and haplotype mean	91
5.1	QTLs associated with various traits under nutrient deficient conditions detected across 12 chromosomes in the GWAS studies; qNPL (panicle length) , qNFLW (flag leaf width) , qNGY (grain yield per plant) under	105

	N deficient trial, qPRL (root length), qPSL (shoot length) and qPSDW (shoot dry weight) under P deficeint trial, qFeNL (number of leaves), qFeSL (shoot length), qFeARD (average root diameter) and qFeRV (root volume) under Fe deficient trial.	

ABBREVIATIONS USED

%	:	Percentage
m	:	Meter
cm	:	Centimeter
g	:	Gram
mg	:	Milligram
ha	:	Hectare
et al.	:	co-workers
spp.	:	Species
eg.	:	Example
etc	:	Etcetera
i.e.	:	That is
viz.	:	Namely
S.E.(m)	:	Standard error mean
CD	:	Critical Difference
N	:	Nitrogen
P	:	Phosphorus
Fe	:	Iron
BAAP	:	Bengal Assam Aus Panel
SNP	:	Single Nucleotide Polymorphism
GWAS	:	Genome Wide Association Studies
FDR	:	False Discovery Rate

ABSTRACT

Rice (*Oryza sativa* L.), the second most cultivated crop nurtures billions of lives including humans and animals. However, the modern high yielding rice genotypes have a low nutrient use efficiency i.e., <50% (of the total applied fertilizers). The breeding activities should focus on developing and/or improving genotypes that can survive under limited inputs utilizing the available resources to the optimum. The Bengal Assam Aus Panel consisting of 298 diverse lines of *aus* rice was used in this study to identify genetic loci involved in nutrient use efficiency with respect to nitrogen, phosphorus and iron. The nitrogen deficient study was conducted in the field where as phosphorus and iron study was conducted in hydroponics. The BAAP with its nearly 2 million SNP sequence information was processed for association studies with the phenotypic data collected in this study. The results highlighted the different traits contributing the maximum variability under nutrient deficient conditions. In case of nitrogen (N) deficient trial there was positive correlation between grain yield, panicle length and leaf width. Similarly, in case of phosphorus (P) and iron (Fe) deficient trial there was a positive correlation between root biomass and shoot growth. The putative candidate gene for enhancing nitrogen use efficiency *via* flag leaf width was; LOC_Os6g41750 (25024626 – 25034551 bp), panicle length; LOC_Os04g09900 (5318060 – 5326427bp), and grain yield per plant; LOC_Os11g39220 (23354149 – 23358318bp). Three accessions; DA24, DM56, and AUS210 were identified as superior donors from the better performing haplotype groups of the above mentioned traits. Phosphorus use efficiency was found to be associated with the putative candidate gene LOC_Os04g45370 (26831005 - 26831867bp) *via* root length, LOC_Os06g41840 (25016001 - 2502001bp) *via* shoot length, and LOC_Os03g25040 (14294507bp - 14297898bp) *via* shoot dry weight. Shada boro, Kele (AUS) and Kada 68-1 were the selected as superior donors for the selected traits. Iron use efficiency was associated with a common peak SNP for root volume and average root diameter, LOC_Os04g54200 (32286661bp to 32291459bp) as a putative candidate gene. The peak SNP for shoot length and number of leaves shared a peak and the putative candidate gene was identified as LOC_Os11g28360 (16316873 – 16321329bp). Only five accessions were common in the superior haplotype across the four traits. Among these five lines, Rata boro had the highest root volume (3.316cm3) and average root diameter (2.19mm). The shoot length was highest in Sada boro G1 (26.05cm) and the average number of leaves were 2.8. The variations explored at the SNP level and amino acids in this study can serve as a guide for further genomic studies. The nonsynomyous substitutions can be validated further through protein expression studies. The SNP haplotypes identified in this study can further be used in haplotype assisted breeding approach while the superior haplotype bearing lines can be used as donor for the respective traits.

INTRODUCTION

Rice (*Oryza sativa* L.), the second most cultivated crop in the world, nurtures billions of lives including humans and animals, and is of prime importance in developing nations, especially in Asia, where it is the sole source of energy (carbohydrate) for a majority of the population. The nutritional content of rice grain is 80% carbohydrates, 7–8% protein, 3% fat, and 3% fibre (Chaudhari *et al*., 2018). India's total rice production was 127.93 million metric tons in the year 2021-2022 (*www.indiastat.com*). As an energy-giving food, the consumption of rice is as high as 103.5 million metric tons in India and 509.87 million metric tons (estimated) worldwide in the crop year 2020-21 (www.fao.org/worldfoodsituation). This data illustrates the high demand for the crop and the ongoing intensive production system. In the coming decades, rice production needs to be carried out sustainably to keep the balance between profitability margins and essential input costs.

The burgeoning population with an increased demand for food grains has compelled farmers to move towards an intensive system of agriculture. This kind of cultivation has introduced practices that heavily exploits soil and water resources with inorganic chemical inputs. This approach gave beneficial results with notable increase in the grain yield of rice in the 1960s but eventually the growth rate declined and was almost stagnant by the mid-1980s (Dobermann *et al*., 2004). This decline can be attributed to multiple reasons. The most significant being the incessant application of fertiliser and the poor nutrient use efficiency of the newly developed varieties. This was the case despite the development of varieties with higher yield potential. The motivated farmers retorted to high blanket application of fertilisers (especially nitrogen) in order to suffice the shortcomings of yield leading to deterioration of soil quality (physical and chemical), declining water table, and also damaging the soil fauna (Srivastava *et al*., 2020). The modern high yielding varieties are bred and adapted to high doses of fertilisers with minimum or no efforts to increase the nutrient use efficiency (NUE), i.e., to make the crop utilise a maximum portion of the externally supplemented fertilisers.

Nutrient utilisation by plants is a complex polygenic trait influenced by a variety of activities such as sensing, absorption, transport and assimilation (Fan *et al*., 2017). Additionally, there are unique features of different nutrients, their region and forms of availability that fluctuate temporally. This can be illustrated by a nutrient such as

phosphorus that is available in the shallow layers of the soil and is limited to the early vegetative stage of the rice plant, later on gets fixed in the soil and is unavailable to the plants while in case of nitrogen, the plant can utilise it throughout its life cycle with the help of longer roots from the deeper soil layers. NUE refers to two different aspects of the plant; nutrient uptake efficiency and nutrient utilisation efficiency. The nutrient uptake efficiency involves the root morphology, transporters and genes involved with the respective nutrients while the utilisation efficiency is a function of the plants' metabolism and the physiological responses leading to it.

In India, fertilizer consumption shows a continuous growing trend, with an yearly increase of 6% since 1970 (Sutton *et al*., 2017). Even after improving agronomic practices the NUE in rice for most of the nutrients remains below fifty percent. For instance, of the total nitrogenous fertiliser applied, the nitrogen recovery efficiency remains less than 30% in the rainfed areas while it ranges between 20-30% under irrigated field conditions (Roberts, 2008). If the trends of the last few decades are probed then it is clear that the use of phosphate fertilisers have tremendously increased, on the contrary the use efficiency has gone down to below 20% (Li *et al*., 2009). The reason behind this can be any of the following occurrences; drainage, volatilisation, leaching loss etc. or fixation in the soil making them unavailable to the plants. At this juncture we have the option either to mend our cultivation practices or to improve the genetic potential of the plant to utilize resources more efficiently. We may either improve our ongoing practices or improve the genetic potential of the plant to make better use of resources. Changes in farming operations, such as tillage, weed management, fertiliser application style, irrigation, and so on, are among the former alternatives. However, this alone will not result in considerable benefits when genotypes are inefficient in their utilisation. The second option is a feasible alternative and a long-term strategy. Thus, breeding priorities for research programmes in the coming decade should include mining genes that improve nutrient consumption or identifying donors that are efficient in nutrient uptake and further utilisation.

Among the macro nutrients, especially nitrogen (N) and phosphorus (P) are the two most important regulators of grain yield in rice. However, with economic development and improved living standard, improving nutritional quality such as micronutrient (iron) contents in grains has become an added goal. The mining of key genes or genomic marker loci that control the growth as well as productivity facilitating higher nutrient uptake

under low N, Fe and P conditions to reveal the underlying molecular mechanisms can be an alluring and important objective in rice breeding. Of all the exogenously supplied nutrients, N is of the highest quantity. This element has an important role to play in tillering, grain filling, root growth and also is an integral part of many biomolecules such as amino acids, nucleic acids, several hormones and most importantly the chlorophyll. Soil with low organic matter, poorly drained wetlands, alkaline and calcareous soils are usually deficient in N content. In India, there has been indiscriminate use of nitrogen fertilisers which have been subsidized highly by the Indian government. On the contrary, the nitrogen use efficiency has gradually decreased from 55% to in 1960 to 35% in 2010 (Móring *et al*., 2021). Thus, it is imperative to minimize the nitrogenous fertilizer use by increasing the efficiency of crop varieties that can sustain and produce more under low levels of N. Several genes and transporters have been reported, that improve the N uptake in rice plants such as *TOND1, OsNRT2.1, OsNRT2.2, OsNAR2.1, OsAMT1, OsAMT2, OsAMT3* etc. (Panda *et al*., 2021b). India doesn't have sufficient rock phosphate reserves, and the deficiency of P is a common phenomenon in Indian soils, hence most of the P consumed is imported from other countries (Dey *et al*., 2017). It is a major growth limiting factor especially in acid upland and degraded lowland soils. Phosphorus uptake has been reportedly enhanced using several genes and transporters like *Phosphorus uptake 1* (*Pup1*) (Gamuyao *et al*., 2012), *OsPHT1* and *OsPHT8* (Jia *et al*., 2011). While Iron deficiency is rarely a problem in lowlands, it is deficient in neutral, calcareous and alkaline upland conditions. Iron uptake is mostly facilitated by *YSL*s (Nozoye *et al*., 2011) and transporters such as *OsTOM1, OsIRT1, OsIRO2, OsIRT2*, and *OsNARMP1* and genes, such as *OsNAS1-3, OsNAAT1, and OsDMAS1* (Mahender *et al*., 2019).

The broad adaptation of rice as a species is associated with large genetic and phenotypic diversity (McNally *et al.*, 2009). Forward genetics, in which many individuals that differ in their genotype are screened for phenotypes of interest, is a powerful tool to address issues that pinpoint traits to its genomic fragment. In general, the genetic differences being screened are obtained from a constructed population; the phenotypic differences identified in the population are connected back to the underlying causative loci via various mapping approaches including Quantitative Trait Locus (QTL) mapping (Korte and Farlow, 2013). A population constructed utilizing accessions from an environmentally defined genetically diverse setting considering diverse trait of interest that are under strong genetic control can be studied for this purpose.

Aus, a distinct subpopulation in the sativa group of cultivated rice, is one such diverse group evolved from the annuals *Oryza nivara* found in Bangladesh, Northern Myanmar and NE India (Kim *et al*., 2016) represents an untapped source of genetic resources. They are phenotypically diverse, photoperiod insensitive, and can contribute towards a number of abiotic stress-tolerance-related genes. Studying such a population can help us explore and unravel genomic segments that can contribute to the increased nutrient use efficiency, specifically for nitrogen, phosphorus and iron in this study. In this perspective, we have used a complementary and powerful tool for connecting the genotype-phenotype map, Genome-Wide Association Studies (GWAS), for the discovery of novel genes and alleles that can be used in marker-assisted selection (MAS). However, there are only a few studies exploiting GWAS to identify genes/QTLs related to nitrogen/ phosphorus/ iron efficiency in rice. The objectives of this study are therefore framed to identify genomic loci significantly associated with nutrient use efficiency exploiting high-density single- nucleotide polymorphisms (SNPs). Genomic information that they provide is expected to bridge the gap between QTLs governing traits and their candidate genes under limited nutrient conditions. The following objective are framed to establish a relation between the genotypic data and the phenotypic data to highlight nutrient use efficiency in the *Aus* population of rice:

1. To assess genetic variability among rice genotypes under low levels of nitrogen, phosphorus and iron
2. To identify genetic loci and superior haplotypes associated with the nutrient use efficiency under low levels of nitrogen, phosphorus and iron
3. To identify suitable donors for tolerance to low levels of nitrogen, phosphorus and iron with adaptive traits

REVIEW OF LITERATURE

Rice enjoys the status of the second most cultivated crop in the world, with more than half of the Asian population depending on it as their sole source of carbohydrates. The nutritional content of rice grain is 80% carbohydrates, 7–8% protein, 3% fat, and 3% fibre (Chaudhari *et al*., 2018). The production of India is 127.93 million metric tonnes (*www.pib.gov.in*), while the consumption rate is as high as 103.5 million metric tons in India against 509.87 million metric tons in the world for the year 2021-22 (*www.statista.com*). These figures clearly indicate the demand for rice and justifies the nature of ongoing intensive cultivation. The external chemical input has increased at an alarming rate from the mid of 20th century, 7kg per hectare during the onset of green revolution (Chand and Pandey, 2008) to 170kgs per hectare in 2018 (*/knoema.com/atlas/India/Fertilizer-consumption*).

The cultivated high-yielding rice varieties at present are well adapted to high-dose fertilizers, and as seen under field conditions, nutrient utilization efficiency is less than 50%. For example, nitrogen recovery efficiency remains less than 30% under rainfed conditions, but ranges from 20-30% under irrigated conditions (Roberts, 2008). Similarly, the trends observed in phosphorus (P) over the last few decades indicate increased reliance on P fertilizers with a reduced P utilization efficiency in the range of 10 -20% (Li *et al*., 2009). This is because a major portion of the nutrients applied, are lost due to drainage, volatilization, leaching, etc., or are fixed in the soil forming complexes inaccessible by the rice plant and remain unavailable. This phenomenon not only increased the input cost towards fertilisers but also lead to soil health deterioration. At this stage, one of the amendments would be to modify cultivation practices or improve the genetic potential of the plant to use resources more efficiently. The former option includes farm management changes starting with cultivation, weed control, fertilization types, irrigation etc. The latter alternative is the only viable option towards sustainable approach. Therefore, plant breeders have an important role to play in this context by identifying genes that improve nutrient utilization, and identifying donors that are efficient in nutrient uptake as well as utilization.

Nutrient utilisation by a plant is a complex polygenic trait involving a plethora of activities that includes sensing of the nutrient complex, absorption, transport and assimilation in the plant cells (Fan *et al*., 2017). The dynamics of different nutrients, their

region, and modes of availability, which alter temporally at every stage of crop growth, add to the complexity. This suggests that different nutrient uptake necessiates different plant morphology and genetic system specifications. Nitrogen (N) and Phosphorus (P) are two major elements required for plant growth. The recovery efficiency of both the nutrients falls below 30%. While nitrogen fertilisers are highly subsidised with a majority of the burden borne by the government, phosphorus fertiliser is extracted from a non-renewable resource that is more likely to get exhausted in near future. Apart from these, the excessive use of these fertilisers increases the input cost and affects the soil health as well. In addition to N and P, Iron (Fe), a micro nutrient essential for plants is abundant in the irrigated system but is deficient in the upland or rainfed cultivated rice fields. Even when in the irrigated system the high levels of N (ammonium form) applied in the field creates an artificial Fe starvation in the rice plant, especially in the seedlings (Zhang *et al*., 2019a). The soil is the primary source of Fe for plants and its optimum availability in the form of Fe2+ is essential for their healthy growth and development. Thus, exploiting rare natural variations in the rice germplasm/ landraces that enhance nutrient use efficiency should be a major objective in plant breeding at the present times. Rice plants, for example, rely on their shallow roots for phosphorus acquisition in the early vegetative stage, while longer roots are better for nitrogen uptake at later stages of growth. Such diverse variations in the morphology do exist as such in nature across cultivated varieties, wild species and sub species of rice. Evaluation of these resources would serve benefits to the cause of harnessing genes for enhancing nutrient use. Therefore, the mining of key genes or genomic marker loci that control plant root and shoot traits facilitating higher nutrient uptake under low nutrient (nitrogen, phosphorus and iron) conditions to reveal the underlying molecular mechanisms can be an important and alluring objective in rice breeding.

2.1 Genetic Variability in rice in relation to nutrient use efficiency

Rice with its rich source of variability has immense scope for selection of traits pertaining to nutrient use efficiency traits. Many studies have reported the important morphological and physiological manifestations that govern the nutrient use efficiency in rice plants. The following collection of research work will highlight the variabilities exploited in rice with respect to nitrogen, phosphorus and iron use separately.

2.1.1 Nitrogen

Nitrogen exists in two different forms in the soil, i.e., in nitrate form (NO_3^-) and ammonium form (NH_4^+) depending on the oxidation status of the soil. In well drained and aerated soils nitrate is the dominant form whereas in the submerged soils ammonium is predominantly present (Panda *et al*., 2021b). In the plant system, N is highly mobile and in times of deficiency in the plant body, N is simply translocated from the older stems and leaves to the younger leaves, while during heading it gets translocated to the panicles to aid in grain filling (Fageria and Baligar, 2005).

Kitajima and Hogan (2003) highlighted the importance of optimum dosage of nitrogen and reported smaller foliage, reduced chlorophyll and crop yield reduction as a consequence of nitrogen deficiency. At the same time, the ill effects of higher doses of nitrogen on the environment when used in excess was also stressed upon. Nitrogen's role in the vegetative growth of the rice plant is more pronounced, Meena *et al*. (2003) reported the enhanced vegetative growth in rice plant reflected in the increased plant height and number of leaves with increased nitrogen application. Namai *et al*. (2009) studied the varietal differences in the physiological nitrogen use efficiency using a wide array of rice varieties, they found wide variations in use efficiency with similar relative dry weight under different nitrogen concentrations.

A study by Fageria *et al*. (2010) dealing nitrogen use efficiency in the upland condition reported that plant height, shoot weight, number of panicle and the N uptake in shoot and grain were positively correlated with the grain yield. They also reported the variation among different genotypes in their N utilisation efficiency and showed a significant quadratic function with grain yield. Mondal *et al*. (2013) observed that optimum application of nitrogen significantly influenced morphological traits like plant height, leaf area index, number of tillers, number of panicles and test weight in rice hybrids. Wang *et al*. (2014) identified the relation between chlorophyll content in the leaves, grain quality and nitrogen use efficiency. They concluded that in order to enhance the grain quality and plant nitrogen use efficiency, nitrogen management and chlorophyll index are two important aspects to be emphasised.

Wang *et al*. (2016) worked on the physiological variations of tillers in response to different levels of nitrogen and concluded that enhanced N levels in the plants can improve the contribution of late emerging tillers to final grain yields. Sujata *et al*. (2019) studied N use efficiency in hydroponic system examining the morpho-physiological traits

and it was reported that shoot length was positively correlated with leaf , root number and root length. Wang *et al*. (2021) screened various rice genotypes at varying N levels in the field. Under the different nitrogen applications, grain yield was highly associated with pre-heading dry matter and harvest index. The yield and NUE of hybrid rice cultivars were more stable than conventional rice cultivars. Leaf chlorophyll and nitrogen dynamics in lowland rice yield for site-specific paddy management was studied by Gholizadeh *et al*. (2017), they concluded that use of a SPAD chlorophyll meter can aid in real-time paddy nitrogen management and influence grain yield. This relation of the SPAD value with leaf N and grain yield was preferably stronger at the panicle initiation stage.

The rice plant has evolved genes/transporters for the uptake of the various forms of nitrogen. The roots assimilate a small fraction of the nitrate form nitrogen and transfer the rest to the shoots, where it is reduced to ammonium. The ammonium form of nitrogen is directly consumed by plant cells after converting it to amino acids (Huang *et al*., 2018a). This conversion to amino acid is facilitated by glutamine synthetase (GS)/glutamine-2-oxoglutarate amino-transferase (GOGAT) cycle (Xu *et al*., 2012).

The gene *Dense and erect panicle 1 (DEP1),* that regulates the panicle architecture is also involved in nitrogen use efficiency in rice. The plants carrying the dep1 allele were sensitive to N at the vegetative stage, which increased the N uptake and utilization. The *DEP1* gene also aids in increasing grain number and grain filling. A study reported that NILs with *DEP1* region exhibited erect panicles and increased number of grains per panicle (Wang *et al*., 2020). A major QTL on chromosome 12, *Tolerance Of Nitrogen Deficiency 1 (TOND1),* was identified in the *indica* cultivar Teqing that responded to low N in the soil (Zhang *et al*., 2015b). The authors studied 75 *indica* and 75 *japonica* cultivars, of which only 41 *indica* cultivars harbored the gene while the rest did not have the gene. Another report suggested a main effect QTL $qRDWN6^{XB}$ on the long arm of chromosome 6 that imparts tolerance to N deficiency in *indica* rice variety XieqingzaoB. The QTLs was fine mapped to a region of 52.3kb flanked by the markers ND-4 and RM19771 containing nine candidate genes (Anis *et al*., 2019). Of these nine genes, there was also a potassium transporter (LOC_Os06g15910) involved in the function of this QTL.

In rice, nitrate uptake is controlled by two different transporter families; nitrate/peptide transporter family (NPF) and Nitrate transporter 2 (*NRT2/NAR2*). Of these,

NPF family is the most explored with 80 genes reported but most of them are low affinity transporters (exception being *OsNPF6.5* which is a dual affinity nitrate transporter). This family of transporters displays a wide range of activities with nitrogen that includes root-shoot transport (*OsNPF2.4*, *OsNPF2.2*), lateral root promotion (*OsNPF8.20*), panicle elongation (*OsNPF4.1*) and increasing grain yield (*OsNPF8.20*) (Huang *et al*., 2018b). The next group of transporters (*NRT2/NPF2*) comes under high affinity class as they play a role in uptake even in low concentration of nitrates in the soil. Out of the five NRT genes discovered so far, *OsNRT2.3b* under high expression levels enhanced not only N, but also Fe and P uptake. Interestingly two other members; *OsNRT2.1*, and *OsNRT2.2* were expressed throughout the root system but couldn't alone work in nitrogen uptake but needed a partner protein like *OsNAR2.1* (Liu *et al*., 2014). Ammonium uptake in rice plants is facilitated by the ammonia transport protein (AMT)/methyl ammonium (MEP)/rhesus (RH) superfamily. At least ten putative *AMT* genes have been operating in rice (three members in each group of *OsAMT1*, *OsAMT2*, and *OsAMT3* and one member of *OsAMT4*) and they are involved mostly in root uptake and root-shoot transport. Therefore, nitrogen use efficiency estimates should take into account uptake, transport as well assimilation efficiency of the rice genotype. Improving any of these can contribute to the nitrogen use efficiency of the rice significantly.

2.1.2 Phosphorus

Phosphorus, besides nitrogen is one of the important nutrients required by rice plant being an important part of nucleic acid, energy conversion process, regulates root growth, proper tillering, flowering and ripening. It has high mobility in the plant system but becomes immobile and inaccessible forming calcium salts and other complexes in the soil (Abel *et al*., 2002). Due to its immobility in the soil, P accumulation in the soil is limited to the shallow layers, hence the surface and subsurface roots have the responsibility of P uptake. This is evident in case of plants with well-developed surface and subsurface root system to be more efficient in their P utilization (Ramaekers *et al*., 2010). Phosphorus deficiency is more pronounced in uplands as it forms poorly soluble complexes in the soil with iron and aluminium in acidic soils and calcium in alkaline soils (Holford, 1997). But gets released from these compounds after flooding the land, regulating the redox potential and pH (Kochian *et al*., 2004; Jensen, 2010).

A number of studies have been undertaken to identify the genetic variations among genotypes in relation in to the P use efficiency in rice. A study to identify P use efficiency

related traits by Fageria *et al*. (1988) used 75 upland rice lines at two different P levels in the field. There was a significant difference at shoot dry matter, grain yield and plant P content based in the soil P level. The study also highlighted that shoot dry matter as most affected by P deficiency. Ni *et al*. (1998) introduced a stress and a normal P environment to five rice genotypes and the results indicated that relative tillering ability, root length and root number are the deciding factors that can be used a selection criterion in deciding the P uptake ability and biomass production for low P tolerance.

The major role of root growth parameters was highlighted by Wissuwa (2003). The study emphasised that minor changes in root morphology can have a bigger impact on the P uptake of rice plants. It was seen that increasing internal efficiency root dry matter production by 22% can enhance P uptake by three times. In addition, Anandan *et al*. (2022) reported that total root surface area is the single major trait contributes 33% of P uptake under low P. Zhang *et al*. (2003) reported that when plants are grown in low P condition, there is an increase in cortical cells and root hair bearing epidermal cells. It was also seen that low P effect could be mimicked by using ethylene precursor, 1-aminocyclopropane-1-carboxylate. Akinrinde and Gaizer (2006) worked on six rice varieties in alfisol at 5 levels (0,50, 100, 150 and 200mg/kg) of P and concluded that root dry weight was influenced the most with phosphorus addition and thus decided root dry weight to be one of the sensitive parameters of P deficiency.

The effect of P deficiency on net photosynthesis have also been highlighted by various studies. Under low levels of P in the soil, photosynthetic efficiency reduced, this was reported to be due to the inefficiency of Pi translocators, an antiporter that exports phosphate from the stroma to the cytosol in exchange for Pi (Stitt and Quick, 1989). Under low P availability, there was a reduction in biomass accumulation that can be attributed to the reduced net photosynthesis as reported by Wissuwa *et al*. (2005). Xu *et al*. (2007) studied the effects of phosphorus deficiency in the rice seedlings and it was concluded that the P deficiency induced a significant reduction in the net photosynthetic rates of the seedlings.

Li *et al*. (2005) used hydroponic set up to study the differences in tolerance level towards low P. There were significant differences of tolerant indices to P deficiency in the test rice genotypes. The traits like relative tiller dry weight, relative shoot dry weight and total relative plant dry weight were suggested as indices for screening low P tolerant lines.

Yong-fu *et al*. (2006) carried out a hydroponic study using a P tolerant genotype, Zhenongda 454 and low P sensitive genotype, Sanyang'ai under low P stress. It was reported that the photosynthetic rates decreased by 16% and 35% respectively. Panigrahy *et al*. (2014) studied the M5 mutants (treated with ethyl methane sulfonate) of Nagina 22 (N22) in low P field. The results showed that seedling growth traits like root weight, root length, root/shoot weight and dry weight are the major selection indices of a low P tolerant genotype. The mutants reportedly gave higher yield than the N22 mutants in P deficient condition.

Saito *et al*. (2015) undertook a trial to assess the variation in 12 upland varieties for P efficiency. The results showed that there was significant variation in aboveground biomass. Mudgo variety was identified with highest aboveground biomass and can be used as a donor for improving early growth under low P soil. Yugandhar *et al*. (2017) used Nagina 22 mutants to screen for low P tolerance and suggested the use of hydroponic with sand as a an alternate to low P field screening and they indicated shoot dry weight and root dry weight as the best parameters for screening a low P tolerant genotypes. Kekulandara *et al*. (2019) screened 48 Sri Lankan varieties at low P and sufficient P levels in hydroponic experiment. The variety Bg 94-1 was identified as a superior genotype with higher biomass and shoot P content.

Deng *et al*. (2020) reported that when plants are grown with normal P input and low P input, plants under low P conditions significantly increased the phosphorus translocation efficiency and internal phosphorus efficiency. The tolerant plants had higher total root length and root oxidation activity. In an attempt to better understand P uptake and its allocation between the root and shoot, Panda *et al*. (2021a) evaluated improved cultivars, landraces of *Oryza sativa*, representatives of *Oryza nivara*, and *Oryza rufipogon*. The results highlighted the traits regulated in a low P tolerant line such as root volume, total root surface area, total dry weight, SPAD value and number of root tips. While, non-destructive (geometric traits by imaging) traits such as minimum enclosing circle, convex hull, and calliper length could be used to differentiate and identify tolerant line under low P in 28-day-old seedling (Bhatta *et al*., 2021).

Due to its immobility in the soil, P accumulation in the soil is limited to the shallow layers, hence the surface and sub-surface roots have the responsibility of P uptake. This is evident in case of plants with well-developed surface and subsurface root

system to be more efficient in their P utilization (Ramaekers *et al*., 2010). *Phosphorus uptake 1* (*Pup1*) a major QTL enhances the early root growth and makes the plant more efficient to P utilization in deficient conditions (Gamuyao *et al*., 2012). This gene is present in one of India's mega variety Swarna, making it more adapted to P deficiency and high use efficiency in Indian soils that are mostly P deficient (Ali *et al*., 2018). Two new donors of *PSTOL1* gene were identified among landraces and improved varieties; a landrace from Assam (IC459373) and a short duration variety Shankar. They performed at par with positive checks Kasalath and Dular (Anandan *et al*., 2021; Bhatta *et al*., 2021). This shows that increasing root growth and root spread at the early stage of seedling growth can be an indirect trait contributing towards P use efficient rice plants. Genotype having *PSTOL1* and higher expression of *PHT1* displayed a significant difference in P concentration and phenotype (Anandan *et al*., 2021). Rice plants can start phosphorus uptake actively as early as 2-3 days after germination, irrespective of the seed P reserves. Two-week old rice seedling needs upto 6ppm of P for normal growth than 30-day old seedling which required 4ppm (Anandan *et al*., 2021). Two such Phosphate transporters have been identified; *OsPHT1* and *OsPHT8* that play a major role in reallocation of P from source to sink organs and help in the nutrient homeostasis in the growing embryo (Jia *et al*., 2011). In rice, 26 *OsPHT* genes have been identified to be involved in P transport, distributed over all the chromosomes but chromosome 7 (Liu *et al*., 2011). Of these 18 are localized in the plasma membrane involved in the core transportation process across the membrane (Mahender *et al*., 2018).

Uptake of phosphorus is done by the transporters located in the root plasma membrane, *OsPHT6*, a transporter gene is located in the root epidermis is a high affinity transporter working well under deficient soil conditions (Ai *et al*., 2009). The P transporter genes have been classified into five groups on the basis of their sequence and localization, namely *PHT1, PHT2, PHT3, PHT4,* and *PHT5* (Wang *et al*., 2017). Of these *PHT1* (a high affinity transporters) is mainly involved in the uptake of P from the soil whereas the rest of the transporters are related to distribution and translocation of P with in the cellular compartments (Młodzińska and Zboińska, 2016). In rice there 13 transporters genes under in *PHT1* are characteristically upregulated in P deprivation conditions (Nussaume *et al*., 2011; Anandan *et al*., 2021). However, the other transporters such as *PHT2, PHT3* and *PHT4* have not been well studied and understood. Studies on genetically modified rice crop has revealed that *OsPHT4* and *OsPHT6* also significantly

increases 1000-grain weight and grain yield as compared to wild type under low Pi concentrations (Zhang *et al*., 2015a; Zhang *et al*., 2014). Interestingly, it was seen that phosphorus use efficiency is not a function only of the Pi transporters as it was seen that P inefficient genotypes were able to uptake Pi from nutrient solution almost at the same rate as that of a tolerant genotype (Wissuwa, 2005). This indicates that the high expression of these transporters might be a part of P starvation response instead of P starvation tolerance (Oono *et al*., 2013). Remobilisation and allocation of the P after uptake can be a major problem, it was seen that overexpression of *OsPT14* comparatively higher P accumulation in the top three leaves only (Wang *et al*., 2017). Thus, the viewpoint here suggests that the focus now should more be on intracellular Pi transporters such that their remobilization and proper distribution can be facilitated. Two sulphate transporters *OsSULTR3;3* and *OsSULTR3;4* have been reported to be involved in P distribution in the plant cells(Zhao *et al*., 2016; Yamaji *et al*., 2017). A mutation studies in these genes resulted in reduced grain P percentage (by 20%) while increasing the P content of the straws (Yamaji *et al*., 2017). This shows their function in remobilization of nutrient during grain filling stage at the later part of crop growth.

2.1.3 Iron

Iron is the most limiting nutrient for rice crop owing to its low solubility of the ferric form (oxidized ion) especially in the aerobic/upland conditions (Zuo and Zhang, 2011). Even though it is abundant in the lithosphere, its bioavailability is limited especially in alkaline soils and oxidized dry aerobic soils. The deficiency symptoms under such conditions can be easily visualized with its typical symptoms like interveinal chlorosis predominantly in younger leaves and reduced root growth. Under severe conditions grain yield reduction as high as 50% have been observed and also complete crop failure at the vegetative stage itself (Mahender *et al*., 2019). Rice like any other *Poaceae*, secretes root exudate or phytosiderophores to facilitate uptake of Fe from the rhizosphere. These phytosiderophores are released as a result of the action of nicotianamine synthase (NAS), nicotianamine aminotransferase (NAAT) and deoxymugineic acid synthase (DMAS) (Shojima *et al*., 1990). However, at the young seedling stage, due to lower levels of secretion of deoxymugineic acid as a result the root tips become chimeric and the epidermal cells become necrotic (Mori *et al*., 1991). These secretions help in forming soluble complexes with Fe in the rhizosphere, a form that can

easily be taken up by roots. The roots pick up these Fe(III)-PS complexes from the soil with help of transmembrane transporters.

The change in root morphology under iron stress have been well documented such as in tomato, there is increased root hair growth under iron deficiencies (Jin *et al*., 2009). Shi *et al*. (2012) assessed aerobic rice's response to iron deficiency in a hydroponic culture system. They reported that aerobic rice was highly sensitive to Fe deficiency and removal of Fe source from the system results in complete chlorosis of the seedlings. The difference in response to Fe nutrition of a rice cultivars is owed to its inherent genotypic characteristics and not the external Fe nutrition (Pal *et al*., 2008). Chen *et al*., (2018) studied two indica rice cultivars; H9405, a Fe enriched cultivar and Yang6, a low Fe seed accumulation cultivar. The genes involved in Fe uptake were up regulated in H9405. Pippal *et al*. (2018) phenotyped the grain iron content in F5 and BC_1F_4 populations of PAU201 x Palman579. It was reported that the iron content was positively correlated with all the traits except plant height in BC_1F_4. The frequency distribution curve for iron content were skewed in the direction of Palman579. However, the grain yield was higher in PAU201. Iron deficiency has been seen to reduce the root meristematic activity but at the same time cell elongation remained uninhibited (Sun *et al*., 2017). Iron use efficiency and Fe harvest index under dry cultivation is reported to be enhanced but the adverse effect was seen in the grain yield and Fe concentration in rice plant and grain (Zhang *et al*., 2019b). Interveinal chlorosis is one of the major symptoms indicating the iron deficiency in a plant leading to seedling death or remarkable losses in grain yield at the later stages. The Fe deficiency is more pronounced in case of rice seedlings because of the low levels of phytosiderophores secreted by the seedlings which completely ceases within 100 days even under Fe deficiency (Mori *et al*., 1991). Apart from interveinal chlorosis various other traits that act as indicators of Fe deficiency are shoot and biomass, number of adventitious roots, photosynthetic rate, and root/shoot ratio (Mongon *et al*., 2017). Of all the iron deficiency phenotyping approaches hydroponic nutrient solution approach has been used widely in crops like rice, wheat, chickpea, and maize (Shen *et al*., 2002; Mahmoudi *et al*., 2007; Silveira *et al*., 2007; Carvalhais *et al*., 2011).

These studies indicate the existence of genetic variability among the rice cultures for nutrient use efficiency. They also suggest that the observed genetic variation can be utilized for breeding elite rice culture with superior grain yield stability under minimal soil nutrient availability, with minimal dependence on fertilizer inputs.

To further improve these identified variations related to nutrient use efficiency one needs to have an idea about different genetic estimations and parameters that govern these variations. In order to assess the genetic variability of a trait one need to have a fair idea of a set of genetic parameters such as genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability estimates. These set of estimates can help a breeder to predict the results of selection for a particular trait.

Karim *et al*. (2007) assessed the genetic parameters for aromatic rice genotypes, it was seen that the phenotypic variance was higher than the genotypic variances for traits like number of panicles per hill, number of filled grains and grain yield indicating a higher effect of environment on these traits. The GCV was high for observed for 1000 grain weight followed by spikelet sterility (%) and grain yield. A similar study was done by Kole *et al*. (2010) and they reported that PCV was higher for panicle number and moderate for grain number per panicle, straw weight, harvest index and grain yield per plant and low for days to flowering , plant height and test weight. While there was high heritability with moderate to high genetic advance for panicle number, grain number, straw weight and grain yield. This indicated a predominance of additive gene action controlling these traits.

Akinwale *et al*. (2011) reported that the GCVs were lower than the corresponding PCVs in all the trait studied, indicating a higher amount of environmental influence on their expressions. High to medium heritability and genetic advance were recorded for number of grain per panicle, grain yield and number of panicles per plant. These figures suggest that the selection for these traits on the basis of their phenotypic performance can be effective.

Ketan and Sarkar (2014) studied 26 indigenous aman rice genotypes. The values for PCV were higher than GCV for all the characters studied. High heritability was observed for days to 50 percent flowering, plant height, 1000 grain weight and panicle length. Number of grain per panicle followed by floret number per panicle displayed the highest genetic advance. Kamlesh *et al*. (2015) studied the genetic parameters for yield and yield attributing traits in rice. A moderate PCV was observed for harvest index, grain yield per hill, biological yield per hill, and panicle length, while test weight, days to 50% blooming, and days to maturity had low estimates of PCV. Biological yield, number of spikelets per panicle, test weight, harvest index, grain yield per hill, days to maturity,

plant height, days to 50% flowering, and number of tillers per hill had high heritability. Konate *et al*. (2016) evaluated 17 recombinant inbred lines along with their parents and a check variety. High estimates of heritability and genetic advance were reported for biomass, days to 50 percent flowering, number of panicles and yield per plant. Sumanth *et al*. (2017) studied 23 rice genotypes for 13 different quantitative traits. High GCV and PCV were observed for seed yield, panicle per plant and spikelets per panicle. High heritability was observed for plant height, spikelets per panicle and panicles per plant. These traits also had high genetic advance, indicating that these traits are controlled by additive gene action. Gyawali *et al*. (2018) studied 7 rice genotypes and local check variety. The estimates of heritability were high for all the traits except panicle length. Highest heritability was found for days to flowering whereas effective tillers per square metre had the highest genetic advance. Gupta *et al*. (2020) studied forty-eight rice germplasm lines for assessment of genetic variability. The results showed high estimates of GCV and PCV for filled grains per panicle followed by total grains per panicle, grain yield per plant and number of effective tillers per plant. plant height, days to 50% flowering and test weight showed high heritability and high genetic advance.

Correlation coefficient is assessed to determine the degree (strength) and direction of association between two or more variable characters and can aid in determining the selection for trait improvement. It indicates the magnitude of linear association between two traits and form the basis of selection index, thereby assisting the breeder in crop improvement programmes. Lakshmi *et al*. (2014) assessed the correlation coefficients of 70 rice genotypes and there was found to be a significant positive association of grain yield per plant with days to maturity, number of productive tiller per plant, plant height and kernel length. Rahman *et al*. (2013) highlighted the importance of flag leaf, it was reported that grain yield was positively correlated with flag leaf length, width and area. Improving flag leaf traits can enhance yield. Flag leaf is one of the important factor for grain as it is involved in photosynthesis as the main source of assimilation needed for plant growth and panicle development (Tian *et al*., 2015). Bhati and Rajput (2015) studied 30 elite rice genotypes and found that grain yield per plant was significantly correlated with harvest index, biological yield per plant, test weight and plant height. Karande *et al*. (2017) used 44 rice genotypes to study the correlation between various agro-morphological traits. Grain yield had significant positive correlation with total tillers per plant, productive tillers per plant, panicle length and grain yield per panicle (Chandra *et*

al., 2009). Rahman *et al*. (2013) studied rice cultivars at the Bangladesh Rice Research Institute and concluded that flag leaf serves as a major photosynthetic energy producing source during grain filling, thus regulating the panicle development and ultimately grain yield in rice.

2.2 SNP haplotypying and Genome Wide Association Studies in rice

The advances in the field of next generation rice genome sequencing have broadened the scope for studying genes at the SNP level and correlating any existing variabilities with the morphology manifested by a genotype. GWAS is such a tool in forward genetics that exploits the whole set of genomes, translated into SNPs and then find associations (if any) with a trait of interest. The basis of association is the linkage disequlibrium (LD) of the population under study, and utilises the available genotyped data along with the phenotypic data. Such an estimation would require a large population with diverse phenotypic variabilities under strong genetic control. These associations are now capable of not only marker identification but also are a method to identify causal variants and putative candidate genes. Genomics-assisted breeding methods have increased the crop improvement efficiency and deliver better results in a short span of time. The present study has used the SNP sequence data of Bengal and Assam Aus Panel to generate associations based on haplotypes that are a result of tightly linked SNPs with a trait of interest. With the advent of next generation sequencing technologies and available of a standard reference genome, GWAS analyses are now being conducted on various rice panels and have led to in depth insight into many important traits pertaining to leaf growth (Yang *et al*., 2015), plant height (Ma *et al*., 2016), flowering time (Huang *et al*., 2012), salt tolerance (Chen *et al*., 2021), drought tolerance (Guo *et al*., 2018), root vigor (Anandan *et al*., 2022), nitrogen deficiency (Shen *et al*., 2021; Xin *et al*., 2021; Li *et al*., 2022), phosphorus deficiency (Nforten and Yoo, 2020; Mai *et al*., 2021), and iron toxicity (Utami *et al*., 2020; Kaewcheenchai *et al*., 2021).

Crowell *et al*. (2016) used 242 tropical rice accessions to study the panicle phenotypes using GWAS. Ten different candidate genes regulating plant architecture were highlighted in the study. The *NAL1-OsKS1* on chromosome 4 was identified as an important locus in improving yield. The gene *NAL1* is known to be involved in cell division, polar auxin transport, flag leaf area, leaf chlorophyll content, panicle size and overall plant architecture.

Xin *et al*. (2021) used 267 japonica varieties that were sequenced to generate 151,202 SNPs to study the N absorption and utilisation at the seedling stage using GWAS. *OsNAC68* was identified as the candidate gene for N utilisation. Overexpression of this genes significantly raised the N use efficiency and grain yield under N deficient condition.

Shen *et al*. (2021) studied a population of 497 lines using Huanghuazhan as the recurrent parent and eight elite lines as the donor parents for grain yield under two different nitrogen levels. 14 QTLs were identified under low N conditions. The study highlighted the role of *qTGW2-1* on chromosome 2 under low and favourable N conditions. Li *et al*. (2022) conducted GWAS using a panel of 230 rice accessions at two different levels of N. They identified 411 genes in 5 QTLs and 2722 differentially expressed genes in relation to low levels of N. They identified *OsNIGT1* as a candidate gene for N utilisation which was reported to inhibit the expression of N transporters such as *NRT2.1, NRT2.4* and *NRT2.1* under sufficient N levels.

Zhong *et al*. (2021) used the Rice Diversity Panel 1 contains 421 rice accessions and identified 23 candidate genes that regulated panicle architecture in rice. They identified Os01g0140100 as a candidate gene associated with yield via florets per panicle in rice. It was highly expressed in pre emergence inflorescence, seed-5 days after pollination and the pistil tissues. Yu *et al*. (2021) identified a gene *NLP4* associated with nitrogen use efficiency, this gene activated *OsNiR* encoding nitrate reductase critical for nitrogen assimilation in rice. They reported that *OsNLP4*-OsNiR increased yield and tiller number through nitrogen assimilation enhancement. Mai *et al*. (2021) studied the effect of inorganic phosphate deficiency using 160 Vietnamese rice landraces in hydroponic medium. Number of crown roots, root length, shoot length, root weight, shoot weight and total weight were studied. Out of 158 genes that were collocated with the defined QTL, the QTL *qRST9.14* was associated all the three weights. Another gene from this region *GLYCEROPHOSPHODIESTER PHOSPHODIESTERASE 13* was found to be involved with Pi transport. Nforten and Yoo (2020) studied 23 genotypes of North Korean rice selected on P deficiency tolerance grown under hydroponic medium. *Pup1* was identified as the QTL that imparted the P tolerance trait in some of the studied rice genotypes. Jang *et al*. (2021) studied 137 rice accessions to identify genes regulating the mesocotyl elongation. Eleven peak SNPs were confirmed to be associated with mesocotyl length and with a LD value of 230kb. Five different candidate genes; Os01g0269800, Os01g0731100, Os08g0136700, Os08g0137800, and Os08g0137900 were found to be significantly associated with phenotypic variation.

MATERIALS AND METHODS

The aim of this research was to explore the genetic variations underneath morphological traits influencing nutrient use efficiency, i.e., N, P and Fe and genetic loci and superior haplotypes associated with it. Thus, three separate experiments were planned to facilitate the study involving each of the above-mentioned nutrients while keeping all other factors constant.

3.1 Plant materials

The experiment material used in this study was the Bengal and Assam Aus Panel (BAAP) developed by Norton *et al*. (2018) that consisted of 298 representatives of rice. The panel consisted of landraces from the aus subpopulation identified by (Travis *et al*. 2015), 19 of the OryzaSNP set (McNally *et al*. 2009), 33 *aus* cultivars from the Rice Diversity Panel 1 (Zhao *et al*. 2011) and released varieties, breeding lines from Bangladesh. These genotypes were subjected to two rounds of single seed descent at International Rice Research Institute (IRRI), Philippines to ensure purity in the panel lines and whole genome sequencing by The Centre for Genome Analysis, Norwich, UK and a SNP database of 2,053,863 SNPs. The ~ 2 million (2,053,863) SNP dataset, generated using skim sequencing at ~4× depth, from this population, is available under the project called "BAAP" (Bengal and Assam Aus Panel) in the SNP-Seek database (http://snp-seek.irri.org/). The list of genotypes used in this study are presented in Table 3.1. The nitrogen, phosphorus and iron deficient trials included 203, 204 and 183 BAAP lines respectively. Additionally, RA23 was added as a check in the iron deficiency experiment.

3.2 Experimental site

The nitrogen deficient trial was undertaken in field condition at ICAR-National Rice Research Institute, Cuttack during *Rabi* 2020 in transplanted system of rice cultivation. The soil was sandy clay loam with 0.53% organic carbon, 240 kg/ha of N, 26.3 kg/ha of P, 164.05 K kg/ha, 51.6% sand, 18% silt, 30.4% clay with bulk density of 1.41 g/cm^3. In order to maintain a nitrogen deficient condition only 50% of the total recommended dose of N was applied. The recommended dose was 80:40:40::N:P_2O_5:K_2O, while the applied fertilizer in the trial was 40:40:40::N:P_2O_5:K_2O. Transplanting was done at 28days after germination in a single row of each genotype in four replications. The planting followed randomized block design in four replications. The spacing was maintained at 20 cm between rows and 15 cm between plants. After each row of entry one

row of Naveen variety was grown to ensure uniform competition in the field. Standard agronomic practices were followed with respect to irrigation schedule and controlling weed in the field. The phosphorus deficient trial and iron deficient trial were conducted in the net house under hydroponic medium. For the hydroponic trial uniform seeds of all the accessions were selected and heat treated in the hot air oven at 50℃ for 45 hrs to break the seed dormancy. Surface sterilization was done with 75% ethanol for one minute and 2.5% sodium hypochlorite for twenty minutes and then rinsed in sterile distilled water to remove any traces of the sterilizing agent. Styrofoam trays with fixed mesh at the bottom were prepared with 12 x 8 holes for seed sowing. Each accession was sown in one row with two replications. Each hole in a row had three seeds and these trays were kept in the dark for 3 days for germination. The Styrofoam with well germinated healthy seedlings were then transferred to trays containing Yoshida solution(Yoshida *et al*. 1971) for Phosphorus experiment containing 0.5 ppm of $NaH_2PO_4.H_2O$ with pH maintained at 4.50-4.55. While the iron deficient trial was done using Yoshida's modified solution devoid of any iron source wih pH of 5.5-5.6. The pH of both the experiments (phosphorus and iron) were adjusted every alternate day and the nutrient solution was changed every 7 days. The composition of the solution used in the hydroponic study for phosphorus deficient trial and iron deficient trial is furnished in Table 3.2.

Traits studied under different nutrient deficient trials

3.2.1 Field condition under nitrogen deficient trial

Six plants were taken from an entry in each replication to record the observations and the mean was calculated using the values recorded from the six plants in each replication for further statistics. A field overview photograph of the nitrogen deficient trial at different growth stages is presented in Figure 1.

3.2.1.1 Chlorophyll Index

Chlorophyll index were recorded using the SPAD 502 chlorophyll meter manufactured by Minolta company, Osaka, Japan. It gives the chlorophyll concentration index. Under the nitrogen deficient trial in the field condition, SPAD index was recorded from the flag leaf at the flowering stage while for phosphorus (28 days after germination) and iron (14 days after germination) from the top second leaf.

3.2.1.2 Days to 50% flowering

This trait was recorded by observing the primary panicles when more than 50 percent of the plants in a variety/row came to flowering stage. The number of days from sowing to flowering were then recorded as the days to 50% flowering.

Table3.1 List of BAAP accessions used in the study

Sl	BAAP ID	Genotype	Sl	BAAP ID	Genotype	Sl	BAAP ID	Genotype	Sl.	BAAP ID	Genotype	Sl.	BAAP ID	Genotype
1	1	ASSAM 4(BORO)	49	61	AUS 298	98	122	Kele(AUS)	147	188	Jabor Sail	196	242	Sada boro
2	2	ARC 5959	50	63	AUS 314	99	123	Chamka	148	189	Dubhi Gora	197	243	Gobir sail
3	3	ARC 5960	51	64	AUS 317	100	124	Kaloshaita	149	190	Nagri	198	244	Dumsia 81
4	4	ARC 5977	52	65	AUS 321	101	125	Kumbi	150	191	DJ 29	199	245	Tulsi boro
5	5	ARC 6000	53	66	AUS 323	102	126	Munshishail	151	193	Lali Boro	200	247	Gul tepi
6	6	ARC 6240	54	68	AUS 335	103	127	Shoni	152	195	Raj Mundo	201	248	Rata boro
7	7	ARC 7325	55	69	AUS 350	104	128	Pura Nukna	153	196	Tepa Boro 508	202	249	Choudhury sail
8	8	ARC 10958	56	70	AUS 353	105	129	Cunail	154	197	W 418	203	250	Bachi boro
9	9	AS 2	57	71	AUS 354	106	130	Faisha Mansa	155	198	White Dubhi	204	253	BR 3
10	11	ARC 11205	58	73	AUS 362	107	131	Kancha Noni	156	199	ARC 7229	205	254	BR 6
11	13	ARC 11600	59	75	AUS 366	108	133	Dhaliboro 105-2	157	200	BJ 1	206	255	BR 16
11	14	AUS Murali	60	76	AUS 369	109	134	Dhaliboro 111-3	158	201	Black Gora	207	256	BRRI dhan 28
12	15	BOWALIA	61	77	AUS 382	110	136	Kaliboro 26	159	202	Dhala Shaitta	208	257	BRRI dhan45
13	16	BOWALIA 2	62	78	AUS 385	111	137	Kaliboro 41-1	160	203	DV85	209	258	BRRI dhan47
14	18	AUS 16	63	79	AUS 391	112	139	Kaliboro 138-2	161	204	DZ78	210	259	BRRI dhan50
15	19	AUS 22	64	80	AUS 401	113	141	Kal Buri	162	205	Jhona 349	211	260	BINA dhan 5
16	21	AUS 29	65	81	AUS 411	114	142	Shada Boro	163	206	Kalamkati	212	261	Iratom 24
17	22	AUS 31	66	82	AUS 414	115	143	Jagal-1640 A	164	207	Kasalath	213	262	IARI 6626
18	23	AUS 37	67	83	AUS 415	116	145	Chandra Mukhi	165	208	T 1	214	263	IARI 7449
19	24	AUS 46	68	84	AUS 417	117	147	Hijli	166	209	T26	215	264	Purple Puttu
20	25	AUS 60	69	86	AUS 435	118	149	Jati AUS	167	210	ARC 6578	216	265	P 79
21	27	AUS 63	70	87	AUS 440	119	150	Kalindi	168	211	CTG 1516	217	266	Polman
22	28	AUS 67	71	88	AUS 453	120	151	Kalo Bira	169	213	DJ 123	218	267	DJ 53
23	29	AUS 68	72	89	AUS 455	121	152	Kele Bari	170	214	DJ 24	219	268	Ziri
24	30	AUS 74	73	90	AUS 462	122	153	Khashia Panja	171	215	DM 43	220	270	P 32
25	31	AUS 77	74	91	AUS 464	123	155	M 136-20	172	216	DM 56	221	271	99216
26	33	AUS 93	75	92	BORO 354	124	156	Nai Dumur	173	217	DM 59	222	272	NP 97
27	34	AUS 96	76	94	BORO	125	157	Padma Sail	174	218	DNJ 140	223	273	DV 118
28	35	AUS 99	77	95	Boro black	126	159	Shete Bhado	175	219	DV 123	224	274	Pachodi 427
29	37	AUS 105	78	96	BCULARN	127	160	Soa Mukhi	176	220	Ghorbhai	225	275	Bowalia
30	38	AUS 125	79	97	AUS Meri	128	165	Deshi Boro	177	221	Goria	226	276	ARC 10303
31	39	AUS 127	80	99	ARC 14855	129	167	Sorishaful	178	222	Jamir	227	278	DA 24
32	41	AUS 131	81	100	ARC 14915	130	168	Kada-176-12	179	223	Kachilon	228	280	Fulbadam
33	42	AUS 136	82	101	ARC 14950	131	170	Gota Lemma	180	224	DZ 193	229	282	Dom Sufid
34	43	AUS 151	83	102	ARC 14965	132	171	Motzhul	181	225	Karkati 87	230	283	Dular
35	45	AUS 154	84	103	ARC 14969	133	172	CN2-175-5-31	182	226	ARC 10376	231	284	Fr 13 A
36	46	AUS 169	85	104	ARC 7098	134	173	Dhingha	183	227	9524	232	285	Ir 64-21
37	47	AUS 175	86	105	Brown Gora S.B. 92	135	174	DA2	184	228	Surjamkuhi	196	286	Li-Jiang-Xin-Tuan-Hei-Gu
38	48	AUS 180	87	106	AUS Paddy(Black)	136	175	Gouerisail	185	229	PTB 30	197	287	M 202
39	49	AUS 204	88	107	AUS Paddy(White)	137	176	Code No BI 93	186	230	Bawoi	198	288	Minghui 63
40	50	AUS 209	89	108	Anjani	138	177	Kali AUS	187	232	Boraya	199	290	N 22
41	51	AUS 210	90	111	AUS Kushi	139	178	Parbatjira	188	233	Bogura	200	292	Pokkali
42	53	AUS 267	91	112	MTU18	140	179	Satha	189	234	Lahaya	201	293	Sadu Cho
43	54	AUS 268	92	113	SLO 19	141	180	DulaAUS	190	235	Deshi boro	202	294	Sanhuangzhan No 2
44	55	AUS 273	93	114	Early Sutarsar 39	142	181	Chinger	191	236	Tupa	203	297	Zhenshan 97 B
45	56	AUS 277	94	116	DA 12	143	183	T 65	192	237	Chhola boro	204	300	Rayada
46	57	AUS 280	95	117	KADA-68-1	144	184	Saita Boro	193	238	Chhola boro(2)			
47	58	AUS 283	96	119	CTG250	145	185	Kuda	194	239	Lafai			
48	60	AUS 294	97	121	Khailaorgoabez	146	187	Kalasu	195	241	Chaili boroi			

Fig.3.1 Field view of Nitrogen deficient trial during transplanting, tillering and grain filling stage

3.2.1.3 Plant height (cm)

Plant height was measured in centimetre from ground level to the tip of the panicle of main culm excluding awns if any at the time of maturity.

3.2.1.4 Flag Leaf length (cm)

The length of the flag leaf was measured in centimetre from the base of leaf to its tip.

3.2.1.5 Flag Leaf width (cm)

Flag leaf width was measured in centimetre at the middle of flag leaf in standing crop.

3.2.1.6 Panicle length (cm)

Panicle length was measured in centimetre from neck node of the panicle to the tip of the uppermost spikelet, excluding awns if any.

3.2.1.7 Number of tillers

Tillers were counted for each entry with randomly selected five plants at the end of active tillering stage.

3.2.1.8 Number of productive tillers

The number of tillers that were bearing panicles were counted at maturity to record the number of productive tillers.

3.2.1.9 Grain yield per plant (g)

Individual plants were hand thrashed, cleaned and dried up to 12% moisture content and then weighed in grams.

3.2.2 Hydroponic trial under phosphorus and iron deficient trial

The observations for phosphorus deficient hydroponic study were taken on the 28th day after germination while for iron deficiency hydroponic study, observations were recorded on the 14th day after germination. Three plants were taken from each replication to record the observations. The mean values of the three plants in each replication were considered for further statistics. Figure 2 shows a representative figure of the hydroponic trial for phosphorus and iron deficient medium.

3.2.2.1 Shoot length (cm)

The length from the base to the tip of the longest leaf were measured as the shoot length.

3.2.2.2 Root length (cm)

The length from the base of the root to the tip of the longest root was recorded as the root length.

3.2.2.3 Number of leaves

Number of leaves in a single seedling were counted and were recorded as the total number of leaves per plant.

3.2.2.4 Number of roots

The total number of roots were counted from the base of the shoot manually to record the number of roots.

3.2.2.5 Shoot dry weight (g)

The shoot samples were dried in an air-forced oven 60°C for 5-6 days and the observation were recorded using weigh balance.

3.2.2.6 Root dry weight (g)

The root samples were oven dried for 5-6 days and the observation was recorded using weigh balance.

The following observations were taken only under iron deficient hydroponic trial samples with the help of WinRHIZO Pro 2013e (LA 2400, Regent Instruments INC.) root scanner for three plants from each replication for every entry in this study: Average root diameter (mm), Root volume (cm^3), Number of root tips, Total root length (cm), Total root projected area (cm^2), and Total root surface area (cm^2).

Table 3.2 Composition of Yoshida's nutrient solution used in this study

Sl. No.	Chemical	Molecular weight	Yoshida's Solution (gL^{-1})	Yoshida's modified Solution (gL^{-1})
1	NH_4NO_3	80.4	91.4	91.40
2.	K_2SO_4	174.26	97.8	71.40
3.	KH_2PO_4	136.10	29.0	23.10
4.	K_2HPO_4	174.18	8.0	4.30
5.	$CaCl_2.2H_2O$	147.02	175	117.0
6.	$MgSO_4.7H_2O$	246.47	324	324.0
7.	$MnCl_2.4H_2O$	197.90	1.5	1.5
8.	$(NH_4)_6.Mo_7O_{24}.4H_2O$	1235.90	0.074	0.074
9.	H_3BO_3	61.83	0.93	0.93
10.	$ZnSO_4.7H_2O$	287.50	0.035	0.035
11.	$CuSO_4.5H_2O$	249.68	0.03	0.03
12.	$FeSO_4$ (fresh)	151.91	2.5	0.0
13.	FeNaEDTA			10.5
14.	$Ca(NO_3)_2$	164.08	2.5	207.13
15.	$NaH_2PO_4.H_2O$	137.99	0.5ppm	

Fig.3.2 Hydroponic trial (view of a single floater with 12 different genotypes) of phosphorus deficient system (left) iron deficient system (right)

3.2.3 Statistical analysis:

Three plants were selected from an entry in each replication and the following statistics were calculated using "variability" package in R and KAUGRAPES (Gopinath *et al*. 2021). PCA analysis was carried out using FactoMine R package (Lê *et al*. 2008) in R 3.6.4. To understand the relationship between different traits, general correlation among all the studied traits were analysed using the corrplot functions from the corrplot package (Wei and Simko 2021) in R 3.6.4.

Mean

On the basis of individual plant observations, the population mean for each character was computed as follows.

$$\bar{X} = \frac{1}{n}\sum_{i=1}^{n} Xi$$

where, $\bar{X}$= population mean

X_i = individual value

n = number of observations

Range

Lowest and highest values for each character were recorded and expressed as range.

Standard Deviation

Standard deviation measures the amount of variability within a sample, it measures deviation of a sample observation from sample mean.

$$SD\ (\sigma) = \sqrt{\frac{\Sigma d^2}{n}}$$

Where, d = deviation of individual value from the mean

n = number of observations

Variance

It is the square of standard devation or sum of the squared deviations of each individual from the mean. $V\ (\sigma^2) = \frac{1}{n-1}\Sigma_{i=1}^{n}(y_j - \bar{y})^2$

where, y_i = individual value

$\bar{y}$ = population value

Co-efficient variation (CV)

It measures the relative dispersion of data points in a data series around the mean.

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

Critical difference (CD)

To test the significance of differences of the estimates, critical differences was calculated as, $S.\ Ed. = \sqrt{\frac{2M_e}{r}}$

$$C.\ D. = S.\ Ed \times t$$

Where, M_e = error mean square

r = number of replications

t = table 't' value for error degree of freedom at 0.0.1 and 0.05 levels of probability

Skewness

Skewness is a distortion or asymmetry in a set of data that deviates from the symmetrical bell curve, or normal distribution. The curve is said to be skewed if it is displaced to the left or right. Skewness can be expressed as a measure of how far a given distribution deviates from a normal distribution. It is calculated by the formulae:

$$Sk_1 = = \frac{X - Mo}{s} \quad \text{and} \quad Sk_2 = = \frac{3 - Md}{s}$$

Where, Sk_1 and Sk_2 are the Pearson's first and second coefficient of skewness

s = standard deviation of the sample

$\bar{X}$ = mean of the sample

Mo = the modal value of the sample

Md = the median value

Kurtosis

Kurtosis is a statistical measure of how much a distribution's tails diverge from the tails of a normal distribution. In other words, kurtosis determines whether a distribution's tails contain extreme values.

Analysis of variance

The data obtained for all the traits studied in the three experiments were subjected separately to analysis of variance as per Panse and Sukhatme (1954). The structure of ANOVA is given below.

Sources of variation	d. f.	MSS	Expected value of MSS	Cal F.
Replication	(r-1)	M_1	--	
Genotype	(g-1)	M_2	$\sigma^2_e + r\,\sigma^2_g$	M_2/M_3
Error	(r-1) (g-1)	M_3	σ^2_e	
Total	(rg-1)			

Co-efficient of variability

Both genotypic and phenotypic coefficients were computed for each character as per the method suggested by (Burton and Devane 1953)

Genotypic coefficient of variability (GCV) = PCV (%) = $\frac{\sigma^2_g}{\bar{x}} \times 100$

Phenotypic coefficient of variability (PCV) = PCV (%) = $\frac{\sigma^2_p}{\bar{x}} \times 100$

Heritability (h^2)

Heritability in broad sense was computed as the ratio of the total genotypic and phenotypic variance as expressed in percentage as given by (Allard 1960).

$$\text{Broad sense Heritability} = \frac{\sigma^2 g}{\sigma^2 p} \text{x } 100$$

Where,

$\sigma^2 g$ = Genotypic variance

$\sigma^2 p$ = Phenotypic variance

As proposed by (Johnson *et al*. 1955), heritability was categorized as follows: <30% : Low, 30 – 60 % : Moderate, >60% : High

Genetic advance

Genetic advance refers to the expected gain in the next generation by selecting the superior individuals under certain amount of selection pressure. From the heritability results, the genetic advance was calculated by the following formula given by (Allard 1960).

$$GA = k\,\sigma p\,H$$

Where, GA = Genetic advance

k = Selection differential at 5% selection intensity

σp = Phenotypic standard deviation

H= Heritability

Correlation

The strength of the linear relationship between two variables, x and y, is measured by correlation coefficients. A positive relationship is shown by a linear correlation coefficient greater than zero. A negative association is indicated by a value less than zero. Finally, a value of 0 implies that the two variables x and y have no relationship.

$$\text{Correlation} = \frac{\text{cov}\ (X,Y)}{(\text{varX})\ (\text{varY})}$$

Principal Component Analysis

The PCA breaks down a big collection of data into a smaller number of components by looking for groups with very strong inter-correlation in a set of variables, with each component explaining a percentage of the overall variability. The first main component contributes the most to overall population variation, followed by subsequent components. And in this study, the principal components with eigen values of more than 1 were considered for further analysis and deductions.

Genome wide association mapping

The BAAP population, that was earlier genotyped was phenotyped for various traits under three different nutrient deficient studies. The panel was then analysed through PIQUE (Norton *et al*. 2018) pipeline to facilitate the preprocessing of the genotype and phenotype data and subjected to EMMAX (Efficient Mixed-Model Association eXpedited) analysis on on individual phenotype in parallel. The threshold level of minor allele frequency was set at 0.5, such that SNPs detected below it were filtered out. The mapping of SNP with the trait values of each genotype of the panel was performed following the EMMA model while simultaneously accounting the cryptic kinship with a significance threshold maintained at $p<0.0001$. The false discovery rate (FDR) of the detected associations were estimated and Benjamini-Hochberg (Benjamini and Hochberg, 1995) adjusted value of prabobilities were derived. In order to identify the putative SNP associations, a significance threshold of 10% FDR was set (McCouch *et al*., 2016) and represented in the Manhattan plots. This FDR was then used to identify QTLs. After GWA, SNPs with $-\log_{10}(P)<4$ were examined to group the SNPs into QTLs. SNPs that were within the genome average LD decay value of 243 Kbp were assumed to be of the same locus.

Clump analysis of significant SNPs

The significant SNPs were bin together into peaks based on the LD decay value using PLINK (Purcell *et al*., 2007) adjusted to parameters; "-clump-p1 0.0001 -clump-p2 0.0001 -clump-r2 0.3 -clump-kb 243". SNPs that were closer than 243 kbp were assumed

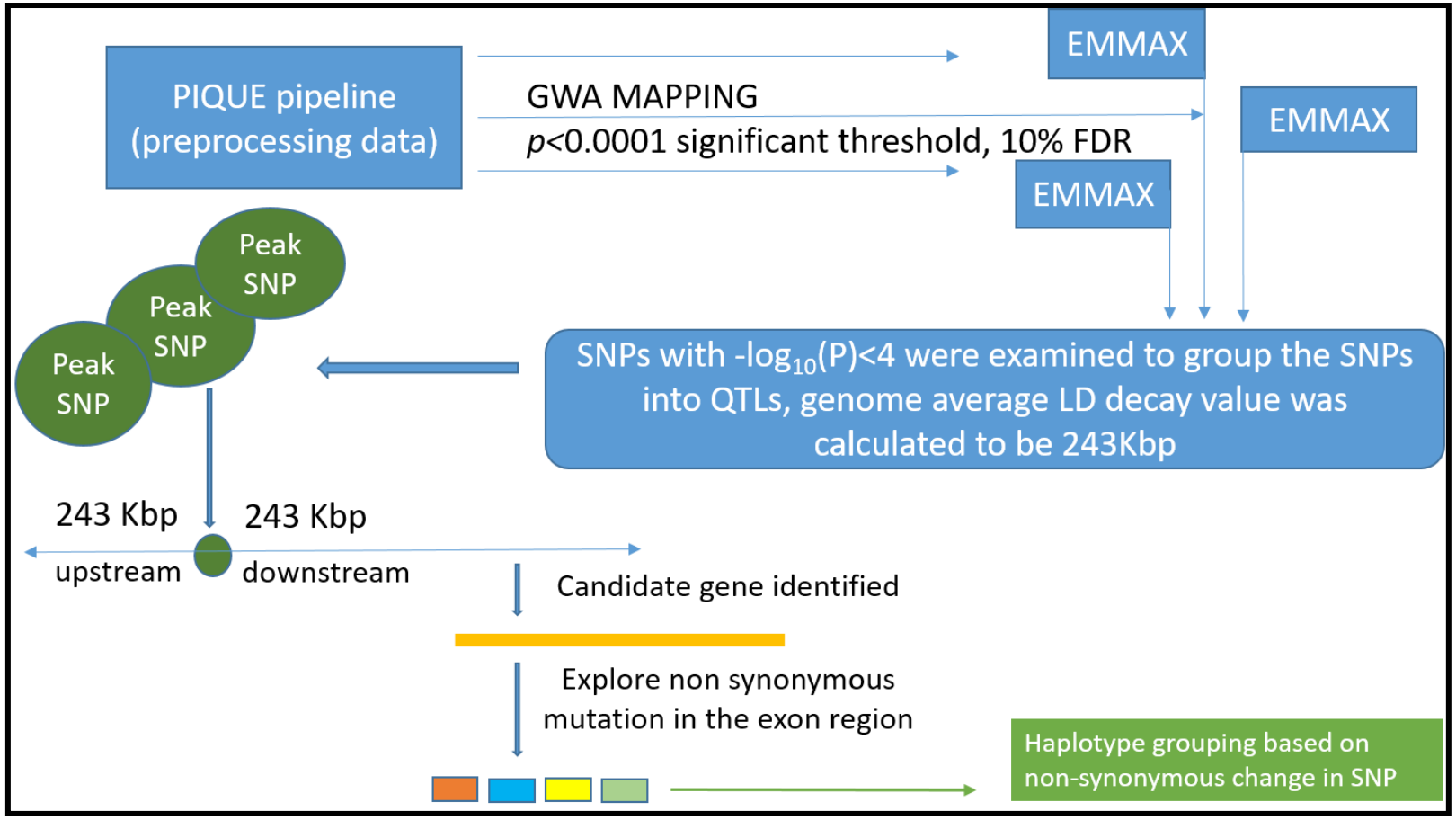


Fig.3.3 Flowchart for highlighting the steps in GWAS and developing haplotypes for a trait

to be part of the same locus. Each trait was treated separately for all the above analyses. Significant SNPs that were singleton were discarded if there was no other SNP in the LD decay window with $p < 0.0001$. The identified clumps of all the traits were put together to examine any similar SNP or overlap of the 243kbp window, any such clumps present were grouped into one. QTLs were reported only when they harboured at least one significant SNP ($p<0.0001$) with a 10% FDR.

Identification of candidate genes and Haplotype analysis

For each significant SNP considered for study, a genomic stretch of 243kbp upstream and downstream positions were calculated. All the genes present in this stretch were annotated using the Rice Genome Annotation Project (RGAP) (http://rice.plantbiology.msu.edu/), release 7. The genes annotated as transposons/ retrotransposons/ hypothetical proteins were excluded from further processes. The RGAP website as well as any other reported works about the genes in the LD region were extensively searched to understand the gene fucntion and ontology. Additionally, the level of expression (in the concerned plant tissue for respective trait) for each of these genes was confirmed from the Rice Expression Profile Database (*RiceXpro*) database, a repository of expression profiles of different genes, a derivative of microarray analysis of tissue specific report. LD heat map representing the peak SNP, putative candidate gene and the LD blocks were prepared using the "LDheatmap", a R package that uses squared Pearson's correlation coefficient (r^2). The SNPs significantly associated with a trait of interest was extracted using PLINK. The non-synonymous and synonymous SNPs within the exonic regions of the candidate genes were identifies on the Nipponbare reference genome from the *snp-seek.irri.org* database. The non-ssynonymous SNPs in the exon regions were used to group the population into haplotypes and the phenotypic mean performance of each haplotype was determined. A precise schematic representation of the GWA mapping and identification of a putative candidate gene have been presented in Fig.3.

RESULTS

The results from the study undertaken to identify the nutrient use efficiency affecting loci for nitrogen, phosphorus and iron are furnished in the following sub-heads.

4.1 Genetic variability among rice genotypes under low levels of nitrogen, phosphorus and iron

Three separate experiments were designed to study the effects of the deficiency of N, P and Fe individually on the morphological manifestation of the different accessions in the Bengal and Assam Aus Panel (BAAP). The N deficient trial was undertaken in the field condition while the P and Fe deficient systems were managed under controlled hydroponic system separately. The following section will describe the results pertaining to the diversity found in the panel, their correlation and principal component analysis (PCA).

4.1.1 Genetic variability among rice genotypes under low levels of nitrogen

In the nitrogen deficient trial, the analysis of variance was carried out for all the traits studied; Chlorophyll Index (SPAD), days to 50% flowering, plant height (cm), flag leaf length (cm), flag leaf width (cm), panicle length (cm), number of tillers, number of productive tillers and grain yield per plant (g) at 5 percent level of significance for 202 accessions in the panel under study. All the traits studied displayed significant differences among the genotypes and there was no significant difference among the replications for these genotypes. The ANOVA for the above mentioned traits in the nitrogen deficient trial is presented in Table 4.1.

Table.4.1 ANOVA of BAAP lines for yield and yield attributing traits under nitrogen deficient trial

	Mean Sum of Squares		
	Genotype (d.f - 202)	**Replication (d.f - 3)**	**Error**
Chlorophyll Index (SPAD)	51.14**	30.08	10.2
Days to 50% flowering	37.41**	14.23	4.06
Plant height (cm)	843.41**	432.28	96.28
Flag Leaf length (cm)	62.09**	10.46	8.81
Flag Leaf width (cm)	0.64**	0.19	0.1
Panicle length (cm)	17.03**	9.56	3.46
Number of tillers	32.38**	22.9	7.9
Number of Productive tillers	15.96**	7.63	4.28
Grain yield per plant (g)	32.33**	20.4	10.31
*, ** Significant at 0.05 and 0.01 levels, respectively			

The mean data of the traits studied in the nitrogen deficient trial is presented in Table 4.2 and the estimates of different variability parameters of the panel under nitrogen deficient condition is presented in Table 4.3 and described below.

4.1.1.1 Chlorophyll Index

The chlorophyll Index was measured using SPAD. The SPAD values ranged from 20.33 in the accession Kele Bari to 42.01 in AUS453. The mean value for chlorophyll index in the panel was 30.95 with a standard deviation of 4.66. The PCV (14.7) was higher than GCV (10.46) with moderate heritability (0.51). The data here shows a skewness of 0.17 and kurtosis of 0.34.

4.1.1.2 Days to 50% flowering

The days to 50% flowering was in the range from 80 (Assam4) to 96.33 (Raj mundo) days. The mean was 87days, the CV (4.97%) was low compared to other traits. The PCV (4.05) was higher than the GCV (3.32) and the heritability was 0.67. The data distribution here shows a skewness of 0.36 and kurtosis of 0.4

4.1.1.3 Plant height (cm)

A considerable amount of variation was seen, as plant height ranged from 81.29cm (Zhenshan 97 B) to 165.02cm (AUS321). The mean plant height was 118.95cm with a standard deviation of 12.23. The PCV (14.14) was higher than the GCV (11.48) and the heritability was 0.67 with a genetic advance value of 19.23. The data distribution here shows almost symmetrical skewness of -0.4.

4.1.1.4 Flag leaf length (cm)

Flag leaf length as a trait displayed variation among the genotypes with the lowest at 22.97cm (Zhenshan 97B) and the highest was 43.87cm (AUS362) with a mean of 31.16cm. The heritability (0.37) was low with a higher PCV value of 17.47 and lower GCV value of 10.56. The data distribution here shows a positive skewness of 0.51.

4.1.1.5 Flag leaf width (cm)

There was a wide variation among the genotypes for flag leaf width ranging from 0.80cm in (AUS paddy white) to 2.98cm (Chhola boro 2). The mean flag leaf width was 1.43cm with a standard deviation of 0.25. There was moderate heritability with 0.47 and genetic advance of 15.58. The data distribution here shows a positive skewness of 0.57.

Table.4.2 Mean data of BAAP lines for different yield and yield attributing traits under nitrogen deficient trial

Sl.	BAAP ID	CULTIVAR NAME	Chlorophyll Index	Days to 50% flowering	Plant height (cm)	Flag Leaf length (cm)	Flag Leaf width (cm)	Panicle length (cm)	Number of tillers	Number of productive tillers	Grain yield per plant (g)
1	1	ASSAM 4(BORO)	33.20	80.00	125.15	30.74	1.30	23.71	14.52	9.48	13.50
2	2	ARC 5959	24.47	87.00	142.96	41.20	1.42	28.32	12.93	9.38	12.79
3	3	ARC 5960	27.23	88.38	139.06	43.80	1.43	27.94	11.97	9.74	14.08
4	4	ARC 5977	33.13	85.13	115.07	28.06	1.29	24.30	12.76	9.67	12.65
5	5	ARC 6000	27.69	83.88	128.93	33.00	1.31	25.81	14.37	10.63	12.80
6	6	ARC 6240	33.83	87.17	119.60	34.87	1.16	25.65	11.50	6.56	11.33
7	7	ARC 7325	40.85	84.81	114.68	29.05	1.28	21.79	14.17	9.99	13.53
8	9	AS 2	32.79	82.19	120.55	27.28	1.24	25.26	16.11	11.05	14.19
9	13	ARC 11600	33.80	89.69	135.17	32.27	1.35	25.13	13.33	11.54	20.36
10	14	AUS MURALI	31.49	83.88	109.83	30.88	1.22	19.90	17.41	9.73	10.75
11	15	BOWALIA	29.95	86.38	123.02	28.91	1.25	24.06	16.01	7.38	18.95
12	16	BOWALIA 2	31.18	90.00	140.92	31.59	1.50	25.57	9.90	9.48	13.40
13	18	AUS 16	33.59	86.99	140.64	29.52	1.70	23.60	11.20	8.59	11.44
14	21	AUS 29	34.39	92.13	109.84	24.97	1.36	25.24	8.60	8.59	11.44
15	23	AUS 37	29.88	87.25	132.19	36.39	1.62	27.91	8.59	4.98	9.81
16	24	AUS 46	32.52	89.06	98.60	26.93	1.41	25.63	10.25	4.92	9.47
17	25	AUS 60	30.45	84.75	111.18	30.22	1.47	24.47	10.36	6.72	11.42
18	27	AUS 63	34.22	83.19	118.97	26.92	1.53	23.78	9.08	4.33	7.69
19	28	AUS 67	28.80	88.25	106.21	30.48	1.56	24.62	14.74	7.68	11.45
20	29	AUS 68	28.80	88.19	111.01	29.71	1.51	25.76	8.60	8.59	11.44
21	30	AUS 74	30.95	84.25	111.38	30.13	1.38	22.97	13.45	7.08	12.11
22	31	AUS 77	26.18	88.00	105.45	32.04	1.50	25.29	15.08	7.64	10.54
23	33	AUS 93	31.27	88.50	126.24	33.00	1.64	26.29	9.11	8.59	11.44
24	34	AUS 96	28.58	86.99	146.85	34.22	1.86	25.62	13.30	6.23	8.18
25	35	AUS 99	34.24	86.99	150.10	34.32	1.90	31.10	7.40	8.59	11.44
26	38	AUS 125	32.52	85.06	113.97	25.05	1.30	22.66	13.14	8.21	6.55
27	41	AUS 131	32.46	81.06	114.33	28.31	1.34	22.33	14.30	11.46	10.03
28	42	AUS 136	27.95	86.06	97.77	28.26	1.52	26.00	9.56	7.03	11.33
29	43	AUS 151	37.16	83.88	118.88	29.94	1.41	25.13	12.91	8.79	10.20
30	45	AUS 154	24.20	80.69	118.59	33.40	1.05	25.98	13.55	9.30	10.58
31	46	AUS 169	27.20	87.56	111.54	29.14	1.38	24.18	11.83	8.33	13.55
32	47	AUS 175	28.58	84.13	110.29	32.46	1.44	23.45	9.71	6.78	9.29
33	50	AUS 209	31.97	95.00	126.42	31.80	2.02	26.14	15.20	8.06	13.99
34	51	AUS 210	30.28	86.99	118.95	31.16	1.42	24.08	13.05	8.59	11.44

35	53	AUS 267	34.75	80.94	95.02	23.70	1.42	18.37	10.90	8.82	11.43
36	54	AUS 268	29.76	88.63	109.13	25.95	1.44	20.27	9.73	8.59	11.44
37	55	AUS 273	30.65	82.81	111.99	31.74	1.55	23.79	11.33	8.78	7.18
38	56	AUS 277	31.34	81.38	108.45	26.93	1.47	25.09	7.80	5.96	9.32
39	57	AUS 280	26.81	86.99	117.28	30.14	1.52	25.20	14.80	9.33	10.33
40	58	AUS 283	31.79	81.38	111.14	32.25	1.53	29.51	7.21	6.34	10.00
41	61	AUS 298	30.53	85.50	115.43	32.02	1.21	24.62	13.54	8.11	7.76
42	63	AUS 314	32.71	88.00	126.35	36.51	1.58	27.20	11.78	7.43	7.89
43	64	AUS 317	30.50	80.69	116.23	28.52	1.24	23.82	11.14	8.07	8.90
44	65	AUS 321	30.70	86.99	165.02	33.10	1.58	24.92	15.20	9.58	11.59
45	69	AUS 350	30.84	85.50	112.84	26.81	1.44	22.33	12.08	8.50	9.33
46	70	AUS 353	31.81	83.25	114.36	32.14	1.47	25.69	10.93	7.15	9.41
47	71	AUS 354	34.02	83.25	102.62	30.39	1.21	22.65	14.80	10.52	10.16
48	73	AUS 362	34.35	85.06	101.03	43.87	1.43	22.49	11.60	7.75	7.82
49	75	AUS 366	37.03	83.56	107.75	27.66	1.44	22.77	12.68	9.30	11.51
50	76	AUS 369	33.66	85.31	104.22	25.82	1.44	21.46	11.63	7.42	11.50
51	77	AUS 382	34.39	88.25	110.01	29.39	1.63	24.49	8.80	5.32	11.75
52	78	AUS 385	31.83	90.69	109.11	27.53	1.59	24.15	12.73	7.86	7.05
53	79	AUS 391	27.21	86.99	101.72	36.42	2.16	28.22	14.60	9.90	13.36
54	80	AUS 401	32.73	86.99	147.96	40.72	1.56	25.72	10.80	7.06	9.66
55	81	AUS 411	30.53	88.94	108.58	25.93	1.57	23.88	17.17	8.74	11.97
56	82	AUS 414	32.32	89.50	108.48	24.66	1.61	22.69	12.90	6.80	10.05
57	83	AUS 415	33.21	89.44	135.11	30.06	1.64	26.42	12.71	6.50	9.13
58	84	AUS 417	32.15	88.00	131.12	27.02	1.56	27.05	13.96	9.48	9.30
59	86	AUS 435	29.43	89.50	122.58	30.19	1.30	24.76	14.50	7.78	11.35
60	87	AUS 440	30.95	90.38	113.77	32.88	1.53	25.62	7.06	4.90	8.07
61	88	AUS 453	42.01	80.69	108.03	28.86	1.58	22.04	9.28	8.59	11.44
62	89	AUS 455	32.39	87.31	111.05	31.55	1.52	24.93	13.61	9.11	10.13
63	90	AUS 462	31.77	86.88	102.70	26.63	1.52	23.97	12.74	7.08	8.97
64	91	AUS 464	32.00	86.88	107.04	29.30	1.52	23.69	13.36	7.26	15.34
65	92	BORO 354	34.56	85.50	136.50	35.94	1.39	23.71	8.64	3.61	6.52
66	94	BORO	31.33	83.88	112.82	37.79	1.25	23.19	14.53	8.59	11.44
67	95	BORO BLACK	29.88	81.69	123.15	31.93	1.35	23.03	18.93	11.61	9.38
68	97	AUSMERI	31.54	85.06	128.94	33.40	1.39	24.06	13.35	5.33	12.91
69	99	ARC 14855	33.99	87.19	125.59	34.79	1.32	25.04	12.80	7.43	15.23
70	100	ARC 14915	28.30	81.38	106.99	26.62	1.55	23.07	8.19	8.59	11.44
71	101	ARC 14950	30.36	88.19	121.76	35.95	1.32	25.25	15.13	9.97	14.82
72	102	ARC 14965	29.38	87.94	105.99	27.21	1.13	22.92	10.96	7.76	10.04

73	103	ARC 14969	27.35	88.25	109.77	26.92	1.13	21.93	11.53	8.58	10.93
74	104	ARC 7098	25.84	86.38	119.34	34.13	1.27	23.98	15.92	12.63	13.98
75	105	BROWN GORA S.B. 92	31.41	86.44	124.31	32.51	1.32	24.11	15.61	8.59	11.44
76	106	AUS PADDY(BLACK)	30.40	83.50	115.21	31.50	1.35	23.48	16.46	10.31	7.73
77	107	AUS PADDY(WHITE)	31.85	90.00	99.44	23.72	0.80	17.58	11.40	9.01	17.01
78	111	AUS KUSHI	25.16	83.94	116.15	28.86	1.32	23.96	12.75	9.86	13.00
79	112	MTU18	23.31	86.99	121.64	32.92	1.79	23.62	9.30	8.59	11.44
80	113	SLO 19	26.92	88.38	116.24	31.71	1.50	21.28	13.99	11.46	11.88
81	114	EARLY SUTARSAR 39	23.89	85.31	112.37	35.35	1.42	23.16	14.40	9.63	11.37
82	116	DA 12	27.83	89.31	134.44	30.43	1.03	22.42	20.65	7.31	12.46
83	121	KHAILAORGOABEZ	32.01	92.00	153.30	34.18	1.74	26.58	9.40	7.64	8.80
84	122	KELE(AUS)	23.01	87.25	106.54	27.57	1.33	20.37	17.29	11.95	16.14
85	123	CHAMKA	26.24	87.17	132.83	33.67	1.28	24.23	15.28	11.17	15.23
86	124	KALOSHAITA	21.64	85.69	112.73	29.84	1.38	22.04	15.28	8.21	9.31
87	126	MUNSHISHAIL	41.83	85.13	139.62	38.23	1.58	26.23	12.43	8.59	11.44
88	127	SHONI	33.11	86.13	99.66	26.85	1.09	19.21	14.28	9.42	15.56
89	128	PURA NUKNA	25.89	82.31	122.86	33.82	1.40	22.84	13.45	8.07	15.72
90	129	CUNAIL	29.00	87.94	105.43	30.94	1.60	25.55	12.43	8.65	12.28
91	130	FAISHA MANSA	31.75	90.00	112.68	32.93	1.61	21.37	11.67	6.81	13.16
92	131	KANCHA NONI	26.84	86.99	118.95	31.16	1.42	24.08	13.05	8.59	11.44
93	133	DHALIBORO 105-2	35.97	85.44	144.26	37.15	1.41	24.34	12.16	6.07	9.64
94	136	KALIBORO 26	28.01	85.06	133.24	32.64	1.40	23.11	10.89	5.76	11.46
95	137	KALIBORO 41-1	34.66	87.81	134.84	33.87	1.41	23.61	12.63	8.59	11.44
96	141	KAL BURI	28.31	86.00	136.94	32.00	1.19	23.18	13.63	8.00	2.00
97	142	SHADA BORO	32.15	88.50	106.74	24.27	0.94	19.67	21.20	9.33	10.16
98	143	JAGAL-1640 A	30.95	86.75	136.14	31.86	1.09	25.12	16.23	8.59	11.44
99	149	JATI AUS	29.53	86.75	127.82	35.21	1.30	21.47	14.75	7.26	15.06
100	150	KALINDI	27.21	84.13	103.40	29.50	1.33	19.98	15.63	9.42	12.50
101	151	KALO BIRA	29.62	87.69	123.61	31.23	1.36	21.88	12.70	7.65	9.29
102	152	KELE BARI	20.33	86.99	135.52	31.84	1.64	26.80	11.40	11.68	14.26
103	155	M 136-20	28.33	89.33	129.83	35.87	1.47	25.22	16.88	12.03	16.05
104	157	PADMA SAIL	33.07	86.13	126.47	41.35	1.92	24.52	12.30	7.22	11.36
105	159	SHETE BHADO	29.27	83.31	121.64	32.50	1.72	22.51	12.58	9.43	12.37
106	160	SOA MUKHI	28.30	93.00	110.38	26.92	1.48	22.02	9.20	8.85	11.88
107	165	DESHI BORO	27.16	87.63	125.65	29.71	1.16	23.68	13.25	6.79	8.22
108	167	SORISHAFUL	31.74	81.06	102.82	24.86	1.32	21.05	16.34	9.88	11.32
109	168	KADA-176-12	28.58	83.81	101.73	25.88	1.27	21.54	16.16	10.00	13.73
110	170	GOTA LEMMA	30.95	88.00	131.76	33.54	1.69	23.88	14.45	10.34	9.11

111	171	MOTZHUL	30.73	95.00	125.54	30.30	1.61	26.25	15.90	11.83	8.93
112	172	CN2-175-5-31	26.38	83.88	111.07	31.10	1.32	22.28	12.28	10.63	13.46
113	173	DHINGHA	27.45	93.00	146.88	28.00	1.44	23.00	16.80	7.46	11.67
114	174	DA2	27.63	91.06	135.92	30.34	1.13	21.98	20.83	12.62	17.13
115	176	CODE NO BI 93	28.70	90.50	138.94	35.95	1.40	25.48	14.41	9.72	15.18
116	177	KALI AUS	33.26	85.31	138.69	32.01	1.40	23.52	16.23	10.48	15.98
117	178	PARBATJIRA	28.97	84.81	125.19	32.94	1.55	24.27	11.53	8.00	9.76
118	179	SATHA	28.06	80.75	124.61	29.91	1.37	22.46	14.94	6.71	7.62
119	180	DULAAUS	33.84	86.99	112.61	26.07	1.59	27.39	11.08	8.59	11.44
120	181	CHINGER	28.95	84.25	118.28	33.85	1.41	22.33	10.06	8.59	11.44
121	184	SAITA BORO	31.88	84.50	123.26	34.35	1.18	20.88	16.80	7.08	9.37
122	185	KUDA	33.46	86.99	97.52	27.05	1.42	20.08	15.12	11.32	15.76
123	187	KALASU	32.97	84.19	111.06	29.56	1.17	20.53	16.34	15.00	15.23
124	188	JABOR SAIL	30.95	89.38	131.77	36.34	1.73	23.11	12.73	11.04	20.19
125	189	DUBHI GORA	27.05	91.50	126.05	33.27	1.48	23.97	8.27	8.59	11.44
126	190	NAGRI	26.55	86.99	139.08	33.34	1.68	26.96	9.20	8.59	11.44
127	191	DJ29	41.20	86.99	127.84	35.44	1.78	28.08	20.20	10.02	14.72
128	193	LALI BORO	31.30	89.88	140.47	35.21	1.45	24.19	10.50	8.00	11.38
129	195	RAJ MUNDO	25.78	96.33	148.91	28.87	1.55	24.41	15.50	11.53	13.31
130	197	W 418	26.14	85.00	106.21	26.21	1.33	23.74	19.13	12.51	11.24
131	198	WHITE DUBHI	26.84	87.94	122.33	33.38	1.44	22.59	11.09	7.13	9.98
132	199	ARC 7229	31.39	86.99	91.30	31.95	1.27	24.10	7.60	8.65	11.44
133	200	BJ 1	27.87	87.50	114.33	32.28	1.10	24.25	15.38	6.63	10.99
134	201	BLACK GORA	28.29	89.00	111.76	25.78	1.25	24.27	15.68	12.56	8.68
135	203	DV85	31.37	83.56	110.01	28.42	1.28	22.92	13.59	10.31	9.61
136	205	JHONA 349	34.82	83.81	115.87	28.43	1.30	23.86	13.28	8.29	10.56
137	206	KALAMKATI	30.95	90.81	118.97	31.21	1.36	27.57	11.30	6.78	10.85
138	207	KASALATH	33.24	90.00	116.02	32.90	1.18	26.09	8.00	4.28	5.46
139	208	T 1	32.16	87.50	130.04	32.50	1.45	27.04	11.48	11.60	16.59
140	209	T26	35.95	85.81	117.74	29.13	1.54	24.79	14.06	8.51	9.87
141	210	ARC 6578	23.66	86.99	119.09	28.31	1.52	22.17	18.70	9.00	9.39
142	211	CTG 1516	30.86	88.42	103.13	28.54	1.34	24.38	9.99	6.48	6.18
143	213	DJ 123	33.93	84.19	105.59	27.60	1.41	24.62	12.52	6.94	8.53
144	214	DJ 24	27.20	89.25	113.55	30.76	1.38	24.80	12.23	6.93	8.15
145	215	DM 43	31.23	86.33	103.84	25.30	1.17	24.52	9.65	5.79	10.64
146	216	DM 56	31.21	83.75	102.94	31.53	1.46	23.97	15.33	10.92	15.52
147	217	DM 59	32.03	89.56	105.55	31.60	1.44	26.16	10.95	5.52	8.20
148	219	DV 123	24.88	88.25	110.29	32.45	1.57	24.84	13.79	6.15	12.13

149	220	GHORBHAI	34.24	89.81	120.28	38.45	1.53	26.28	15.30	10.11	11.82
150	221	GORIA	27.85	81.31	115.94	33.46	1.43	24.83	14.18	8.44	10.09
151	222	JAMIR	28.40	87.88	117.41	32.86	1.32	23.39	12.60	8.33	13.27
152	225	KARKATI 87	32.32	84.81	114.26	29.21	1.27	23.93	10.36	6.92	8.01
153	226	ARC 10376	28.77	83.81	107.46	28.22	1.36	21.23	11.01	5.47	4.54
154	228	SURJAMKUHI	30.95	88.00	108.65	29.58	1.54	22.66	12.56	9.01	4.75
155	229	PTB 30	28.13	85.69	108.25	28.31	1.52	26.77	18.72	9.30	11.31
156	230	BAWOI	34.55	85.56	121.03	41.43	1.22	25.51	11.71	7.53	16.97
157	233	BOGURA	36.02	86.94	126.93	30.34	1.15	23.63	16.78	9.53	13.22
158	234	LAHAYA	30.58	88.00	132.57	33.19	1.45	25.35	11.80	7.39	9.80
159	235	DESHI BORO	31.34	85.00	104.68	25.64	1.54	20.48	11.60	8.59	12.81
160	236	TUPA	33.44	88.69	141.00	34.17	1.29	24.45	11.83	9.81	12.68
161	237	CHHOLA BORO	34.85	85.38	127.07	30.34	1.22	23.50	19.39	11.75	11.85
162	238	CHHOLA BORO(2)	30.88	88.63	131.34	31.58	2.98	24.37	14.64	9.47	12.42
163	239	LAFAI	32.27	87.38	132.78	31.23	1.25	22.77	14.98	8.95	7.47
164	241	CHAILI BOROI	28.84	88.25	132.23	36.41	1.23	23.74	11.47	7.28	12.96
165	242	SADA BORO G1	32.96	83.50	125.50	35.32	1.21	22.94	13.14	11.04	9.06
166	243	GOBIR SAIL	30.11	86.38	147.79	28.31	1.33	25.92	15.20	10.05	11.37
167	244	DUMSIA 81	28.32	86.75	130.84	30.52	1.21	24.81	17.99	13.94	15.61
168	245	TULSI BORO	31.01	87.31	114.47	32.50	1.23	22.47	12.21	10.25	9.10
169	247	GUL TEPI	33.35	84.50	138.90	36.33	1.31	22.93	13.73	9.54	13.73
170	248	RATA BORO	33.42	87.19	127.36	31.41	1.25	22.70	13.21	10.29	10.07
171	249	CHOUDHURY SAIL	30.89	86.99	123.32	24.38	1.44	21.76	20.80	8.44	11.44
172	250	BACHI BORO	32.66	88.69	126.49	36.88	2.19	24.89	10.55	7.33	12.93
173	253	BR 3	33.80	94.00	86.71	25.67	1.35	22.13	15.54	11.86	12.79
174	254	BR 6	29.15	90.81	99.02	26.34	1.21	24.55	13.28	8.30	12.25
175	255	BR 16	31.39	87.50	97.41	25.31	1.27	25.55	10.79	6.39	10.93
176	256	BRRI DHAN 28	34.37	90.69	102.51	28.81	1.27	23.99	13.73	11.83	16.03
177	257	BRRI DHAN45	28.70	89.13	96.37	26.07	1.39	23.55	14.33	10.97	17.34
178	258	BRRI DHAN47	32.61	94.00	103.68	30.78	1.55	24.12	9.19	6.24	11.19
179	259	BRRI DHAN50	38.21	85.13	120.90	31.14	1.35	23.67	12.91	8.36	11.31
180	260	BINA DHAN 5	36.70	86.00	106.61	27.48	1.51	24.81	11.68	6.83	10.69
181	261	IRATOM 24	28.23	93.00	85.91	28.20	1.39	22.98	16.18	12.26	11.82
182	262	IARI 6626	31.99	88.67	117.15	32.56	1.82	24.80	17.15	12.66	13.99
183	264	PURPLE PUTTU	33.25	89.00	112.25	42.27	1.29	29.59	9.64	5.06	10.35
184	265	P 79	30.41	88.31	118.59	32.55	1.60	22.73	13.53	7.82	12.41
185	266	POLMAN	27.09	88.63	128.10	34.59	1.46	27.93	10.06	5.89	11.87
186	267	DJ 53	37.51	88.00	119.44	35.65	1.64	24.43	12.18	6.69	12.79

187	268	ZIRI	29.37	88.00	117.42	32.21	1.43	25.60	10.73	8.93	17.41
188	270	P 32	30.70	95.00	153.62	27.80	1.56	23.52	13.00	9.50	6.70
189	271	99216	33.76	91.00	124.23	32.83	1.59	27.30	14.88	11.17	13.37
190	272	NP 97	33.65	85.19	120.89	32.76	1.39	25.71	9.61	5.28	12.34
191	273	DV 118	23.44	87.69	120.63	36.74	1.80	25.51	11.29	5.83	10.80
192	274	PACHODI 427	27.70	85.33	148.92	33.37	1.53	24.39	12.80	7.69	7.74
193	275	BOWALIA	37.36	94.00	102.75	27.15	1.43	20.66	13.40	10.29	18.40
194	276	ARC 10303	34.48	87.38	129.07	30.05	1.14	25.43	13.14	9.37	10.07
195	278	DA 24	33.68	88.00	125.22	35.15	1.83	24.59	16.46	10.66	16.43
196	280	FULBADAM	34.45	84.56	111.28	29.93	1.27	25.40	10.69	7.48	9.53
197	285	IR 64-21	36.62	93.50	86.74	25.61	1.40	24.15	10.83	7.36	11.24
198	286	LI-JIANG-XIN-TUAN-HEIGU	36.27	87.25	105.42	30.43	1.25	21.69	11.16	3.61	5.76
199	288	MINGHUI 63	28.01	90.50	122.68	35.51	1.49	24.48	12.55	6.85	10.57
200	292	POKKALI	30.95	91.67	150.72	38.30	1.68	29.05	7.20	4.83	10.20
201	293	SADU CHO	38.64	90.75	123.86	27.48	1.14	22.57	12.41	7.50	13.50
202	294	SANHUANGZHAN NO2	38.55	86.00	89.53	28.46	1.34	23.56	13.64	10.34	8.12
203	297	ZHENSHAN 97 B	27.61	88.56	81.29	22.97	1.30	20.54	15.42	10.42	9.20

Table 4.3 Estimates of variability of BAAP lines for yield and yield attributing traits under nitrogen deficient trial

Traits	RANGE	MEAN	SD	CD (%)	CV (%)	GCV	PCV	h^2_{bs}	GA	SKEWNESS	KURTOSIS
Chlorophyll Index	20.33 – 42.01	30.95	4.6	4.44	14.97	10.46	14.7	0.51	15.34	0.17	0.34
Days to 50% flowering	80.00 – 96.33	87.00	3.53	2.79	4.05	3.32	4.05	0.67	5.61	0.36	0.4
Plant height (cm)	81.29 – 165.02	118.95	12.23	13.62	10.31	11.48	14.14	0.66	19.23	-0.04	0.71
Flag Leaf length (cm)	22.97 - 43.87	31.16	5.55	6.05	17.85	10.56	17.47	0.37	13.14	0.51	0.42
Flag Leaf width (cm)	0.80 - 2.98	1.43	0.25	18	17.46	11.07	16.19	0.47	15.58	0.57	1.94
Panicle length (cm)	17.58 - 31.10	24.08	2.68	2.58	11.13	7.65	10.88	0.49	11.08	0.72	1.05
Number of tillers	7.06 - 21.20	13.05	2.89	3.9	19.28	18.96	20.7	0.44	25.81	0.39	0.2
Number of Productive tillers	3.61-15.00	8.59	1.82	2.87	18.28	19.88	21.23	0.41	26.05	0.28	-0.33
Grain yield per plant (g)	2.00 - 20.36	11.45	2.67	4.46	17.98	20.49	34.78	0.35	24.9	0.39	0.03

4.1.1.6 Panicle length (cm)

Panicle length ranged from 17.58cm (AUS paddy white) to 31.10cm (AUS99). The mean was 24.08cm with a cv of 11.13%. The heritability (0.49) was moderate. The distribution of data for this trait in the panel is positively skewed (0.72).

4.1.1.7 Number of tillers

Number of tillers for the panel ranged from a minimum of 7.06 (AUS440) to a maximum of 21.20 (Shada boro). The mean number of tillers per plant was 13.05 with a CV of 19.28%. The heritability was 0.44 and the genetic advance was 25.81. The data distribution here shows symmetrical distribution with a skewness of 0.39.

4.1.1.8 Number of productive tillers

Number of productive tillers ranged from 3.61 in LI-JIANG-XIN-TUAN-HEI-GU to 15 in KALASU. The mean number of productive tillers per plant was 8.59 and the CV was 18.28%. The heritability was moderate with 0.41. The data distribution here shows an almost symmetrical skewness of 0.28.

4.1.1.9 Grain yield per plant (g)

Grain yield per plant showed a wide range of values with the lowest being 2gms (AUS paddy white) to as high as 20.36gms (ARD11600). The mean grain yield per plant in the panel was 11.45gms with a CV of 17.98%. The heritability (0.35) was low and genetic advance of 24.90. The data distribution here shows an almost symmetrical skewness of 0.39.

Correlation analysis and Principal component analysis

The Pearson's correlation of the above discussed traits under the nitrogen deficient condition highlighted the significant positive correlation of grain yield per plant with flag leaf width (0.43), panicle length (0.30) and number of productive tillers (0.37). There was also a significant positive correlation of Days to 50% flowering with number of productive tillers (0.22) and grain yield per plant (0.15). Flag leaf width had a significant negative correlation (-0.21) with number of tillers. The results of the correlation analysis for the BAAP lines under nitrogen deficient trial are presented in Table 4.4.

The PCA results further highlighted the amount variation contributed by each trait and the nature of relationship the traits studied (Table 4.5). Only three PCs had eigen values of more than one and thus three main axes were considered for further deductions. PC1 explained 28.5% of the variation while PC2 explained 20.3% of the

variation between the genotypes in the panel and individual traits. Panicle length (25.62) contributed to the highest variation followed by flag lead width (22.03) in PC1. Number of productive tillers (31.93) and chlorophyll Index (38.46) contributed to the highest variation in PC2 and PC3 respectively. The PCA biplot for yield and yield attributing traits under nitrogen deficient trial is presented in Fig.4.1.

Table 4.4 Estimates of correlation among yield and yield attributing traits under nitrogen deficient trial

	Chlorophyll Index	Days to 50% flowering	Plant height	Flag Leaf length	Flag Leaf width	Panicle length	Number of tillers	Number of productive tillers
Days to 50% flowering	0.125	1						
Plant height	0.012	-0.042	1					
Flag Leaf length	0.029	-0.044	0.558**	1				
Flag Leaf width	0.071	-0.013	0.053	0.119	1			
Panicle length	0.016	-0.073	0.149*	0.161*	0.585**	1		
Number of tillers	0.020	0.074	0.116	-0.077	-0.211**	-0.039	1	
Number of productive tillers	0.087	0.222**	-0.007	-0.043	-0.136	0.113	0.247**	1
Grain yield per plant	0.030	0.153*	0.057	0.082	0.436**	0.307**	0.079	0.377**

Table 4.5 Estimates of eigen values and contribution of yield and yield attributing traits towards the major principal components under nitrogen deficient trial

Traits	PC1	PC2	PC3
Eigen values	2.27	1.77	1.14
Chlorophyll Index	0.47	4.51	38.46
Days to 50% flowering	0.56	2.26	31.24
Plant height (cm)	16.85	8.51	8.72
Flag Leaf length (cm)	16.56	5.36	1.16
Flag Leaf width (cm)	22.03	0.61	10.75
Panicle length (cm)	25.62	0.14	0.003
Number of tillers	5.91	30.31	7.57
Number of Productive tillers	2.4	31.93	0.07
Grain yield per plant (g)	0.12	16.33	1.99

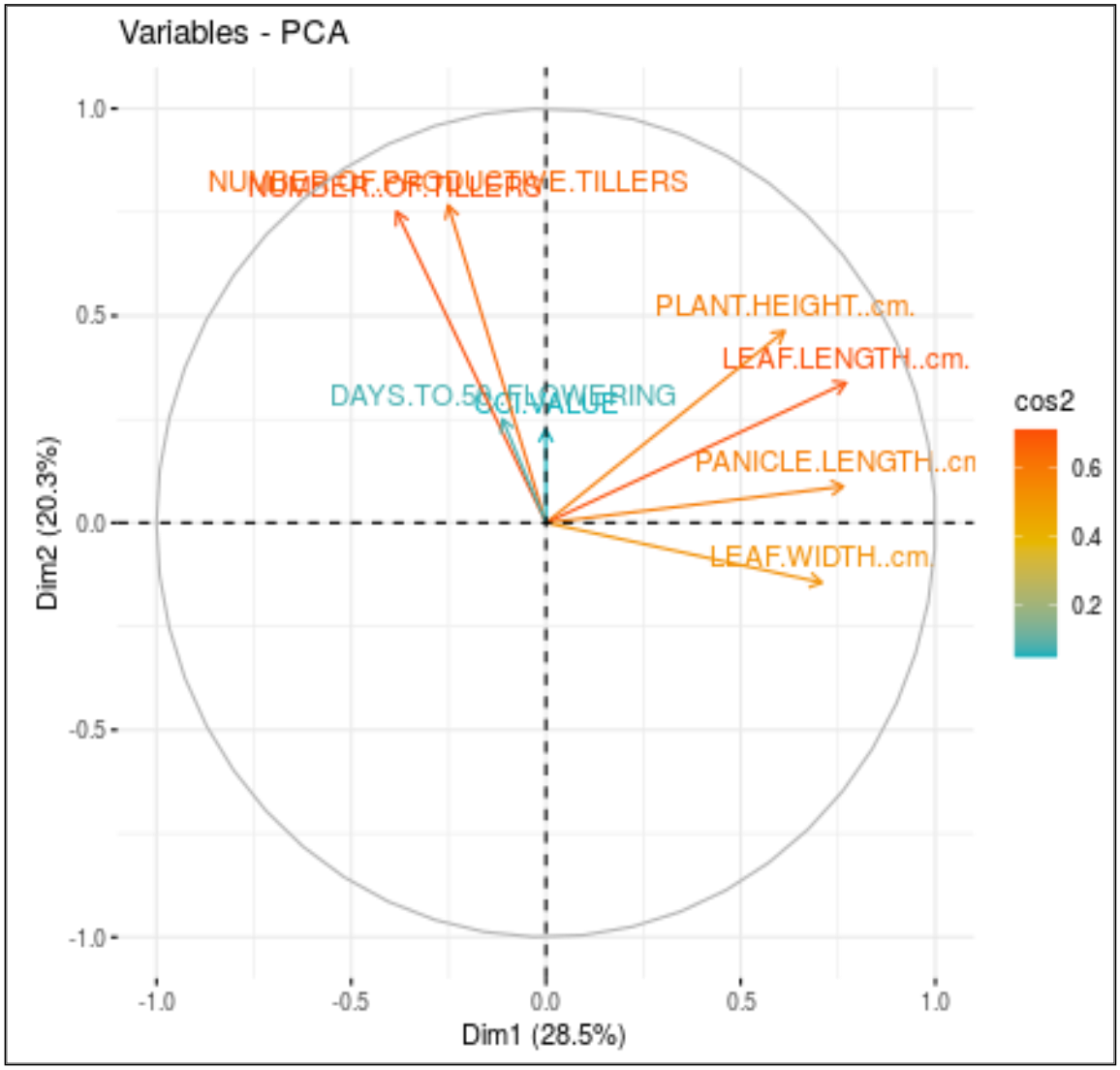


Fig.4.1 PCA biplot for yield and yield attributing traits under nitrogen deficient trial

4.1.2 Genetic variability among rice genotypes under low levels of phosphorus

The phosphorus deficient experiment was undertaken in a controlled condition of nutrient source and pH for a duration of 28 days and the following observations were taken: chlorophyll index, shoot length (cm), root length (cm), number of leaves, number of crown roots, shoot dry weight, root dry weight and shoot dry weight: root dry weight ratio for a total of 204 accessions in the BAAP population.

The analysis of variance for the vegetative stage characters studied under phosphorus deficient condition is presented in Table 4.6. All the traits studied were significantly different from each other while there was no significant difference between the replications. The mean data of the traits studied in the P deficient trial is presented in Table 4.7 and the estimates of different variability parameters of the panel under P deficient condition is presented in Table 4.8 and described below.

Table 4.6 ANOVA of BAAP lines for the vegetative stage traits studied under phosphorus deficient trial

	Mean Sum of Squares		
	Treatment (d.f - 203)	**Replication (d.f - 1)**	**Error**
Chlorophyll Index	21.20**	0.19	0.13
Shoot length (cm)	153.81**	15.92	6.39
Root length (cm)	32.91**	4.42	9.99
Number of leaves	0.51**	0.02	0.04
Number of crown roots	15.92**	4.63	3.9
Shoot dry weight (g)	0.0095**	0.00033	0.00014
Root dry weight (g)	0.00023**	0.000016	0.00008
SDW:RDW ratio	31.49**	0.99	17.36
*, ** Significant at 0.05 and 0.01 levels, respectively			

Table 4.7 Mean data of BAAP lines for vegetative stage traits under phosphorus deficient trial

BAAP ID	Cultivar Name	Chlorophyll Index	Shoot length (cm)	Root length (cm)	Number of leaves	Number of roots	Shoot dry weight (g)	Root dry weight (g)
1	ASSAM 4(BORO)	28.90	29.60	12.40	4.00	13.00	0.0320	0.0140
2	ARC 5959	23.91	27.95	9.25	4.50	14.67	0.0325	0.0161
3	ARC 5960	25.77	24.40	8.57	4.33	14.00	0.0460	0.0145
4	ARC 5977	37.06	54.28	12.08	5.17	16.17	0.0840	0.0086
5	ARC 6000	22.13	53.20	15.85	4.83	11.67	0.0596	0.0044
6	ARC 6240	28.41	42.90	18.28	5.17	17.50	0.0711	0.0267
7	ARC 7325	25.53	52.47	17.12	4.83	13.33	0.0716	0.0145
8	ARC 10958	23.00	47.50	17.95	5.50	17.00	0.0730	0.0100
9	AS 2	27.29	54.30	12.77	5.17	17.33	0.0871	0.0084
13	ARC 11600	29.40	30.70	6.93	5.00	12.83	0.0358	0.0105
14	AUS MURALI	26.44	39.78	17.43	5.00	16.00	0.0628	0.0256
15	BOWALIA	27.59	40.67	20.98	5.00	18.00	0.0683	0.0272
15	BOWALIA	28.20	47.23	18.63	5.33	14.50	0.0913	0.0114
16	BOWALIA 2	30.46	56.98	10.47	4.83	16.33	0.0800	0.0070
19	AUS 22	25.34	33.55	7.60	4.33	17.33	0.0371	0.0051
21	AUS 29	28.91	29.48	6.92	4.83	14.00	0.0527	0.0127
23	AUS 37	25.07	36.00	11.82	5.00	12.83	0.0707	0.0120
24	AUS 46	30.66	41.68	15.47	4.83	15.83	0.0705	0.0298
25	AUS 60	28.20	42.20	13.82	3.50	13.67	0.0509	0.0260
30	AUS 74	28.11	49.62	17.97	5.00	11.17	0.0957	0.0321
31	AUS 77	23.59	36.18	11.95	4.33	13.83	0.0499	0.0215
33	AUS 93	20.56	43.38	35.13	4.83	14.83	0.0590	0.0069
38	AUS 125	30.74	34.83	9.08	4.50	14.58	0.0428	0.0101
39	AUS 127	29.29	36.53	8.62	4.83	16.50	0.0541	0.0075
41	AUS 131	28.59	33.82	7.97	4.17	14.83	0.0578	0.0162
43	AUS 151	30.91	38.45	11.35	5.00	14.50	0.0600	0.0250
45	AUS 154	25.09	45.55	18.12	5.00	15.83	0.0750	0.0281

46	AUS 169	26.21	34.98	11.27	4.00	12.17	0.0772	0.0256
47	AUS 175	29.36	36.47	6.62	4.67	14.83	0.0557	0.0195
48	AUS 180	32.30	44.60	11.50	5.00	17.00	0.0660	0.0250
49	AUS 204	25.24	48.43	10.78	5.00	16.17	0.0608	0.0078
51	AUS 210	28.00	37.00	12.70	5.00	10.00	0.0670	0.0330
53	AUS 267	29.20	31.22	9.70	4.50	9.33	0.0491	0.0128
54	AUS 268	28.34	32.25	8.58	4.00	14.83	0.0840	0.0330
55	AUS 273	27.04	28.67	7.35	4.17	9.33	0.0577	0.0128
60	AUS 294	29.34	48.60	12.62	5.00	17.83	0.0951	0.0401
61	AUS 298	25.59	41.80	18.43	4.50	14.83	0.0633	0.0262
63	AUS 314	27.89	30.63	6.00	4.17	12.33	0.0514	0.0216
64	AUS 317	33.83	44.83	12.07	4.33	14.50	0.0807	0.0105
65	AUS 321	29.00	49.60	12.42	4.83	19.00	0.0769	0.0089
68	AUS 335	27.81	41.93	15.47	5.00	16.67	0.1037	0.0468
69	AUS 350	26.51	29.42	7.32	4.00	13.67	0.0390	0.0183
70	AUS 353	29.44	32.63	6.15	4.00	13.33	0.0489	0.0141
71	AUS 354	29.96	34.35	7.00	4.00	12.67	0.0394	0.0123
72	AUS 361	24.11	45.58	16.20	4.83	15.33	0.0760	0.0280
73	AUS 362	25.49	36.18	7.97	4.00	11.67	0.0542	0.0138
75	AUS 366	36.14	40.10	12.58	4.00	11.17	0.0723	0.0292
76	AUS 369	25.81	31.90	6.00	4.00	10.50	0.0391	0.0115
77	AUS 382	26.31	31.83	5.00	5.00	11.67	0.0445	0.0122
78	AUS 385	25.37	29.30	5.83	3.50	13.33	0.0368	0.0080
81	AUS 411	23.71	26.53	6.50	3.83	16.17	0.0264	0.0098
82	AUS 414	19.40	43.10	8.80	4.67	14.00	0.0367	0.0191
83	AUS 415	23.29	35.98	13.30	4.17	12.33	0.0566	0.0231
84	AUS 417	24.57	36.43	14.85	4.00	13.33	0.0457	0.0194
86	AUS 435	27.54	43.97	16.75	4.50	14.17	0.0672	0.0259
87	AUS 440	27.47	41.25	10.78	4.67	13.83	0.0594	0.0106

88	AUS 453	27.22	34.95	10.20	5.00	13.00	0.0213	0.0175
89	AUS 455	25.63	49.45	16.33	5.00	17.17	0.0812	0.0327
90	AUS 462	23.51	41.97	9.48	5.00	14.70	0.0273	0.0060
91	AUS 464	28.99	43.08	16.62	4.33	12.83	0.0750	0.0320
92	BORO 354	27.49	41.68	13.83	4.83	18.00	0.0711	0.0282
94	BORO	28.01	45.20	10.73	4.83	17.50	0.0817	0.0332
95	Boro Black	28.79	46.37	18.65	5.17	15.17	0.0698	0.0271
97	AUS Meri	27.46	40.40	11.85	5.00	14.00	0.0566	0.0234
99	ARC 14855	29.10	28.82	7.75	4.42	11.75	0.0512	0.0205
100	ARC 14915	27.71	39.30	12.90	5.00	14.00	0.0888	0.0292
101	ARC 14950	27.20	31.50	8.10	5.00	16.00	0.0420	0.0190
102	ARC 14965	28.69	32.32	9.77	5.00	18.17	0.0503	0.0202
103	ARC 14969	29.29	48.00	15.33	5.00	19.00	0.1073	0.0469
104	ARC 7098	27.61	37.66	11.51	4.17	12.00	0.0625	0.0143
106	AUS PADDY(BLACK)	26.19	41.42	15.27	4.83	16.67	0.0578	0.0241
107	AUS PADDY(WHITE)	27.40	43.10	14.55	5.00	11.33	0.0544	0.0062
108	ANJANI	23.46	48.35	14.73	4.83	12.50	0.0801	0.0076
111	AUS KUSHI	25.37	42.22	12.97	4.67	14.83	0.0657	0.0281
113	SLO 19	29.70	47.83	14.50	4.67	16.00	0.0945	0.0381
114	EARLY SUTARSAR 39	29.60	40.00	16.53	5.17	14.83	0.0895	0.0340
116	DA 12	21.87	38.18	14.35	4.83	11.17	0.0758	0.0322
117	KADA-68-1	27.50	48.72	12.10	5.00	14.67	0.0848	0.0099
121	KHAILAORGOABEZ	23.04	43.05	16.90	5.17	12.00	0.0544	0.0482
122	KELE(AUS)	22.24	44.38	14.03	5.17	16.83	0.0863	0.0092
123	CHAMKA	32.20	38.50	13.00	6.00	14.80	0.0720	0.0280
124	KALOSHAITA	27.91	44.55	15.42	5.00	17.33	0.0903	0.0115
125	KUMBI	32.06	45.37	11.42	5.00	14.50	0.1011	0.0335
126	MUNSHISHAIL	25.93	42.87	12.58	5.00	12.17	0.0647	0.0070
127	SHONI	22.37	48.03	17.58	5.00	13.83	0.0829	0.0086

128	PURA NUKNA	23.63	47.53	15.57	5.00	14.80	0.0937	0.0320
129	CUNAIL	24.99	28.85	9.55	4.67	14.17	0.0406	0.0042
130	FAISHA MANSA	28.83	36.30	13.35	4.83	18.50	0.0632	0.0298
133	DHALIBORO 105-2	31.60	48.77	13.47	5.00	18.50	0.0598	0.0271
136	KALIBORO 26	24.59	46.63	25.25	3.50	19.00	0.0547	0.0223
137	KALIBORO 41-1	28.09	51.83	13.35	4.67	24.17	0.0791	0.0300
139	KALIBORO 138-2	31.37	46.38	15.87	5.00	18.83	0.0988	0.0393
141	KAL BURI	25.99	49.47	14.58	5.00	18.08	0.0852	0.0384
142	SHADA BORO	28.00	49.73	14.53	5.83	18.00	0.0872	0.0271
143	JAGAL-1640 A	27.73	54.93	16.60	4.83	14.83	0.0900	0.0086
145	CHANDRA MUKHI	26.80	45.55	12.15	5.00	12.67	0.0808	0.0136
147	HIJLI	24.87	49.38	14.15	4.83	13.83	0.0469	0.0045
149	JATI AUS	29.30	45.65	15.28	5.00	18.83	0.0808	0.0279
150	KALINDI	28.96	44.45	12.90	5.00	19.00	0.1001	0.0294
151	KALO BIRA	26.89	46.82	16.93	5.00	15.33	0.0939	0.0241
153	KHASHIA PANJA	28.14	36.08	11.17	5.00	12.33	0.0706	0.0248
155	M 136-20	22.16	46.23	15.52	5.00	13.67	0.0942	0.0367
157	PADMA SAIL	31.14	33.83	12.43	6.00	14.00	0.0770	0.0247
159	SHETE BHADO	27.64	47.87	10.92	4.50	14.17	0.0615	0.0065
160	SOA MUKHI	24.01	49.25	15.12	5.00	16.67	0.0899	0.0108
165	DESHI BORO	23.40	27.30	11.60	4.00	9.00	0.0220	0.0300
167	SORISHAFUL	23.10	40.33	13.17	4.83	14.00	0.0693	0.0111
168	KADA-176-12	27.26	49.78	15.60	4.83	13.33	0.0830	0.0329
171	MOTZHUL	25.50	53.23	14.60	4.67	12.33	0.0812	0.0105
172	CN2-175-5-31	22.54	42.12	17.32	5.00	16.50	0.0804	0.0354
173	DHINGHA	22.09	53.40	14.22	4.83	15.83	0.0743	0.0061
174	DA2	25.90	35.70	9.30	4.00	10.00	0.0420	0.0140
175	GOUERISAIL	26.04	42.77	13.17	4.17	10.00	0.0558	0.0058
176	CODE NO BI 93	24.36	41.53	11.97	5.00	12.83	0.0715	0.0314

177	KALI AUS	28.59	50.53	12.17	4.67	17.00	0.0600	0.0280
178	PARBATJIRA	23.23	51.23	15.35	5.00	15.33	0.0999	0.0380
179	SATHA	25.80	54.00	16.00	6.00	14.80	0.1240	0.0700
180	DULAAUS	29.84	34.48	14.52	5.00	15.33	0.0837	0.0437
181	CHINGER	23.00	51.85	19.03	5.00	14.83	0.0827	0.0101
183	T 65	25.16	45.58	14.38	5.00	13.00	0.0606	0.0054
184	SAITA BORO	39.80	49.03	15.07	5.00	16.50	0.0766	0.0227
185	KUDA	31.86	35.15	8.98	4.83	20.67	0.0460	0.0056
187	KALASU	26.44	49.58	15.67	5.00	15.33	0.0771	0.0268
188	JABOR SAIL	25.56	45.40	18.55	5.17	13.17	0.0691	0.0104
191	DJ29	26.76	44.33	12.60	4.83	12.83	0.0944	0.0125
195	RAJ MUNDO	29.59	55.00	14.78	5.83	17.50	0.0881	0.0086
197	W 418	30.04	47.63	12.78	4.67	15.67	0.1014	0.0348
198	WHITE DUBHI	23.41	41.22	13.43	4.67	13.00	0.0610	0.0073
200	BJ 1	25.99	28.80	9.85	5.67	20.33	0.0543	0.0173
201	BLACK GORA	29.50	51.82	15.43	5.00	15.83	0.0852	0.0122
202	DHALA SHAITTA	27.31	39.00	9.80	4.67	17.50	0.0512	0.0292
203	DV85	29.10	28.40	10.10	5.00	14.00	0.0420	0.0110
204	DZ78	24.36	47.40	13.75	5.00	17.00	0.1021	0.0450
205	JHONA 349	26.50	32.00	11.00	3.00	14.00	0.0230	0.0150
206	KALAMKATI	26.51	31.43	9.85	4.83	18.50	0.0605	0.0315
207	KASALATH	28.56	33.05	9.10	5.00	16.00	0.0520	0.0160
208	T 1	28.09	28.52	6.10	3.83	9.83	0.0265	0.0070
209	T26	28.09	31.12	8.88	4.33	13.50	0.0421	0.0129
211	CTG 1516	24.24	40.05	11.62	4.83	12.50	0.0566	0.0082
213	DJ 123	27.53	31.08	8.92	5.00	17.33	0.0513	0.0205
214	DJ 24	25.49	36.68	12.53	5.00	13.00	0.0420	0.0241
215	DM 43	24.64	25.95	9.25	4.67	12.50	0.0345	0.0155
216	DM 56	26.96	26.13	5.78	4.50	13.83	0.0142	0.0468

217	DM 59	24.67	29.63	7.43	5.00	15.67	0.0416	0.0201
218	DNJ 140	27.10	22.80	7.00	4.00	11.00	0.0300	0.0140
219	DV 123	26.00	26.13	9.52	4.83	15.17	0.0398	0.0082
220	GHORBHAI	27.43	27.68	9.48	4.00	13.83	0.0350	0.0208
221	GORIA	26.36	27.30	10.12	3.17	12.67	0.0510	0.0178
222	JAMIR	29.59	38.00	10.07	4.67	13.00	0.0473	0.0273
223	KACHILON	25.89	28.87	9.33	3.83	12.67	0.0332	0.0095
224	DZ 193	25.47	48.90	16.70	5.50	13.17	0.0925	0.0108
225	KARKATI 87	28.43	25.05	9.18	3.50	5.67	0.0401	0.0096
226	ARC 10376	25.67	44.78	17.97	4.67	13.50	0.0567	0.0087
228	SURJAMKUHI	25.17	23.98	6.90	3.83	11.83	0.0287	0.0133
229	PTB 30	30.83	24.18	7.27	4.50	13.33	0.0358	0.0097
230	BAWOI	22.69	48.97	15.32	5.00	15.85	0.0826	0.0113
233	BOGURA	25.43	32.27	12.53	4.00	12.08	0.0586	0.0204
234	LAHAYA	28.36	26.23	6.23	4.33	12.83	0.0315	0.0160
236	TUPA	28.80	32.38	8.08	4.83	9.92	0.0463	0.0190
237	CHHOLA BORO	27.10	21.10	7.40	4.00	13.00	0.0450	0.0170
238	CHHOLA BORO(2)	26.54	27.09	6.58	4.00	15.00	0.0282	0.0091
239	LAFAI	27.04	33.15	9.77	4.33	12.00	0.0430	0.0138
241	CHAILI BOROI	26.56	42.17	8.95	5.33	24.33	0.0516	0.0170
242	SADA BORO G1	26.20	38.40	5.50	5.00	21.00	0.0430	0.0160
243	GOBIR SAIL	27.41	38.80	9.12	5.00	19.00	0.0402	0.0104
244	DUMSIA 81	28.37	29.42	7.30	5.00	12.00	0.0326	0.0080
245	TULSI BORO	24.74	31.85	8.70	4.17	12.00	0.0490	0.0188
247	GUL TEPI	27.10	39.05	8.57	5.17	20.17	0.0621	0.0087
248	RATA BORO	24.60	29.40	9.50	4.00	16.00	0.0390	0.0150
250	BACHI BORO	30.41	50.45	11.97	6.00	15.33	0.0827	0.0097
253	BR 3	16.60	31.85	10.78	5.00	19.83	0.0545	0.0135
254	BR 6	30.61	21.30	9.10	5.00	12.67	0.0624	0.0197

255	BR 16	20.43	28.30	12.62	4.83	13.67	0.0572	0.0095
256	BRRI DHAN 28	31.86	23.78	8.70	5.00	13.33	0.0495	0.0123
257	BRRI DHAN45	28.73	24.13	5.93	5.00	14.50	0.0390	0.0170
258	BRRI DHAN47	34.19	34.25	10.55	5.00	21.17	0.0794	0.0372
259	BRRI DHAN50	27.66	34.23	11.18	4.00	13.33	0.0590	0.0202
260	BINA DHAN 5	30.30	28.12	6.98	5.00	19.83	0.0488	0.0210
261	IRATOM 24	28.81	21.27	6.67	5.58	16.00	0.0368	0.0102
262	IARI 6626	34.50	36.20	12.30	5.00	22.00	0.0920	0.0280
264	PURPLE PUTTU	23.13	28.07	7.78	4.50	11.83	0.0533	0.0253
265	P 79	27.31	33.15	8.75	4.50	14.17	0.0603	0.0193
266	POLMAN	27.50	36.30	8.50	4.00	15.00	0.0460	0.0200
267	DJ 53	29.01	27.42	7.08	4.00	15.00	0.0314	0.0130
268	ZIRI	27.66	36.13	6.65	5.00	14.33	0.0545	0.0210
270	P 32	25.80	56.63	15.65	4.83	20.50	0.0803	0.0100
271	99216	28.81	25.72	9.25	4.83	16.00	0.0258	0.0158
272	NP 97	27.09	29.14	8.59	3.75	10.58	0.0418	0.0228
273	DV 118	25.56	33.18	8.50	4.83	12.83	0.0475	0.0123
274	PACHODI 427	25.64	50.87	13.85	5.00	12.67	0.0693	0.0086
276	ARC 10303	30.33	35.30	8.43	4.83	13.67	0.0580	0.0290
278	DA 24	28.03	31.27	10.27	5.00	17.00	0.0142	0.0024
280	FULBADAM	27.22	31.85	8.75	4.00	11.50	0.0595	0.0330
282	DOM SUFID	32.27	34.50	7.40	4.50	18.50	0.1100	0.0360
283	DULAR	30.42	35.65	8.50	5.00	18.00	0.0740	0.0245
284	FR 13 A	27.94	43.38	15.62	5.00	17.33	0.1105	0.0376
285	IR 64-21	32.80	25.50	7.90	5.17	16.00	0.0363	0.0156
286	LI-JIANG-XIN-TUAN-HEI-GU	28.50	36.35	8.48	4.33	16.00	0.0455	0.0196
288	MINGHUI 63	18.99	43.12	12.68	5.17	13.67	0.0722	0.0083
290	N 22	22.09	44.78	18.58	4.50	12.67	0.0583	0.0092
292	POKKALI	28.24	56.58	10.85	5.00	17.50	0.1052	0.0109

293	SADU CHO	29.70	42.60	8.50	4.83	17.17	0.0606	0.0177
294	SANHUANGZHAN NO 2	33.64	42.30	7.58	5.17	15.33	0.0309	0.0153
297	ZHENSHAN 97 B	35.46	27.85	8.35	5.00	19.50	0.0700	0.0250

Table 4.8 Estimates of variability of BAAP lines for the vegetative stage traits studied under phosphorus deficient trial

	RANGE	MEAN	SD	CD (5%)	CV	GCV	PCV	h^2_{bs}	GA	SKEWNESS	KURTOSIS
Chlorophyll Index	16.60 - 39.8	27.21	3.25	0.74	1.32	11.92	11.99	0.98	24.42	0.05	2.66
Shoot length (cm)	21.10 - 56.98	38.86	8.77	1.78	6.99	22.09	23.03	0.92	43.65	0.01	-0.98
Root length	5.0 - 35.13	11.84	4.05	6.23	18.62	28.58	39.1	0.53	43.02	1.18	1.4
Number of leaves	3.00 - 6.00	4.72	0.51	0.43	5.08	10.19	11.22	0.82	19.09	-0.54	0.9
Number of crown roots	5.667 - 24.33	14.8	2.81	3.89	14.53	20.96	23.9	0.61	29.83	0.41	0.81
Shoot dry weight (g)	0.0141 - 0.124	0.062	0.02	0.024	22.68	35.93	38.94	0.78	62.56	0.2	-0.56
Root dry weight (g)	0.002 - 0.07	0.02	0.01	0.0113	16.42	51.21	53.15	0.89	97.44	0.98	1.38
SDW:RDW ratio	0.303 - 44.58	4.49	1.12	2.94	23.1	59.10	63.28	0.87	93.85	0.51	0.68

4.1.2.1 Chlorophyll Index

The chlorophyll index as measured by the SPAD meter ranged from 16.60 (BR3) to 39.80 (Saita Boro). The mean index value was 27.2 with a CV values of 1.32. The heritability was high (0.98), the PCV (23.03) was higher than GCV (22.09) and the genetic advance was 24.42.

4.1.2.2 Shoot length (cm)

Shoot length displayed a wide variation with the minimum value of 21.10cm (Chhola Boro) and the highest shoot length was 56.98cm (Bowalia2). The mean shoot length in the studied population was 38.86cm with a CV of 6.99%. There was high heritability (0.92) and genetic advance of 43.65. The data distribution here shows a symmetrical skewness of 0.05.

4.1.2.3 Root length (cm)

Root length exhibited a range of variation from 5.00cm (AUS3825) to 35.13cm (AUS93). The mean root length was 11.84cm. There was moderate heritability (0.53) for the trait with genetic advance of 43.02. The data was positively skewed with a value of 1.18.

4.1.2.4 Number of leaves

The number of leaves ranged between 3 (Jhona349) to 6 (Chamka, Padma Sail, Satha). The mean number of leaves was 4.72 with a CV value of 5.08. The heritability was high (0.82) and skewness of -0.54.

4.1.2.5 Number of crown roots

The number of roots displayed a great deal of variation from values as low as 5.67 (Karkati 87) to a maximum of 24.33 (Chaili boro). The mean number of roots for the panel under study was 14.8 with a moderate heritability (0.61). The data distribution here shows a skewness of 0.41.

4.1.2.6 Shoot dry weight (g)

The shoot biomass or the dry weight ranged from 0.0142 (DM56) to 0.1240 (Satha). The mean shoot dry weight 0.062, PCV (38.94) was higher than the GCV (35.93) and heritability of 0.78. The data distribution here shows a symmetrical distribution with a skewness of 0.2.

4.1.2.7 Root dry weight (g)

The root dry weight ranged from 0.0024gms (DA24) to 0.0700gms (Satha). The mean root dry weight was 0.062gms. The data distribution was positively skewed (0.98). There was high heritability (0.89) for the trait.

4.1.2.8 SDW : RDW ratio

The index value derived from root dry weight and shoot dry weight ranged from 0.30 (DM56) to 13.44 (ARC6000). The mean index value was 4.49. The heritability was 0.87 with genetic advance value of 93.85. The data distribution here shows a skewness of 0.51.

The correlation analysis (presented in Table 4.9) among the traits studied under phosphorus deficient hydroponic condition significant positive correlation of shoot dry weight with shoot length (0.71), root length (0.55), number of leaves (0.46) and number of roots (0.30). The chlorophyll index had significant negative correlation with root length (-0.23) and positive correlation with number of roots (0.18). The principal component analysis was done to reduce the data dimensionality and identify major contributors to the variability in the studied panel. The PCA results are presented in Table 4.10. There were three major dimensions with eigen values of more than unity. Shoot length and shoot dry weight had the highest contribution towards PC1 with 26.19 and 25.08 respectively. Root dry weight had highest contribution in PC2, i.e., 39.08. The PCA biplot for the vegetative stage traits studied under phosphorus deficient trial is presented in Fig.4.2.

Table 4.9 Estimates of correlation among the vegetative stage traits studied under phosphorus deficient trial

	Chlorophyll Index	Shoot length	Root length (cm)	Number of leaves	Number of crown roots	Shoot dry weight (g)	Root dry weight (g)
Shoot length (cm)	-0.061	1					
Root length (cm)	-0.231**	0.661**	1				
Number of leaves	0.093	0.426**	0.288**	1			
Number of crown roots	0.189**	0.284**	0.131	0.404**	1		
Shoot dry weight (g)	0.099	0.714**	0.552**	0.466**	0.301**	1	
Root dry weight	0.163*	0.12	0.201**	0.146*	0.188**	0.444**	1
SDW:RDW ratio	-0.182**	0.482**	0.258**	0.229**	-0.007	0.243**	-0.656**

Table 4.10 Estimates of eigen values and contribution of the vegetative stage traits studied towards the major principal components under phosphorus deficient trial

	PC1	PC2	PC3
Eigen values	2.98	1.85	1.17
Chlorophyll Index	0.002	12.94	32.9
Shoot length (cm)	26.19	1.92	0.41
Root length (cm)	18.59	0.97	16.16
Number of leaves	14.76	0.4	11.14
Number of crown roots	7.32	5.59	20.17
Shoot dry weight (g)	25.08	1.72	0.67
Root dry weight (g)	2.68	39.08	12.28
SDW:RDW ratio	5.35	37.34	6.24

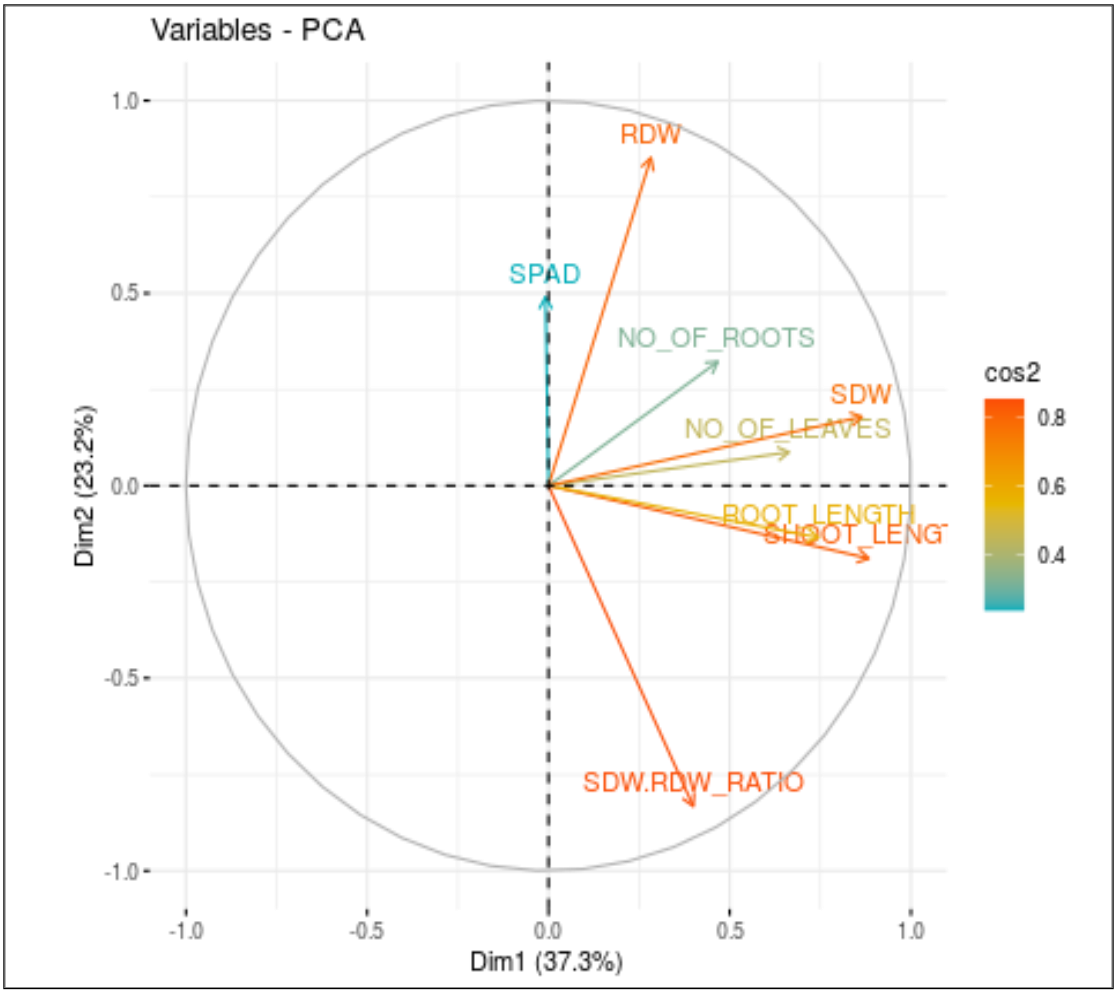


Fig.4.2 PCA biplot for the vegetative stage traits studied under phosphorus deficient trial

4.1.3 Genetic variability among rice genotypes under low levels of iron

The study to identify genetic loci regulating the plant morphological trait under iron deficient condition was undertaken in a controlled condition in the net house. The nutrient source and the pH was controlled and adjusted regularly. Under iron deficient hydroponic trial, the following traits were studied: chlorophyll index, shoot length (cm), root length (cm), number of leaves, number of roots, average root diameter (mm), root volume (cm^3), number of root tips, total root length (cm), total root projected area (cm^2) and total root surface area (cm^2) for a total of 183 accession in the BAAP population. The analysis of variance (presented in Table 4.11) shows no significant difference among the replications while there was significant difference among the genotypes of the panel. The mean data of the traits studied in the Fe deficient trial is presented in Table 4.12 and the estimates of different variability parameters presented in Table 4.13 and described below.

4.1.3.1 Chlorophyll index

The chlorophyll index as measured by the SPAD value ranged from 1.25 (Kalasu) to 28.27 (BR3) with a mean value of 7.66 and RA23 had a mean value of 11.57. The heritability was high with a value of 0.86 and genetic advance was 9.23. The kurtosis indicated a symmetrical distribution of 0.49.

4.1.3.2 Shoot length (cm)

Shoot length had a wide range of variation with a minimum value of 5.46cm (DA24) to a maximum of 33.63cm (Kalburi) with a mean of 18.13cm. The heritability was 0.69 with a preponderance of PCV (33.27) over GCV (31.31). The skewness was 1.41 and kurtosis was 2.34.

4.1.3.3 Root length (cm)

The root length had a wide range of values, with the lowest value at 4.36cm (Bina Dhan 5) and the highest was 35cm (Kada-68-1). The mean root length was 11.42cm with a CV of 16.46. The heritability was 0.39 and the kurtosis was -0.62.

4.1.3.4 Number of leaves

The range of number of leaves was 2 - 3.67 while the average number of leaves for the panel was 2.53 with a standard deviation of 0.41. The heritability was moderate (0.64) with kurtosis of 4.77. The mean number of leaves in RA23 was 4.

Table 4.11 ANOVA of BAAP lines for the vegetative stage traits studied under iron deficient trial

	Mean Sum of Squares		
	Treatment (d.f - 182)	**Replication (d.f - 1)**	**Error**
Chlorophyll Index	43.64**	12.65	0.95
Shoot length (cm)	63.91**	7.85	4.17
Root length (cm)	36.66**	2.2	15.44
Number of leaves	0.33**	0.12	0.64
Number of crown roots	6.07**	0.06	0.94
Average root diameter (mm)	0.57**	0.43	0.26
Root volume (cm^3)	1.52**	0.34	0.81
Number of root tips	80584.00**	34401	35664
Total root length (cm)	697.62**	210.25	141.1
Total root projected area (cm^2)	4.01**	1.29	1.5
Total root surface area (cm^2)	16.68**	10.48	5.65
*, ** Significant at 0.05 and 0.01 levels, respectively			

4.1.3.5 Number of crown roots

The number of roots ranged from 2.00 (Lahaya, DV123) to 12.00 (Bowalia 2) with 5.43 mean number of roots for the panel. The heritability was 0.71 and the genetic advance was 2.91. The kurtosis for this trait was -1.09.

4.1.3.6 Average root diameter (mm)

The average root diameter was measured using the root scanner and it ranged from 0.21mm (Gota Lemma) to 2.19mm (Rataboro) with a mean value of 0.62mm. There was moderate heritability (0.49) and the kurtosis was 0.82.

4.1.3.7 Root volume (cm^3)

The root volume ranged from 0.001cm^3 (ARC14855) to 3.39cm^3 (BR16) and the mean root volume for the panel was 0.58cm^3. The heritability was 0.43 and a genetic advance of 0.46. The skewness and kurtosis were 1.79 and 2.48 respectively.

4.1.3.8 Number of root tips

The number root tips ranged from 68.50 (ARC14855) to 982.33 (AUS294) while the panel mean was 320.46 with a CV value of 18.15. The heritability for this trait was 0.29. The kurtosis for this trait was 5.21 in the panel.

4.1.3.9 Total root length (cm)

The total root length was ranging from 16.50cm (ARC14855) to 139.04cm (AUS294) and the mean was 55.81cm. The heritability was 0.57 and the genetic advance was 21.14. The skewness was 1.17.

Table.4.12 Mean data of BAAP lines for vegetative stage traits under iron deficient trial

BAAP ID	CULTIVAR NAME	Chlorophyll Index	Shoot length (cm)	Root length (cm)	Number of leaves	Number of crown roots	Average root diameter (mm)	Root volume (cm^3)	Number of root tips	Total length (cm)	Total root projected area (cm^2)	Total root surface area (cm^2)
1	ASSAM 4(BORO)	3.63	25.42	9.18	2.00	5.83	0.44	0.08	219.00	40.33	1.90	5.97
2	ARC 5959	6.60	8.20	18.60	2.00	4.00	0.38	0.18	491.33	77.73	3.87	12.17
4	ARC 5977	4.70	19.15	9.15	2.00	5.17	0.30	0.03	281.67	41.95	1.33	4.18
5	ARC 6000	10.50	18.55	10.85	2.17	5.00	0.42	0.09	187.33	37.60	1.91	6.00
6	ARC 6240	6.90	26.40	8.92	2.17	6.00	0.34	0.03	199.33	29.24	0.99	3.13
7	ARC 7325	10.37	20.45	11.55	2.00	3.83	0.32	0.04	255.00	41.95	1.35	4.25
8	ARC 10958	8.03	6.34	17.65	2.75	3.83	0.29	0.02	100.00	25.62	0.74	2.33
9	AS 2	6.77	25.61	11.63	2.00	6.17	0.34	0.04	149.33	39.62	1.36	4.28
13	ARC 11600	6.87	14.92	7.83	2.50	4.67	0.28	0.01	176.33	24.03	0.66	2.08
15	BOWALIA	16.80	26.57	10.90	2.50	8.00	0.74	0.41	321.33	59.52	4.01	12.59
16	BOWALIA 2	13.00	26.75	10.32	3.00	12.00	1.20	0.75	276.67	50.65	5.95	18.70
19	AUS 22	11.93	10.65	23.28	2.33	3.50	0.65	0.72	371.33	66.44	7.32	22.99
21	AUS 29	3.90	10.68	7.40	2.00	7.42	0.33	0.02	174.00	25.68	0.84	2.64
23	AUS 37	15.50	18.49	9.51	3.00	11.17	0.46	0.07	176.67	41.74	1.93	6.06
24	AUS 46	5.83	12.65	7.00	2.50	8.35	0.47	0.07	209.50	48.30	1.60	5.02
25	AUS 60	4.27	15.63	9.76	2.00	6.00	0.53	0.17	244.33	51.54	6.37	20.01
27	AUS 63	3.57	16.97	7.72	2.83	6.83	0.41	0.05	157.33	34.37	1.43	4.49
30	AUS 74	5.07	19.32	10.37	2.00	6.42	1.22	1.03	279.00	21.47	0.76	2.38
31	AUS 77	2.57	19.15	9.20	2.17	4.83	0.34	0.02	164.67	50.85	3.49	10.96
33	AUS 93	12.13	26.35	10.17	2.17	10.83	0.40	0.05	262.33	41.01	1.60	5.04
38	AUS 125	3.40	17.50	10.73	2.17	6.83	1.58	1.37	332.67	52.40	7.35	23.09
39	AUS 127	5.27	10.90	16.20	2.00	4.00	0.43	0.27	470.00	82.10	4.84	15.20
41	AUS 131	3.70	17.27	9.72	2.00	6.50	0.65	0.31	284.67	52.86	4.25	13.35
43	AUS 151	3.73	10.50	20.50	3.00	4.50	0.72	0.55	642.50	100.33	8.32	26.13
45	AUS 154	3.65	30.77	14.17	3.00	7.83	0.51	0.22	422.00	69.63	4.04	12.68
46	AUS 169	9.30	16.07	10.35	2.25	3.58	0.40	0.05	262.33	40.70	1.64	5.15
47	AUS 175	16.90	23.05	11.08	2.50	8.00	0.78	0.72	429.33	82.95	7.44	23.39
49	AUS 204	12.13	8.33	20.50	2.00	2.70	0.38	0.14	479.00	79.15	3.44	10.81
53	AUS 267	15.23	28.60	13.45	2.83	9.17	0.40	0.07	276.67	57.48	2.32	7.30
54	AUS 268	8.60	9.23	13.97	2.33	2.30	0.28	0.03	276.00	40.88	1.14	3.58
55	AUS 273	3.80	15.83	10.18	2.50	6.67	0.31	0.02	113.67	29.02	0.92	2.88
58	AUS 283	1.70	10.65	14.43	2.83	2.33	0.57	0.26	561.67	71.37	4.58	14.40
60	AUS 294	4.17	7.49	19.53	2.42	4.08	0.82	1.17	982.33	139.04	13.66	42.90
61	AUS 298	3.90	27.17	12.35	3.00	6.17	1.60	1.52	455.67	78.14	12.12	38.08
63	AUS 314	10.57	13.75	8.15	2.67	5.33	0.34	0.04	347.33	41.50	1.40	4.40

64	AUS 317	9.80	25.10	14.28	2.17	4.00	0.41	0.17	717.33	101.21	4.43	13.93
65	AUS 321	6.80	25.02	10.13	2.83	7.33	0.72	0.38	236.00	47.31	4.31	13.54
68	AUS 335	6.63	7.75	19.80	3.00	5.00	0.78	0.86	845.33	109.50	10.08	31.66
69	AUS 350	3.00	14.30	10.70	2.17	5.50	0.31	0.02	102.33	31.43	0.98	3.08
70	AUS 353	5.77	17.83	16.53	2.00	5.17	1.95	2.56	291.67	50.21	10.44	32.81
72	AUS 361	4.50	11.63	20.08	2.67	3.17	0.67	0.67	455.00	72.97	7.07	22.22
73	AUS 362	13.00	21.67	12.20	2.67	6.00	0.30	0.05	292.00	67.91	1.99	6.24
75	AUS 366	2.13	21.87	13.47	2.83	6.50	0.45	0.10	289.33	57.07	2.62	8.24
76	AUS 369	6.10	13.68	21.62	2.83	3.67	0.30	0.02	163.00	31.54	0.94	2.95
79	AUS 391	9.83	18.23	12.82	3.00	6.50	0.38	0.07	362.67	63.93	2.33	7.31
81	AUS 411	5.73	11.50	7.23	2.00	6.67	0.60	0.20	242.00	59.29	3.72	11.70
82	AUS 414	7.43	16.12	11.82	3.17	3.83	0.30	0.05	334.67	67.96	2.03	6.38
83	AUS 415	2.80	21.22	14.77	3.17	6.33	0.26	0.02	309.33	45.28	1.19	3.75
86	AUS 435	5.87	18.82	11.27	2.50	3.67	0.26	0.01	216.00	27.07	0.70	2.19
87	AUS 440	4.80	18.25	10.08	2.17	3.67	0.36	0.02	109.00	20.62	0.70	2.19
89	AUS 455	2.77	20.77	7.67	2.00	7.50	0.28	0.02	187.33	72.27	9.04	28.39
90	AUS 462	3.50	12.35	6.75	2.00	5.67	0.90	0.67	362.67	34.88	2.11	6.64
91	AUS 464	5.70	16.85	10.28	2.33	3.50	0.60	0.17	176.00	40.58	1.80	5.66
92	BORO 354	6.17	24.78	11.45	2.67	5.33	0.33	0.03	188.67	35.81	1.14	3.58
94	BORO	4.03	12.60	9.35	2.00	5.00	0.39	0.05	153.00	45.63	4.39	13.78
95	BORO BLACK	1.50	20.22	7.62	2.17	7.00	0.31	0.02	161.67	36.70	1.33	4.18
97	AUSMERI	8.47	15.18	10.02	2.00	7.17	0.29	0.06	407.33	65.62	1.90	5.97
99	ARC 14855	4.70	15.10	18.60	2.83	5.33	0.31	0.01	68.50	16.50	0.52	1.64
101	ARC 14950	2.77	18.65	6.77	2.17	5.17	0.33	0.02	157.67	20.56	0.66	2.06
102	ARC 14965	7.57	15.93	8.12	3.00	5.00	0.26	0.02	128.67	32.02	0.86	2.70
103	ARC 14969	2.35	18.73	7.95	2.33	6.00	0.37	0.04	234.00	34.65	1.34	4.20
104	ARC 7098	2.95	15.32	7.27	2.83	4.00	0.40	0.05	215.33	37.89	1.50	4.71
106	AUS PADDY(BLACK)	3.70	16.91	7.22	2.00	6.58	0.53	0.22	269.67	44.51	4.28	13.43
107	AUS PADDY(WHITE)	9.90	18.52	9.22	2.00	6.67	0.74	0.21	178.00	31.77	1.06	3.35
108	ANJANI	7.57	12.43	7.23	2.67	5.33	0.38	0.03	133.00	22.94	0.94	2.96
111	AUS KUSHI	5.20	16.07	11.80	2.00	6.17	0.67	0.25	309.33	79.45	2.76	8.67
113	SLO 19	13.80	25.35	11.93	2.50	6.00	0.26	0.03	276.67	48.35	1.27	4.00
114	EARLY SUTARSAR 39	7.17	21.05	9.68	2.67	6.17	0.37	0.03	211.00	45.01	2.97	9.32
116	DA 12	9.63	20.30	11.97	2.50	4.17	0.39	0.06	215.00	46.32	1.81	5.67
117	KADA-68-1	5.27	29.52	35.00	3.00	8.67	0.84	0.92	512.00	58.86	4.76	14.96
121	KHAILAORGOABEZ	3.83	17.63	7.08	2.17	7.67	0.37	0.04	162.67	34.20	1.25	3.91
122	KELE(AUS)	4.43	18.18	7.50	2.33	8.67	0.46	0.15	445.67	88.33	4.04	12.70
123	CHAMKA	23.20	17.07	9.17	2.83	9.00	0.96	0.53	348.67	55.65	4.33	13.59
124	KALOSHAITA	8.07	25.12	19.00	3.17	6.17	0.37	0.06	362.33	60.23	2.19	6.89
125	KUMBI	4.37	18.73	6.30	2.17	5.67	0.41	0.05	201.00	40.37	1.63	5.12
126	MUNSHISHAIL	3.87	13.42	8.98	2.50	6.50	0.55	0.20	397.67	83.44	4.55	14.30

127	SHONI	6.87	23.83	10.03	2.50	3.00	0.27	0.04	275.00	62.69	1.67	5.26
128	PURA NUKNA	4.77	24.23	19.18	3.17	5.00	0.43	0.25	560.67	85.51	4.81	15.10
129	CUNAIL	6.50	13.97	5.52	2.33	5.33	0.34	0.03	165.50	40.41	1.64	5.17
130	FAISHA MANSA	18.47	15.52	6.75	2.67	6.67	0.59	0.18	204.00	37.33	0.94	2.95
133	DHALIBORO 105-2	5.87	13.40	12.65	3.00	3.67	0.44	0.06	179.00	36.56	1.65	5.19
136	KALIBORO 26	4.87	20.25	6.70	2.00	3.83	0.25	0.02	260.67	36.73	0.92	2.90
137	KALIBORO 41-1	8.53	26.83	12.33	2.17	5.00	0.32	0.04	209.00	43.11	1.39	4.37
141	KAL BURI	9.50	33.63	15.48	3.00	9.17	0.70	0.51	282.33	33.32	0.94	2.96
143	JAGAL-1640 A	5.00	24.08	8.03	2.00	7.83	0.86	0.54	457.67	56.52	1.82	5.72
145	CHANDRA MUKHI	1.97	15.35	6.87	2.17	4.33	0.42	0.04	189.00	94.17	8.58	26.97
147	HIJLI	7.40	20.43	9.73	2.17	6.00	0.40	0.04	140.67	27.37	0.86	2.69
150	KALINDI	8.03	24.95	16.38	3.17	4.17	0.56	0.39	519.67	46.41	3.13	9.84
151	KALO BIRA	4.07	20.80	12.58	3.17	5.33	0.34	0.10	695.00	114.25	6.98	21.92
155	M 136-20	4.33	21.37	10.00	2.00	6.00	0.43	0.13	559.67	99.41	3.56	11.20
157	PADMA SAIL	4.97	11.58	11.15	2.00	5.33	0.53	0.05	100.50	93.78	3.99	12.54
159	SHETE BHADO	9.97	17.88	12.65	2.50	5.50	0.74	1.07	557.67	22.98	1.21	3.79
160	SOA MUKHI	15.17	28.10	12.40	2.67	6.33	0.31	0.03	231.67	101.55	9.79	30.76
165	DESHI BORO	7.60	7.20	8.95	2.17	3.00	0.48	0.15	271.00	43.09	1.21	3.79
167	SORISHAFUL	9.03	17.42	8.43	2.00	3.50	0.29	0.02	179.00	41.11	2.32	7.30
170	GOTA LEMMA	12.97	12.93	17.17	2.50	3.50	0.21	0.01	244.67	30.24	0.92	2.88
171	MOTZHUL	8.10	20.83	10.07	2.00	6.83	0.46	0.11	295.67	29.61	1.01	3.16
172	CN2-175-5-31	4.83	15.38	7.56	2.00	7.67	0.93	0.85	274.33	64.97	2.99	9.38
173	DHINGHA	12.27	29.75	14.07	2.83	8.50	1.47	1.54	517.00	68.42	7.29	22.90
174	DA2	6.80	30.25	12.17	2.83	7.33	0.43	0.07	276.00	91.20	11.56	36.30
175	GOUERISAIL	5.27	10.97	10.10	2.00	6.33	0.37	0.04	122.00	54.59	1.95	6.13
176	CODE NO BI 93	4.60	12.68	11.72	2.33	5.83	0.57	0.25	372.67	36.22	1.36	4.28
177	KALI AUS	5.27	20.60	10.08	2.17	6.67	0.32	0.06	346.00	58.70	3.36	10.55
178	PARBATJIRA	2.05	17.30	7.32	2.00	8.00	1.53	1.39	394.33	74.87	11.35	35.64
179	SATHA	17.10	24.48	8.65	3.00	5.33	0.27	0.03	352.00	51.00	1.41	4.42
180	DULAAUS	7.03	16.27	10.90	2.00	5.17	0.37	0.04	246.33	26.93	0.90	2.84
181	CHINGER	17.93	14.22	8.75	2.83	4.17	0.32	0.03	162.00	40.28	1.59	4.99
183	T 65	5.50	15.92	8.33	2.33	5.67	0.30	0.02	132.33	30.30	0.89	2.80
184	SAITA BORO	3.27	23.58	11.48	2.00	6.67	0.70	0.28	324.67	59.24	4.34	13.62
185	KUDA	7.40	13.67	6.57	2.00	6.67	0.45	0.08	202.33	44.42	2.12	6.65
187	KALASU	1.25	25.05	13.58	2.67	8.50	1.16	0.92	315.67	74.42	9.20	28.90
188	JABOR SAIL	4.47	25.92	11.20	3.00	8.83	1.06	0.71	325.67	78.94	9.92	31.15
191	DJ29	20.80	22.42	14.65	2.00	3.83	0.39	0.08	292.00	46.85	2.23	6.99
195	RAJ MUNDO	4.40	25.53	11.45	2.67	5.33	0.40	0.04	208.33	33.59	1.35	4.24
197	W 418	5.57	24.95	13.15	2.50	4.50	0.28	0.03	306.67	46.70	1.28	4.01
198	WHITE DUBHI	1.43	22.97	11.97	2.00	6.17	1.17	1.24	303.67	43.95	6.51	20.45
200	BJ 1	6.47	19.83	12.97	2.17	5.83	0.34	0.03	100.33	24.93	0.88	2.77

201	BLACK GORA	15.57	13.82	12.95	2.50	3.83	0.48	0.09	310.67	50.34	2.44	7.67
203	DV85	5.47	15.48	8.02	3.00	3.67	0.26	0.04	388.33	62.40	1.77	5.57
205	JHONA 349	7.40	22.95	11.45	3.00	4.50	0.68	0.57	569.33	79.10	6.39	20.08
206	KALAMKATI	3.30	14.61	10.33	3.17	4.17	0.50	0.20	509.67	79.35	4.36	13.70
208	T 1	19.37	12.98	4.87	2.75	2.42	0.32	0.03	253.33	38.93	1.29	4.04
209	T26	4.53	20.13	7.50	3.33	3.17	0.98	1.34	643.33	94.86	9.97	31.32
211	CTG 1516	6.67	13.15	9.05	2.17	2.67	0.39	0.06	306.50	38.78	1.57	4.94
213	DJ 123	3.40	25.60	17.00	3.00	6.50	0.35	0.11	560.67	105.62	3.72	11.70
214	DJ 24	7.30	15.00	8.25	2.00	3.50	0.33	0.05	367.50	57.72	1.91	6.00
215	DM 43	4.80	25.00	10.35	3.50	5.33	1.45	3.06	745.00	102.28	15.43	48.48
216	DM 56	12.97	28.30	13.25	2.83	5.17	0.33	0.04	234.67	49.28	1.63	5.12
217	DM 59	4.30	13.83	9.68	3.00	2.17	0.33	0.03	214.50	34.32	1.15	3.60
218	DNJ 140	18.47	8.70	18.60	3.00	3.83	0.81	1.01	762.67	125.37	12.09	37.98
219	DV 123	2.55	13.33	10.00	2.00	2.00	0.56	0.20	286.00	40.84	3.21	10.08
220	GHORBHAI	1.83	18.35	9.23	3.17	4.17	0.50	0.15	347.00	49.42	3.00	9.44
221	GORIA	2.87	18.35	10.52	3.00	4.17	0.33	0.03	313.33	45.95	1.30	4.07
222	JAMIR	8.77	17.38	5.05	3.00	6.00	0.42	0.11	527.00	81.79	3.33	10.47
224	DZ 193	7.87	23.80	12.87	3.67	4.67	0.34	0.04	228.00	44.44	1.47	4.62
225	KARKATI 87	3.77	20.48	10.83	3.00	3.83	0.65	0.75	670.67	98.94	8.96	28.15
226	ARC 10376	4.40	10.87	6.03	2.00	5.67	0.29	0.02	194.67	34.05	0.98	3.07
228	SURJAMKUHI	7.33	18.85	8.72	2.67	3.50	0.34	0.06	387.00	63.73	2.17	6.81
229	PTB 30	7.00	12.50	8.03	3.33	3.83	0.37	0.08	411.67	74.85	2.75	8.65
230	BAWOI	3.90	17.59	9.78	2.92	4.25	1.87	2.49	236.00	33.85	7.34	23.06
233	BOGURA	8.23	10.48	18.95	2.83	4.17	0.25	0.01	144.67	26.44	0.67	2.10
234	LAHAYA	10.07	8.30	11.77	3.00	2.00	0.46	0.09	319.00	50.46	2.33	7.33
236	TUPA	5.10	21.43	10.72	2.83	5.67	0.70	0.18	250.33	47.02	3.23	10.15
237	CHHOLA BORO	9.33	27.03	7.43	3.00	6.83	1.24	1.28	357.33	58.22	8.14	25.56
239	LAFAI	10.10	25.13	7.92	2.50	8.17	1.77	2.65	544.33	91.89	16.41	51.56
241	CHAILI BOROI	14.07	29.45	10.05	2.50	6.00	1.99	2.83	314.67	59.52	13.28	41.73
242	SADA BORO G1	8.13	26.05	10.32	2.75	5.08	0.62	0.59	668.00	111.76	8.67	27.25
243	GOBIR SAIL	10.40	18.15	9.20	2.83	5.83	0.51	0.09	171.00	43.13	2.25	7.08
244	DUMSIA 81	8.50	17.17	4.92	2.83	4.67	1.68	1.72	446.00	82.67	12.96	40.73
245	TULSI BORO	11.53	23.42	6.08	2.50	5.83	1.67	2.25	420.00	71.58	11.71	36.80
247	GUL TEPI	16.23	11.00	21.18	2.33	2.50	0.77	0.90	549.00	72.59	8.24	25.87
248	RATA BORO	10.17	21.07	8.85	2.50	6.17	2.19	3.32	508.00	85.05	18.30	57.49
250	BACHI BORO	12.90	18.05	7.35	2.67	5.33	1.68	2.19	605.67	93.82	15.55	48.84
253	BR 3	28.27	15.92	9.00	3.00	6.67	1.83	2.67	605.33	105.98	18.76	58.94
254	BR 6	6.83	15.00	17.25	3.00	3.17	0.50	0.14	340.00	62.32	3.08	9.68
255	BR 16	20.40	13.65	8.80	2.83	5.83	1.90	3.39	636.50	94.90	18.12	56.93
256	BRRI DHAN 28	11.80	14.67	8.38	2.67	5.17	0.67	0.29	225.67	43.53	3.74	11.75
257	BRRI DHAN45	7.57	7.65	4.88	2.67	4.00	0.31	0.02	275.33	37.25	0.98	3.07

258	BRRI DHAN47	10.30	8.70	22.32	2.17	4.00	0.58	0.25	307.00	59.28	4.14	13.02
259	BRRI DHAN50	13.23	15.43	7.72	2.67	3.50	0.99	0.93	447.67	85.35	9.52	29.90
260	BINA DHAN 5	3.40	9.45	4.37	3.00	6.33	0.42	0.04	123.33	28.95	1.27	3.98
261	IRATOM 24	15.23	16.25	9.50	2.83	5.83	1.53	2.32	730.67	122.46	18.30	57.48
262	SWARNA	9.17	10.15	21.32	2.67	3.17	0.25	0.02	226.00	31.22	0.90	2.84
265	P 79	6.50	23.77	15.67	3.00	3.83	2.15	2.36	367.00	53.33	9.01	28.29
267	DJ 53	6.43	12.35	7.97	2.17	4.50	0.82	0.32	293.67	54.64	4.36	13.71
268	ZIRI	4.83	19.57	12.58	3.00	5.50	0.79	0.64	422.67	76.59	6.64	20.86
270	P 32	10.47	19.68	8.92	2.17	5.33	0.33	0.04	164.33	46.71	1.53	4.80
271	99216	6.37	15.43	11.95	3.00	5.33	0.82	0.49	359.33	63.03	6.11	19.20
272	NP 97	7.40	20.70	13.33	2.50	3.17	0.90	0.58	362.00	63.64	6.36	19.97
273	DV 118	8.60	19.63	13.12	3.00	6.67	0.47	0.05	142.33	28.92	1.39	4.35
274	PACHODI 427	2.40	26.97	12.93	2.33	6.50	0.52	0.10	184.00	41.48	2.24	7.04
275	BOWALIA	4.50	23.63	15.58	3.33	3.17	0.65	0.19	274.33	42.47	3.04	9.56
276	ARC 10303	6.50	25.10	30.32	2.00	3.33	0.59	0.13	166.00	34.78	2.37	7.46
278	DA 24	13.53	5.47	13.97	2.67	4.33	0.27	0.02	211.00	37.90	1.03	3.25
280	FULBADAM	6.60	19.05	14.25	2.83	5.67	1.42	1.24	372.33	70.39	10.33	32.45
282	DOM SUFID	12.80	12.67	17.42	2.50	3.83	0.36	0.02	117.67	17.87	0.66	2.08
285	IR 64-21	2.50	14.33	6.40	2.83	7.65	0.45	0.08	215.00	50.46	2.32	7.28
286	LI-JIANG-XIN-TUAN-HEI-GU	6.07	23.48	8.83	2.00	5.33	1.59	1.12	304.67	58.60	8.65	27.17
288	MINGHUI 63	3.73	10.11	15.11	2.17	3.08	0.24	0.02	266.33	45.66	1.08	3.38
290	N 22	5.30	13.00	9.82	2.00	5.00	0.33	0.03	133.00	31.27	1.04	3.26
292	POKKALI	3.27	19.57	8.23	2.33	7.33	1.18	1.24	497.33	78.65	10.15	31.88
293	SADU CHO	3.97	17.70	10.67	2.33	6.67	0.33	0.05	243.33	58.03	1.95	6.11
294	SANHUANGZHAN NO 2	10.33	10.62	6.60	2.17	5.50	0.84	0.67	251.33	58.52	5.98	18.80
CHECK	RA23	11.57	14.73	10.55	4.00	6.67	0.52	0.19	318.00	64.46	3.62	11.37

Table.4.13 Estimates of variability of BAAP lines for the vegetative stage traits studied under iron deficient trial

	RANGE	MEAN	SD	CD (5%)	CV	GCV	PCV	H^2_{bs}	GA	SKEWNESS	KURTOSIS
Chlorophyll Index	1.25-28.26	7.66	1.48	1.92	18.32	60.56	61.92	0.86	9.23	0.49	0.15
Shoot length (cm)	5.46-33.63	18.13	3.42	4.03	11.6	31.31	33.27	0.69	11	1.41	2.34
Root length (cm)	4.36-35	11.42	1.88	7.75	16.46	31.3	46.5	0.39	4.96	0.15	-0.62
Number of leaves	2.00-3.66	2.53	0.41	0.49	10.62	15.11	18.12	0.64	0.65	1.7	4.77
Number of roots	2.00-12.00	5.43	0.78	1.94	14.36	30.25	35.14	0.71	2.91	0.19	-1.09
Average root diameter (mm)	0.21-2.19	0.62	0.1	0.82	16.12	52.69	97.39	0.49	0.36	0.62	0.82
Root volume (cm3)	0.01-3.38	0.58	0.43	1.45	13.24	11.49	23.64	0.43	0.46	1.79	2.48
Number of root tips	68.5-982.33	320.46	74.19	303.22	18.15	38.187	70.22	0.29	11.55	2.35	5.21
Total root length (cm)	16.49-139.03	55.81	33.25	19.07	7.44	33.13	43.97	0.57	21.14	1.17	1.7
Total root projected area (cm_2)	0.52-18.76	4.23	5.52	1.96	19.71	16.57	27.67	0.36	1.12	1.19	1.445
Total root surface area (cm_2)	1.64-58.94	13.29	17.34	3.81	17.1	18.1	28.86	0.39	2.47	0.4	0.24

Table 4.14 Estimates of correlation among the vegetative stage traits studied under iron deficient trial

	Chlorophyll Index	Shoot length	Root length	Number of leaves	Number of roots	Average root diameter	Root volume	Number of root tips	Total length	Total root projected area	Total root surface area
Chlorophyll Index	1										
Shoot length	0.012	1									
Root length	0.044	0.018	1								
Number of leaves	0.149*	0.173*	0.166*	1							
Number of roots	0.041	0.459**	-0.208**	-0.027	1						
Average root diameter	0.161*	0.202**	-0.047	0.115	0.212**	1					
Root volume	0.216**	0.162*	-0.013	0.165*	0.13	0.946**	1				
Number of root tips	0.133	0.048	0.221**	0.31**	-0.062	0.418**	0.517**	1			
Total root length	0.118	0.075	0.121	0.243**	0.045	0.375**	0.438**	0.821**	1		
Total root projected area	0.198**	0.139	0.02	0.203**	0.121	0.803**	0.849**	0.649**	0.748**	1	
Total root surface area	0.164*	0.205**	0.027	0.173*	0.079	0.291**	0.386**	0.596**	0.805**	0.410**	1

Table.4.15 Estimates of eigen values and contribution of the vegetative stage traits studied towards the major principal components under iron deficient trial

	PC1	PC2	PC3
Eigen values	4.75	1.61	1.24
Chlorophyll Index	1.37	0.08	1.28
Shoot length (cm)	0.97	36.94	31.44
Root length (cm)	0.12	17.89	16.56
Number of leaves	1.94	3.52	31.07
Number of roots	0.53	20.06	6.92
Average root diameter (mm)	14.76	4.8	3.85
Root volume (cm^3)	16.47	1.71	3.48
Number of root tips	12.05	9.64	1.63
Total root length (cm)	12.59	5.31	0.87
Total root projected area (cm^2)	10.69	15.88	3.8
Total root surface area (cm^2)	11.7	0.26	1.16

4.1.3.10 Total root projected area (cm^2)

The total root projected area was ranging from 0.52cm^3 (ARC14855) to 18.76cm^3 (BR3) while the mean value for the panel was 4.23cm^3. The heritability was low with a value of 0.36. The skewness was 1.19 and the kurtosis was 1.44.

4.1.3.11 Total root surface area (cm^2)

The total root surface area ranged from 1.64cm^3 (ARC14855) to 58.94cm^3 (BR3) and the mean was 13.29cm^3. The heritability was low (0.39), with a high PCV (28.86) and low GCV (18.10). The skewness was 0.4 and the kurtosis was 0.24.

The correlation analysis (Table 4.14) represents the association of different vegetative traits studied under iron deficient hydroponic condition. Shoot length has significant positive correlation with number of leaves (0.173), number of roots (0.45), average root diameter (0.20), root volume (0.16) and total root surface area (0.20). The number of roots were negatively correlated with number of roots (-0.20). Chlorophyll index had significant positive relation with number of leaves (0.149), root volume (0.21) and total root projected area (0.20). These traits subjected to PCA and the results are furnished in Table 4.15 and the biplot for PCA is presented in Fig.4.3. There were three major components with eigen values more than unity. The PC1 explained 32.4% of the total variation while PC2 explained 18.1% of variation observed in the panel under iron deficient trial. Root volume and average root diameter has the highest values in PC1, 16.47 and 14.76 respectively. Shoot length has the highest value (36.94) in PC2 while number of leaves had the highest value (31.07) in PC3 after shoot length.

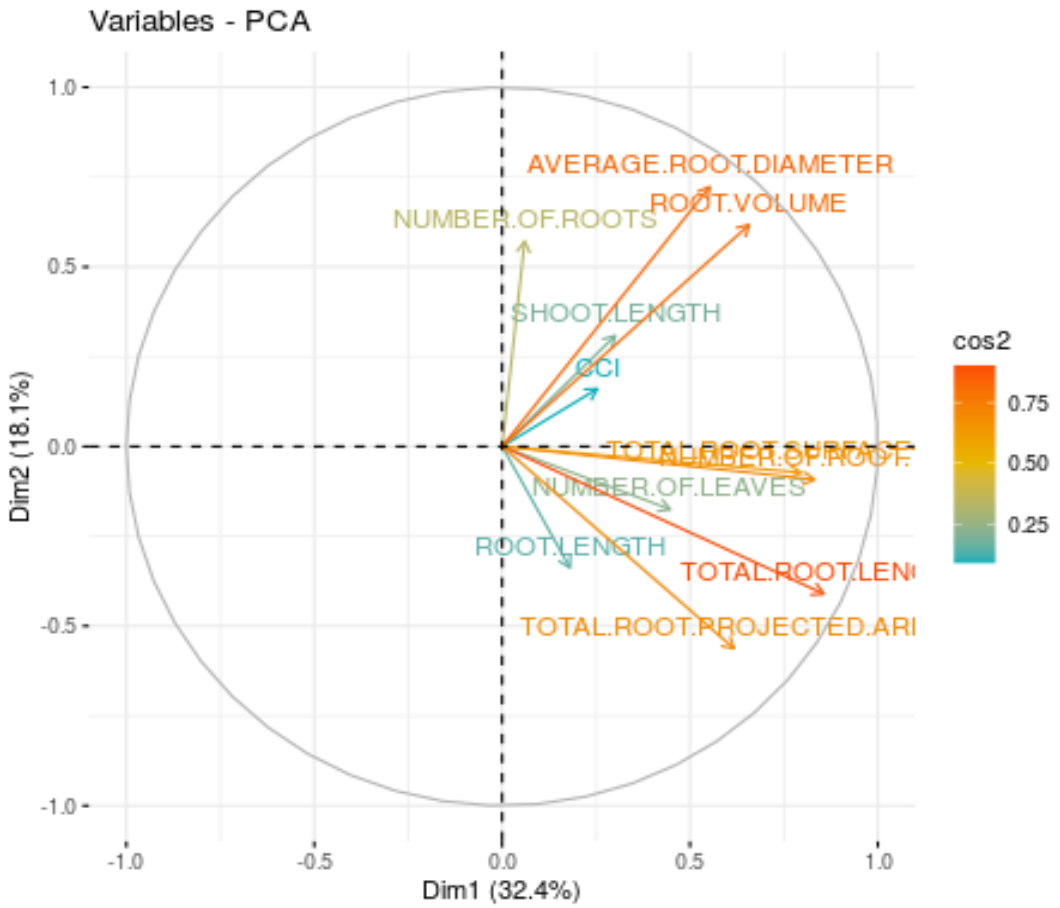


Fig.4.3 PCA among the vegetative stage traits studied under iron deficient condition

2.1 Identification of genetic loci, superior haplotypes and superior donors associated with nitrogen use efficiency

Three were chosen from the nitrogen deficient trial to study genome wide association, identification of haplotype and donors based on correlation with grain yield per plant. They were grain yield per plant along with leaf width and panicle length. The PCA results also supported the above assertion. The clump analysis highlighted significant SNP positions associated with each trait (Table 4.16). Flag leaf width had 10 QTLs, panicle length had 17 QTLs and grain yield per plant had 5 QTLs associated significantly in the BAAP. The SNP with the highest association was chosen for further analysis and identification of a putative causal gene for the associated trait. Manhattan plots for these three traits highlighting the peak SNPs across chromosomes are presented in Fig.4.4.

The peak SNP associated with flag leaf width as revealed from the clump analysis was at 25266590bp on chromosome 6. After taking into consideration the population LD decay value, the genomic region from 25023590bps to 25509590bps was chosen for identifying a putative candidate gene influencing flag leaf width. 46 genes were present in this region excluding the hypothetical proteins, transposable and retrotransposable elements (https://shigen.nig.ac.jp/rice/oryzabase). The gene LOC_Os6g41750 (25024626 – 25034551 bp), annotated as OsAPC3 (Anaphase Promoting Complex3) was selected as the putative candidate gene regulating flag leaf width (Figure.4.5). This gene's expression was evident in the vegetative stage leaves as per the microarray analysis reported in the *RiceXpro* database. The gene has been previously reported to be regulating leaf growth. This gene harboured 35 SNPs from BAAP 2million SNP database, of these two SNPs were having nonsynonymous mutation. These SNPs were located at 25026948bp (C/T polymorphism) and 2520766 (T/C polymorphism) that resulted in the amino acid substitutions from valine (V) to alanine (A) and isoleucine (I) to leucine (L) respectively. In addition to this there were five synonymous SNPs within this region. The two nonsynonymous SNPs grouped the whole panel into three groups with three different haplotypes; hap A (n=38), hap B (n=124) and hap C (n=18). Hap A (1.52cm) and Hap C (1.53cm) were having high mean and significantly different from the inferior group Hap B (1.38cm).

Table 4.16 List of QTLs and their position detected in the present study under nitrogen deficient trial

CHR	QTL	POSITION	FLAG LEAF WIDTH	PANICLE LENGTH	GRAIN YIELD PER PLANT
2	*qNPL_2.1*	9.40 - 9.89		9.65	
2	*qNPL_2.2*	12.29 - 12.78		12.53	
2	*qNPL_2.3*	13.25 - 13.73		13.49	
2	*qNPL_2.4*	32.06 - 32.54		32.30	
2	*qNFLW_2.1*	32.06 - 32.54	32.30		
2	*qNPL_2.5*	35.03 - 35.52		35.27	
2	*qNFLW_2.2*	8.67 - 9.16	8.91		
3	*qNPL_3.1*	22.91 - 23.40		23.16	
4	*qNFLW_4.1*	16.99 - 17.47	17.23		
4	*qNFLW_4.2*	30.12 - 30.61	30.36		
4	*qNFLW_4.3*	5.00 - 5.49	5.24		
4	*qNPL_4.1*	5.00 - 5.49		5.24	
4	*qNPL_4.2*	6.68 - 7.17		6.93	
4	*qNFLW_4.4*	6.76 - 7.24	7.00		
4	*qNPL_4.3*	7.59 - 8.07		7.83	
5	*qNPL_5.1*	4.11 - 4.59		4.35	
6	*qNGY_6.1*	5.07 - 5.55			5.31
6	*qNPL_6.1*	24.00 - 24.48		24.24	
6	*qNFLW_6.1*	25.02 - 25.51	25.26		
6	*qNFLL_6.2*	29.46 - 29.95	29.70		
7	*qNGY_7.1*	0.00 -0.47			0.23
8	*qNPL_8.1*	24.23 - 24.72		24.47	
8	*qNPL_8.2*	27.98 - 28.46		28.22	
9	*qNGY_9.1*	6.48 - 6.96			6.72
9	*qNFLL_9.1*	14.58 - 15.07	14.83		
9	*qNPL_9.1*	21.68 - 22.17		21.93	
10	*qNFLW_10.1*	13.41 - 13.89	13.65		
11	*qNGY_11.1*	22.91 - 23.40			23.15
11	*qNPL_11.1*	24.53 - 25.01		24.77	
11	*qNPL_11.2*	25.40 - 25.88		25.64	
12	*qNPL_12.1*	3.63 - 4.12		3.87	
12	*qNGY_12.1*	6.75 - 7.24			7.00

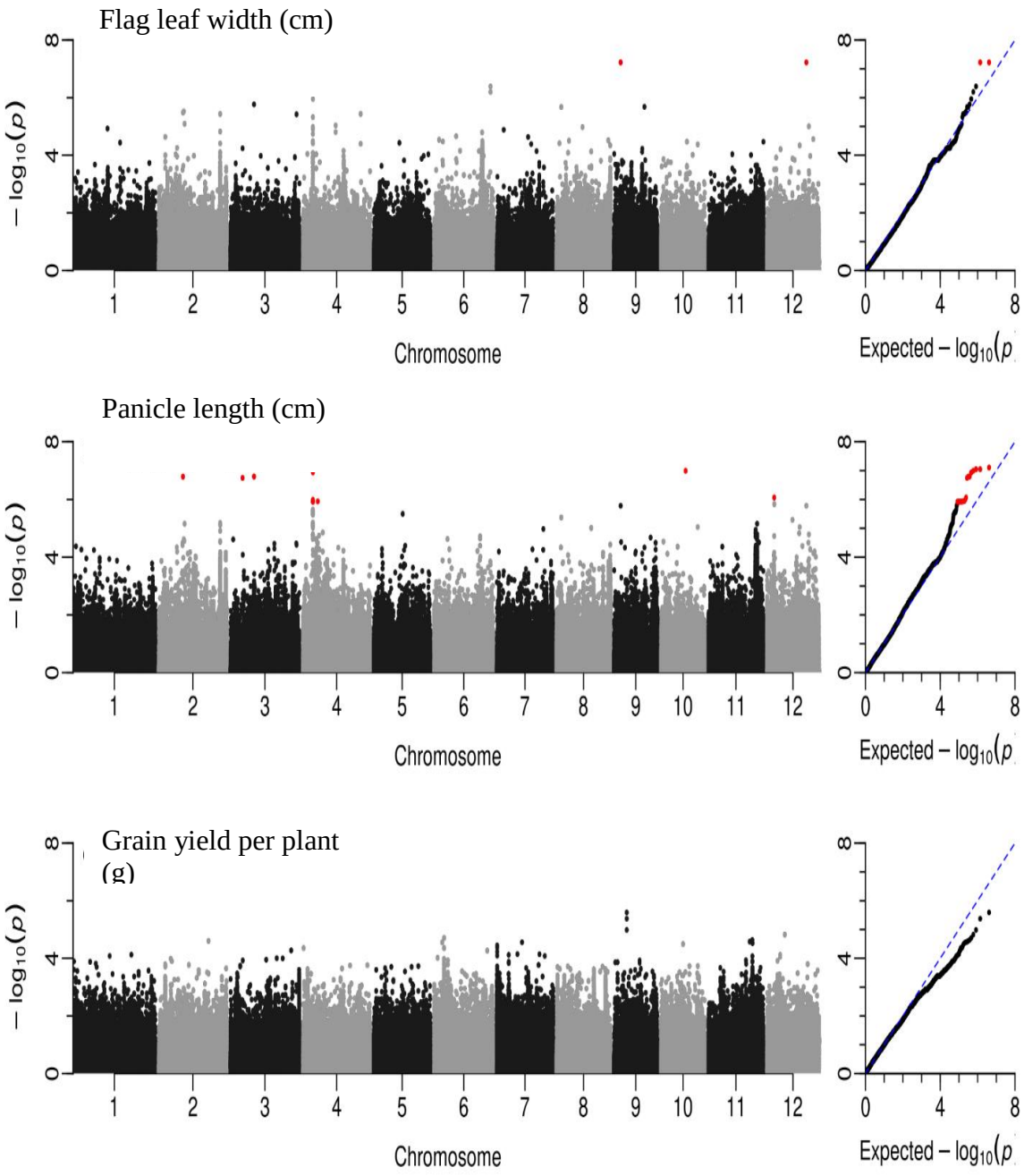
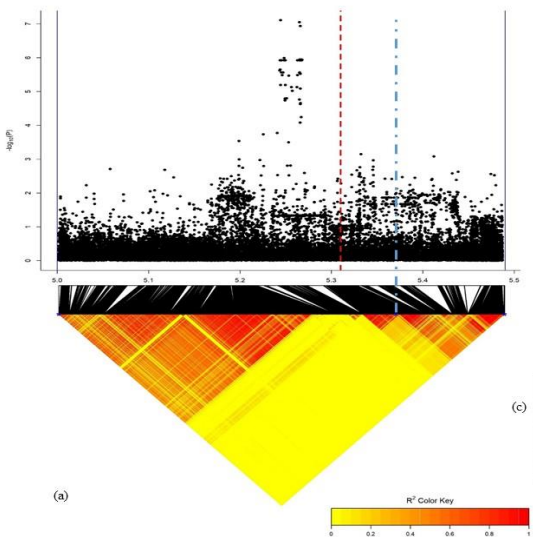
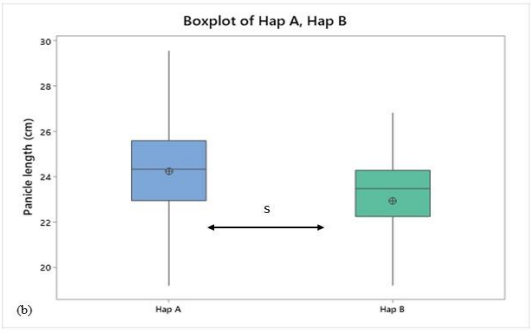


Fig.4.4 Manhattan plots from GWA mapping of flag leaf width, panicle length and grain yield per plant under nitrogen deficient condition of BAAP population.

The peak SNP associated with panicle length was at 5244473bp on chromosome 4. The LD value of 243kbp was taken into consideration and the genomic region from 5001473bp to 5487473bp was searched to identify a putative candidate gene (Figure.4.6). The genes in this region were annotated as per the information available from *oryzabase* database. This region harboured 33 genes excluding hypothetical proteins and retro/transposable elements. There were 121 genes in this region. The gene LOC_Os04g09900 (5318060 – 5326427bps); *OsCPS4*/ GA1 was selected as the putative candidate gene regulating panicle length. The putative candidate gene carries, six nonsynonymous SNPs. These mutations were located at 5323194bp (C/T), 5325054bp (C/T), 5325237bp (G/C), 5325403bp (T/C), 5325413bp (C/A) and 5325832bp (G/A) resulting in changes from alanine to valine, serine to leucine, leucine to phenyl alanine, cysteine to arginine, serine to tyrosine and glutamate to lysine respectively. The nonsynonymous SNPs grouped the whole panel into two groups; Hap A (24.34cm) was superior and significantly different than Hap B (22.91cm).

Grain yield was associated with a SNP centred at 23121131bp on chromosome 11. The LD decay value highlighted a genomic region from 22878131bp to 23364131bp. The region harboured 45 genes excluding hypothetical proteins and retro/transposable elements according to the annotations from *Oryzabase* database. The gene locus LOC_Os11g39220 (23354149 – 23358318bp) was selected as the putative causal gene (Fig.4.7), annotated as *OsACX2*. A total of 75 number of SNPs were highlighted in this stretch from the sequencing of BAAP 2 million SNP database. Of these, two were nonsynonymous changes at the SNP level located at 23358183bp and 23358203bp with G/A polymorphism and T/G polymorphism respectively. The mutation from G/A led to a change in amino acid from Glycine to Glutamate while the mutation from T/G lead to a change in amino acid from Aspartate to Glutamate. These mutations classified the panel into three different classes Hap A (10.21g) and Hap B (10.26g) were statistically significant from the superior haplotype Hap C (11.73g). The table 4.17 shows the different haplotypes and the SNP polymorphisms for the three traits subjected to GWAS under nitrogen deficient trial.

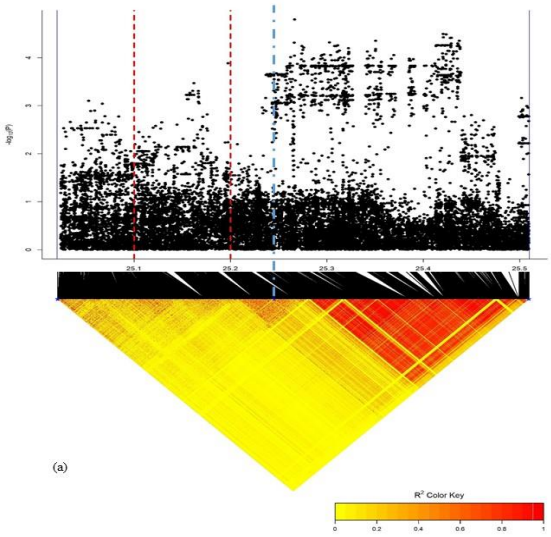


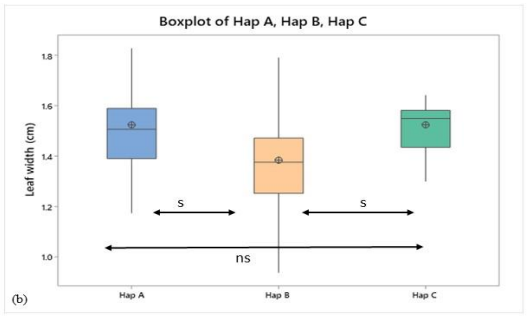


(c)

Position(bp)	5323194	5325054	5325237	5325403	5325413	5325832	Phenotype
Codon	gCg/gTg	tCg/tTg	ttG/ttC	Tgc/Cgc	tCc/tAc	Gaa/Aaa	Panicle length (cm)
Amino acid	A/V	S/L	L/F	C/R	S/Y	E/K	
Nipponbare	C	C	G	T	C	G	
Hap A	C	C	G	T	C	G	24.23
Hap B	T	T	C	C	A	A	22.91

Fig.4.5 Significant association for flag leaf width on chromosome 6 at 25266590bp a) Local Manhattan plot (top) and LD heat map (bottom) of QTL on Chromosome 6 (243 kbp upstream and downstream of the peak SNP) and blue dash line represents the candidate gene *OsAPC3* (LOC_Os6g41750); b) Boxplot and significance test representation of haplotypes for flag leaf width (c) Nonsynonymous SNPs in the candidate gene *OsAPC3* significantly associated with flag leaf width, and amino acid variations and haplotype mean

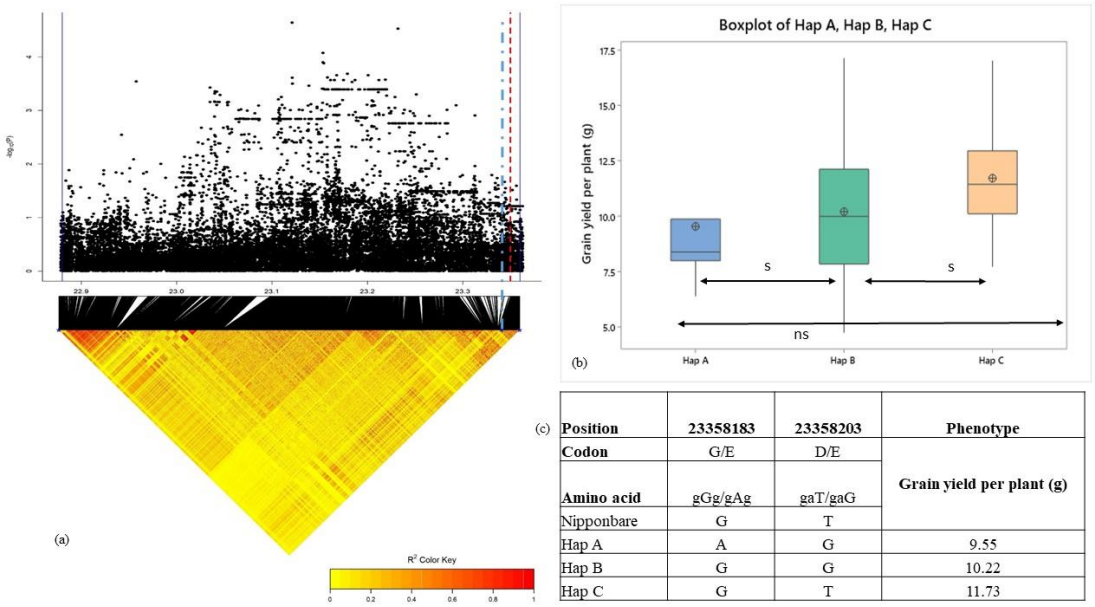




(c)

Position(bp)	25026948	25028000	Phenotype
Codon	gTt/gCt	Ata/Tta	Flag leaf width (cm)
Amino acid	V/A	I/L	
Nipponbare	T	A	
Hap A	C	A	1.52
Hap B	T	A	1.22
Hap C	T	T	1.52

Fig.4.6 Significant association for panicle length on chromosome 4 at 5244473bp a) Local Manhattan plot (top) and LD heat map (bottom) of QTL on Chromosome 4 (243 kbp upstream and downstream of the peak SNP) and blue dash line represents the candidate gene *OsCPS4/GA1* (LOC_Os04g09900); b) Boxplot and significance test representation of haplotypes for flag leaf width (c) Nonsynonymous SNPs in the candidate gene *OsCPS4/GA1* significantly associated with flag leaf width, and amino acid variations and haplotype mean



(c)

Position	23358183	23358203	Phenotype
Codon	G/E	D/E	Grain yield per plant (g)
Amino acid	gGg/gAg	gaT/gaG	
Nipponbare	G	T	
Hap A	A	G	9.55
Hap B	G	G	10.22
Hap C	G	T	11.73

Fig.4.7 Significant association for grain yield per plant on chromosome 11 at 23121131bp a) Local Manhattan plot (top) and LD heat map (bottom) of QTL on Chromosome 11 (243 kbp upstream and downstream of the peak SNP) and blue dash line represents the candidate gene *OsACX2* (LOC_Os11g39220); b) Boxplot and significance test representation of haplotypes for flag leaf width (c) Nonsynonymous SNPs in the candidate gene *OsACX2* significantly associated with grain yield per plant, and amino acid variations and haplotype mean

Table.4.17 SNP polymorphisms and haplotype group for leaf width, panicle length and grain yield per plant under nitrogen deficient trial.

Leaf width			Panicle length						Grain yield per plant		
	Position (bp)			Position (bp)						Position (bp)	
BAAP ID	25026948	25028000	BAAP ID	5323194	5325054	5325237	5325403	5325413	5325832	BAAP ID	23358183	23358203
Nip	TT	AA	Nip	CC	CC	GG	TT	CC	GG	Nip	GG	TT
18	CC	AA	15	CC	CC	GG	TT	CC	GG	179	A/G	GG
28	CC	AA	16	CC	CC	GG	TT	CC	GG	15	AA	GG
33	CC	AA	18	CC	CC	GG	TT	CC	GG	100	AA	GG
126	CC	AA	28	CC	CC	GG	TT	CC	GG	102	AA	GG
3	CC	AA	30	CC	CC	GG	TT	CC	GG	176	AA	GG
63	CC	AA	33	CC	CC	GG	TT	CC	GG	46	AA	GG
113	CC	AA	57	CC	CC	GG	TT	CC	GG	95	AA	GG
149	CC	AA	73	CC	CC	GG	TT	CC	GG	128	AA	GG
155	CC	AA	78	CC	CC	GG	TT	CC	GG	141	AA	GG
195	CC	AA	81	CC	CC	GG	TT	CC	GG	151	AA	GG
265	CC	AA	126	CC	CC	GG	TT	CC	GG	172	AA	GG
278	CC	AA	187	CC	CC	GG	TT	CC	GG	173	AA	GG
130	CC	AA	188	CC	CC	GG	TT	CC	GG	266	AA	GG
157	CC	AA	6	CC	CC	GG	TT	CC	GG	270	AA	GG
266	CC	AA	7	CC	CC	GG	TT	CC	GG	272	AA	GG
272	CC	AA	58	CC	CC	GG	TT	CC	GG	230	AA	GG
4	CC	AA	61	CC	CC	GG	TT	CC	GG	34	AA	GG
21	CC	AA	63	CC	CC	GG	TT	A/C	GG	69	AA	GG
25	CC	AA	64	CC	CC	GG	TT	CC	GG	124	AA	GG
27	CC	AA	65	CC	CC	GG	TT	CC	GG	189	AA	GG
31	CC	AA	82	CC	CC	GG	TT	CC	GG	280	AA	GG
38	CC	AA	83	CC	CC	GG	TT	CC	GG	208	AA	GG
42	CC	AA	86	CC	CC	GG	TT	CC	GG	209	AA	GG
50	CC	AA	87	CC	CC	GG	TT	CC	GG	57	GG	GG
77	CC	AA	91	CC	CC	GG	TT	CC	GG	78	GG	GG
79	CC	AA	94	CC	CC	GG	TT	CC	GG	81	GG	GG
271	CC	AA	100	CC	CC	GG	TT	CC	GG	3	GG	GG
205	CC	AA	106	CC	CC	GG	TT	CC	GG	6	GG	GG
206	CC	AA	107	CC	CC	GG	TT	CC	GG	58	GG	GG
207	CC	AA	114	CC	CC	GG	TT	CC	GG	64	GG	GG
208	CC	AA	170	CC	CC	GG	TT	CC	GG	82	GG	GG
209	CC	AA	178	CC	CC	GG	TT	CC	GG	83	GG	GG
213	CC	AA	195	CC	CC	GG	TT	CC	GG	84	GG	GG
214	CC	AA	267	CC	CC	GG	TT	CC	GG	86	GG	GG
216	CC	AA	273	CC	CC	GG	TT	CC	GG	91	GG	GG
219	CC	AA	276	CC	CC	GG	TT	CC	GG	92	GG	GG
220	CC	AA	278	CC	CC	GG	TT	CC	GG	105	GG	GG
225	CC	AA	2	CC	CC	GG	TT	CC	GG	112	GG	GG
237	TT	AA	24	CC	CC	GG	TT	CC	GG	143	GG	GG
242	TT	AA	43	CC	CC	GG	TT	CC	GG	155	GG	GG
15	TT	AA	46	CC	CC	GG	TT	CC	GG	178	GG	GG

16	TT	AA	51	CC	CC	GG	TT	CC	GG	195	GG	GG
30	TT	AA	53	CC	CC	GG	TT	CC	GG	273	GG	GG
57	TT	AA	56	CC	CC	GG	TT	CC	GG	278	GG	GG
73	TT	AA	80	CC	CC	GG	TT	CC	GG	51	GG	GG
133	TT	AA	88	CC	CC	GG	TT	CC	GG	56	GG	GG
179	TT	AA	89	CC	CC	GG	TT	CC	GG	80	GG	GG
187	TT	AA	90	CC	CC	GG	TT	CC	GG	89	GG	GG
188	TT	AA	104	CC	CC	GG	TT	CC	GG	90	GG	GG
245	TT	AA	111	CC	CC	GG	TT	CC	GG	104	GG	GG
248	TT	AA	116	CC	CC	GG	TT	CC	GG	111	GG	GG
255	TT	AA	127	CC	CC	GG	TT	CC	GG	116	GG	GG
1	TT	AA	128	CC	CC	GG	TT	CC	GG	157	GG	GG
6	TT	AA	130	CC	CC	GG	TT	CC	GG	174	GG	GG
7	TT	AA	141	CC	CC	GG	TT	CC	GG	239	GG	GG
9	TT	AA	151	CC	CC	GG	TT	CC	GG	27	GG	GG
13	TT	AA	157	CC	CC	GG	TT	CC	GG	31	GG	GG
14	TT	AA	173	CC	CC	GG	TT	CC	GG	35	GG	GG
47	TT	AA	270	CC	CC	GG	TT	CC	GG	38	GG	GG
58	TT	AA	21	CC	CC	GG	TT	CC	GG	41	GG	GG
61	TT	AA	23	CC	CC	GG	TT	CC	GG	55	GG	GG
64	TT	AA	25	CC	CC	GG	TT	CC	GG	79	GG	GG
65	TT	AA	27	CC	CC	GG	TT	CC	GG	129	GG	GG
87	TT	AA	29	CC	CC	GG	TT	CC	GG	137	GG	GG
92	TT	AA	31	CC	CC	GG	TT	CC	GG	152	GG	GG
94	TT	AA	35	CC	CC	GG	TT	CC	A/G	167	GG	GG
100	TT	AA	38	CC	CC	GG	TT	CC	GG	211	GG	GG
101	TT	AA	41	CC	CC	GG	TT	CC	GG	214	GG	GG
102	TT	AA	42	CC	CC	GG	TT	CC	GG	217	GG	GG
103	TT	AA	50	CC	CC	GG	TT	CC	GG	225	GG	GG
105	TT	AA	54	CC	CC	GG	TT	CC	GG	228	GG	GG
106	TT	AA	69	CC	CC	GG	TT	CC	GG	229	GG	GG
107	TT	AA	70	CC	CC	GG	TT	CC	GG	242	GG	TT
112	TT	AA	71	CC	CC	GG	TT	CC	GG	16	GG	TT
114	TT	AA	75	CC	CC	GG	TT	CC	GG	18	GG	TT
121	TT	AA	76	CC	CC	GG	TT	CC	GG	28	GG	TT
122	TT	AA	124	CC	CC	GG	TT	CC	GG	30	GG	TT
136	TT	AA	131	CC	CC	GG	TT	CC	GG	33	GG	TT
143	TT	AA	167	CC	CC	GG	TT	CC	GG	73	GG	TT
170	TT	AA	181	CC	CC	GG	TT	CC	GG	126	GG	TT
176	TT	AA	190	CC	CC	GG	TT	CC	GG	133	GG	TT
178	TT	AA	268	CC	CC	GG	TT	CC	GG	187	GG	TT
267	TT	AA	280	CC	CC	GG	TT	CC	GG	188	GG	TT
273	TT	AA	191	CC	CC	GG	TT	CC	GG	248	GG	TT
274	TT	AA	271	CC	CC	GG	TT	CC	GG	255	GG	TT
275	TT	AA	206	CC	CC	GG	TT	CC	GG	1	GG	TT
276	TT	AA	211	CC	CC	GG	TT	CC	GG	7	GG	TT
236	TT	AA	213	CC	CC	GG	TT	CC	GG	9	GG	TT
238	TT	AA	214	CC	CC	GG	TT	CC	GG	13	GG	TT
2	TT	AA	216	CC	CC	GG	TT	CC	GG	14	GG	TT
5	TT	AA	217	CC	CC	GG	TT	CC	GG	47	GG	TT
24	TT	AA	219	CC	CC	GG	TT	CC	GG	63	GG	TT
43	TT	AA	220	CC	CC	GG	TT	CC	GG	87	GG	TT

45	TT	AA	221	CC	CC	GG	TT	CC	GG	94	GG	TT
46	TT	AA	226	CC	CC	GG	TT	CC	GG	101	GG	TT
51	TT	AA	228	CC	CC	GG	TT	CC	GG	106	GG	TT
53	TT	AA	229	CC	CC	GG	TT	CC	GG	107	GG	TT
56	TT	AA	288	CC	CC	GG	TT	CC	GG	113	GG	TT
95	TT	AA	203	TT	TT	C/G	C/T	A/C	AA	114	GG	TT
97	TT	AA	237	TT	TT	CC	CC	AA	AA	121	GG	TT
99	TT	AA	242	TT	TT	CC	CC	AA	AA	122	GG	TT
104	TT	AA	133	TT	T/C	CC	CC	AA	AA	136	GG	TT
111	TT	AA	179	TT	TT	CC	CC	AA	AA	149	GG	TT
116	TT	AA	245	TT	TT	CC	CC	AA	AA	265	GG	TT
127	TT	AA	248	TT	TT	CC	CC	AA	AA	267	GG	TT
128	TT	AA	255	TT	TT	CC	CC	AA	AA	274	GG	TT
141	TT	AA	1	TT	TT	CC	CC	AA	AA	275	GG	TT
150	TT	AA	3	TT	TT	CC	C/T	A/C	AA	236	GG	TT
151	TT	AA	9	TT	TT	CC	CC	AA	AA	238	GG	TT
160	TT	AA	13	TT	TT	CC	CC	AA	AA	2	GG	TT
165	TT	AA	14	TT	TT	CC	CC	AA	AA	5	GG	TT
172	TT	AA	47	TT	TT	CC	CC	AA	AA	43	GG	TT
174	TT	AA	84	TT	TT	CC	CC	AA	AA	45	GG	TT
177	TT	AA	92	TT	TT	CC	CC	AA	AA	53	GG	TT
264	TT	AA	101	TT	TT	CC	CC	AA	AA	88	GG	TT
230	TT	AA	102	TT	TT	CC	CC	AA	AA	99	GG	TT
233	TT	AA	103	TT	TT	CC	CC	AA	AA	127	GG	TT
239	TT	AA	105	TT	TT	CC	CC	AA	AA	130	GG	TT
241	TT	AA	112	TT	TT	CC	CC	AA	AA	150	GG	TT
23	TT	AA	113	TT	TT	CC	CC	AA	AA	165	GG	TT
29	TT	AA	121	TT	TT	CC	CC	AA	AA	177	GG	TT
35	TT	AA	122	TT	TT	CC	CC	AA	AA	233	GG	TT
41	TT	AA	136	TT	TT	CC	CC	AA	AA	4	GG	TT
54	TT	AA	143	TT	TT	CC	CC	AA	AA	21	GG	TT
55	TT	AA	149	TT	TT	CC	CC	AA	AA	23	GG	TT
69	TT	AA	155	TT	TT	CC	CC	AA	AA	25	GG	TT
70	TT	AA	176	TT	T/C	CC	C/T	A/C	AA	29	GG	TT
71	TT	AA	265	TT	TT	CC	CC	AA	AA	42	GG	TT
75	TT	AA	274	TT	TT	CC	CC	AA	AA	50	GG	TT
76	TT	AA	275	TT	TT	CC	CC	AA	AA	54	GG	TT
123	TT	AA	236	TT	TT	CC	CC	AA	AA	71	GG	TT
124	TT	AA	238	TT	T/C	CC	CC	AA	AA	75	GG	TT
129	TT	AA	5	TT	TT	CC	CC	AA	AA	76	GG	TT
137	TT	AA	45	TT	TT	CC	CC	AA	AA	77	GG	TT
142	TT	AA	95	TT	TT	CC	CC	AA	AA	123	GG	TT
152	TT	AA	97	TT	TT	CC	CC	AA	AA	131	GG	TT
159	TT	AA	99	TT	TT	CC	CC	AA	AA	142	GG	TT
167	TT	AA	150	TT	TT	CC	CC	AA	AA	159	GG	TT
168	TT	AA	160	TT	T/C	CC	CC	AA	AA	168	GG	TT
180	TT	AA	165	TT	TT	CC	CC	AA	AA	180	GG	TT
181	TT	AA	172	TT	TT	CC	CC	AA	AA	181	GG	TT
184	TT	AA	174	TT	TT	CC	CC	AA	AA	185	GG	TT
185	TT	AA	177	TT	TT	CC	CC	AA	AA	190	GG	TT
189	TT	AA	264	TT	TT	CC	CC	AA	AA	191	GG	TT
190	TT	AA	266	TT	TT	CC	CC	AA	AA	193	GG	TT

268	TT	AA	272	TT	TT	CC	CC	AA	AA	198	GG	TT
280	TT	AA	230	TT	TT	CC	CC	AA	AA	271	GG	TT
191	TT	AA	233	TT	TT	CC	CC	AA	AA	199	GG	TT
193	TT	AA	239	TT	TT	CC	CC	AA	AA	207	GG	TT
198	TT	AA	241	TT	TT	CC	CC	AA	AA	210	GG	TT
199	TT	AA	4	TT	TT	CC	CC	AA	AA	213	GG	TT
200	TT	AA	34	TT	TT	CC	CC	AA	AA	215	GG	TT
201	TT	AA	55	TT	TT	CC	CC	AA	AA	216	GG	TT
203	TT	AA	77	TT	TT	CC	CC	AA	AA	219	GG	TT
210	TT	AA	79	TT	TT	CC	CC	AA	AA	220	GG	TT
215	TT	AA	123	TT	TT	CC	CC	AA	AA	221	GG	TT
217	TT	AA	129	TT	TT	CC	CC	AA	AA	222	GG	TT
221	TT	AA	137	TT	TT	CC	CC	AA	AA	226	GG	TT
222	TT	AA	142	TT	TT	CC	CC	AA	AA	288	GG	TT
226	TT	AA	152	TT	TT	CC	CC	AA	AA	237	GG	G/T
288	TT	AA	159	TT	TT	CC	CC	AA	AA	245	GG	G/T
229	TT	T/A	168	TT	TT	CC	CC	AA	AA	61	GG	G/T
78	TT	TT	180	TT	TT	CC	CC	AA	AA	65	GG	G/T
81	TT	TT	184	TT	TT	CC	CC	AA	AA	103	GG	G/T
82	TT	TT	185	TT	TT	CC	CC	AA	AA	170	GG	G/T
83	TT	TT	189	TT	TT	CC	CC	AA	AA	276	GG	G/T
84	TT	TT	193	TT	TT	CC	CC	AA	AA	24	GG	G/T
86	TT	TT	197	TT	TT	CC	CC	AA	AA	97	GG	G/T
91	TT	TT	198	TT	TT	CC	CC	AA	AA	160	GG	G/T
80	TT	TT	199	TT	TT	CC	CC	AA	AA	264	GG	G/T
88	TT	TT	200	TT	TT	CC	CC	AA	AA	241	GG	G/T
89	TT	TT	201	TT	TT	CC	CC	AA	AA	70	GG	G/T
90	TT	TT	205	TT	TT	CC	CC	AA	AA	184	GG	G/T
173	TT	TT	207	TT	TT	CC	CC	AA	AA	268	GG	G/T
270	TT	TT	208	TT	TT	CC	CC	AA	AA	197	GG	G/T
34	TT	TT	209	TT	TT	CC	CC	AA	AA	200	GG	G/T
131	TT	TT	210	TT	TT	CC	CC	AA	AA	201	GG	G/T
197	TT	TT	215	TT	TT	CC	CC	AA	AA	203	GG	G/T
211	TT	TT	222	TT	TT	CC	CC	AA	AA	205	GG	G/T
228	TT	TT	225	TT	TT	CC	CC	AA	AA	206	GG	G/T

The three haplotypes were studied together (Table 4.17) and the accessions that were common among the three were selected. 23 accessions were common for the superior haplotypes. Of which, DA24 (leaf width – 1.83cm, panicle length – 24.59cm, grain yield per plant – 16.43g), DM56 (leaf width – 1.46cm, panicle length – 23.97cm, grain yield per plant – 15.52g) and AUS210 (leaf width – 2.02cm, panicle length – 26.14cm, grain yield per plant – 13.39g) were the selected as superior donors for the selected traits; flag leaf width, panicle length and grain yield per plant.

4.2.2 Identification of genetic loci, superior haplotypes and superior donors associated with phosphorus use efficiency

The variability studies and PCA results identified three traits with a maximum contribution to the overall variability in the panel, they were root length, shoot length and shoot dry weight. The data collected for these traits were further subjected to Genome Wide Association studies. The clump results from obtained indicate the QTLs associated with each of these traits (Table 4.18), this distribution of associated SNPs and the peak is presented in the manhattan plots in Fig 4.8. Shoot length had 6 QTLs, root length had 9 QTLs and shoot dry weight had 6 QTLs associated significantly. The SNP with the highest association was chosen for further analysis and identification of a putative causal gene for the associated trait.

Table 4.18 List of QTLs and their position detected in the present study under phosphorus deficient trial

CHR	QTL	POSITIONS	SHOOT LENGTH	ROOT LENGTH	SHOOT DRY WEIGHT
3	qPSDW_3.1	13.97 - 14.45			14.21
4	qPRL_4.1	10.10 - 10.58		10.34	
4	qPRL_4.2	26.62 - 27.10		26.86	
4	qPSL_4.1	26.62 - 27.10	26.86		
5	qPRL_5.1	29.06 - 29.55		29.30	
5	qPRL_5.2	29.49 - 29.97		29.73	
6	qPSDW_6.1	1.35 - 183			1.59
6	qPSL_6.1	10.61 - 11.10	10.85		
6	qPSL_6.2	25.02 - 25.50	25.26		
6	qPSDW_6.2	25.27 - 25.76			25.52
7	qPSL_7.1	1.56 - 2.05	1.80		
7	qPRL_7.1	18.95 - 19.43		19.19	
7	qPSL_7.2	19.36 - 19.85	19.60		
7	qPRL_7.2	25.72 - 26.21		25.97	
8	qPRL_8.1	9.25 - 9.73		9.49	
10	qPRL_10.1	0.10 - 0.59		0.35	
10	qPRL_10.2	11.41 - 11.90		11.66	
11	qPSDW_11.1	14.72 - 15.21			14.97
11	qPSDW_11.2	24.03 - 24.51			24.27
11	qPSL_11.1	24.47 - 24.96	24.71		
12	qPSDW_12.1	18.96 - 19.45			19.20

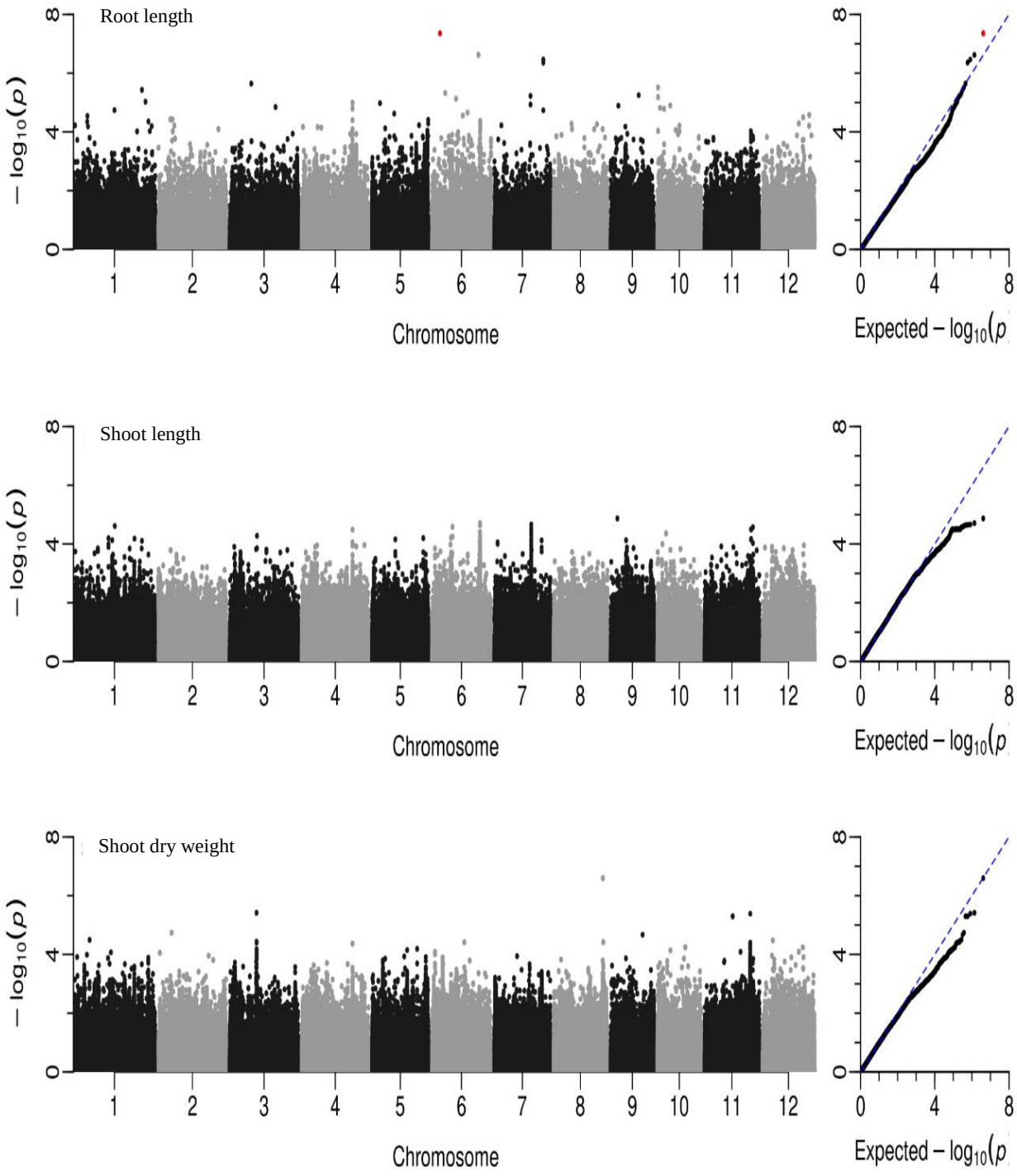
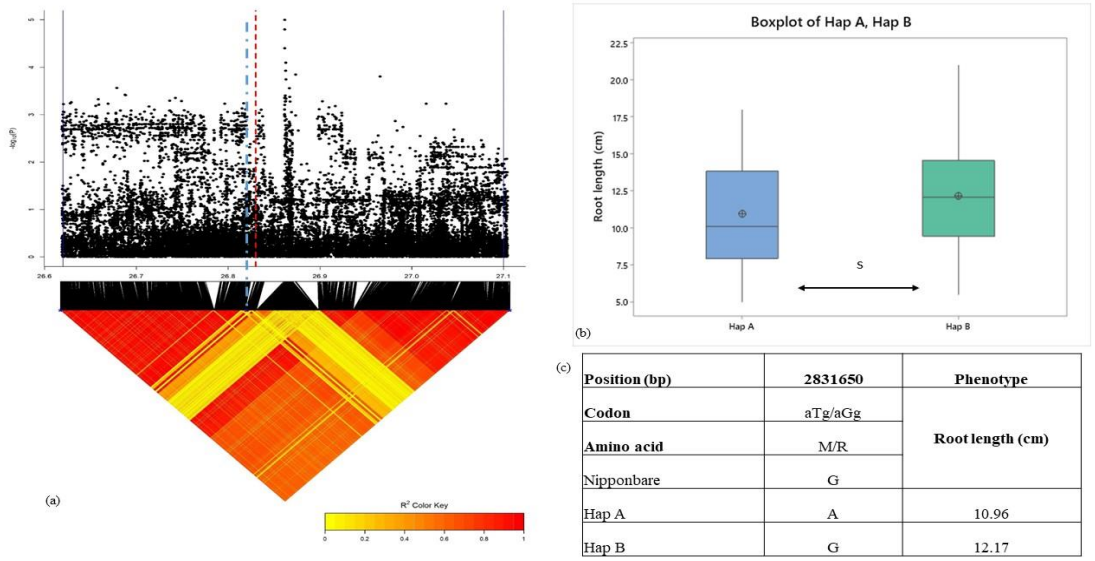


Fig.4.8. Manhattan plots from GWA mapping of root length, shoot length and shoot dry weight under phosphorus deficient condition of BAAP population.

Root length was one of the traits chosen for GWAS to understand the effect of phosphorus deficiency in rice seedlings. The peak SNP associated with this trait was at 26861688 on chromosome 4. The LD value highlighted a genomic region from 26618688 to 27104688bps with a total 48 number of genes excluding hypothetical proteins and retrotransposons. LOC_Os04g45370, annotated as *OsSAUR19* was chosen as the putative candidate gene stretching from 26831005 to 26831867bp. There were three SNPs from this region sequenced in 2 million BAAP database, of which one was a nonsynonymous mutation at 26831650bps (G/T polymorphism). This change resulted in the amino acid polymorphism from methionine (aTG) to arginine (aGg). The panel was grouped into two haplotypes on the basis of the nonsynonymous SNP; Hap A (10.96cm) was significantly different from superior haplotype Hap B (12.17cm). The peak SNP associated with shoot length under phosphorus deficient trial was located on chromosome 6 at 25259001bp. Taking the LD value into consideration a genomic region from 25016001 to 2502001bp was selected. This region harboured a total of 45 genes excluding the hypothetical proteins and retro/transposable elements. The genes were annotated using the *Oryzabase* database. The gene LOC_Os06g41840, annotated as CCR7 was chosen as the putative candidate gene for shoot length. The BAAP sequencing of this gene harboured 13 SNPs of which one was nonsynonymous located at 25088223bp (A/T polymorphism) leading to a change in amino acid from arginine (agA) to serine (agT). This polymorphism grouped the whole panel into two haplotypes; Hap A (40.10cm) and Hap B (37.33cm). Hap A was found to be significantly different and superior than Hap A. The peak SNP identified from the clump analysis for shoot dry weight was located at 14212541bp on chromosome 3. The genomic region from 13969541bp to 14455541bp was identified considering the LD value of the panel. This region harboured a total of 64 genes excluding the hypothetical proteins and retro/transposable elements. These genes were annotated using the *Oryzabase* database. Out of these genes, LOC_Os03g25040 was chosen as the putative candidate gene, ranging from 14294507bps to 14297898bps. This region harboured a total of 118 SNPs available in the 2 million SNP BAAP database, of which were five SNPs; 14294575bp, 14296148bp, 14296472bp, 14296486bp and 14297565bp had nonsynonymous mutation, resulting in the change in amino acids from serine to proline (Tca/Cca), alanine to valine (gCt/gTt), lysine to arginine (aAg/aGg), serine to proline (Tct/Cct) and methionine to threonine (aTg/aCg). On the basis of theses

nonsynonymous SNPs the panel was group into three different haplotypes; Hap A (0.065mg), Hap B (0.052mg) and Hap C (0.054mg). Among the three haplotypes, Hap A was significantly different and superior than Hap B and Hap C.

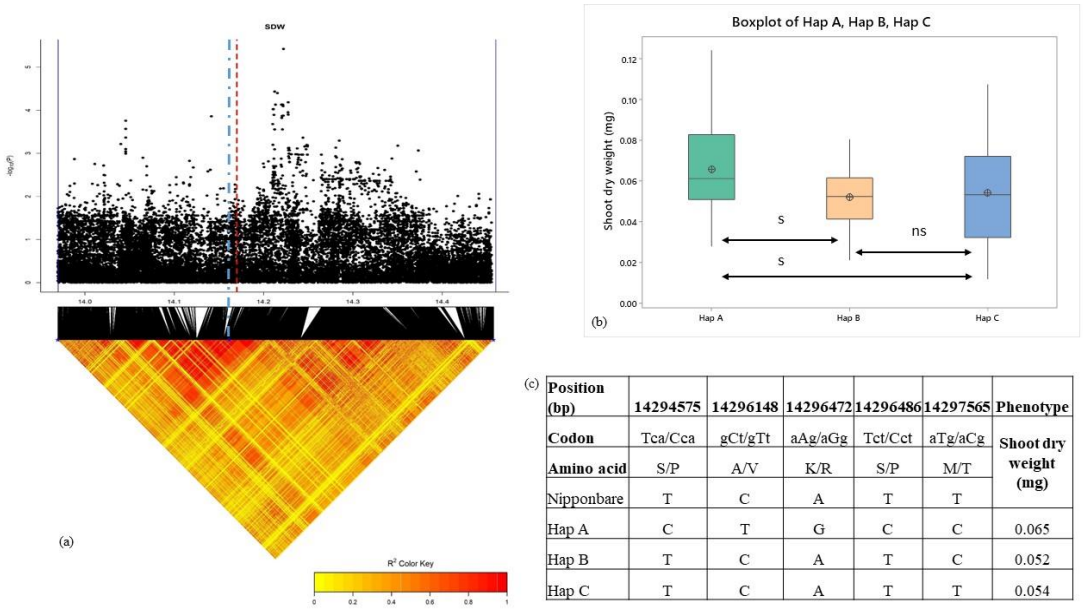
The three haplotypes were studied together (Table 4.19) and the accessions that were common among the three were selected and then sorted on the basis of biomass accumulation. Twenty accessions were common for the superior haplotypes among the three traits. Of which, Shada boro (shoot dry weight – 0.087mg, root length – 14.53cm, shoot length – 49.73cm), Kele (AUS) (shoot dry weight – 0.086mg, root length – 14.03cm, shoot length – 44.38cm) and Kada 68-1 (shoot dry weight – 0.084mg, root length – 12.10cm, shoot length – 48.72cm) were the selected as superior donors for the selected traits; root length, shoot length and shoot dry weight.



(c)

Position (bp)	2831650	Phenotype
Codon	aTg/aGg	Root length (cm)
Amino acid	M/R	
Nipponbare	G	
Hap A	A	10.96
Hap B	G	12.17

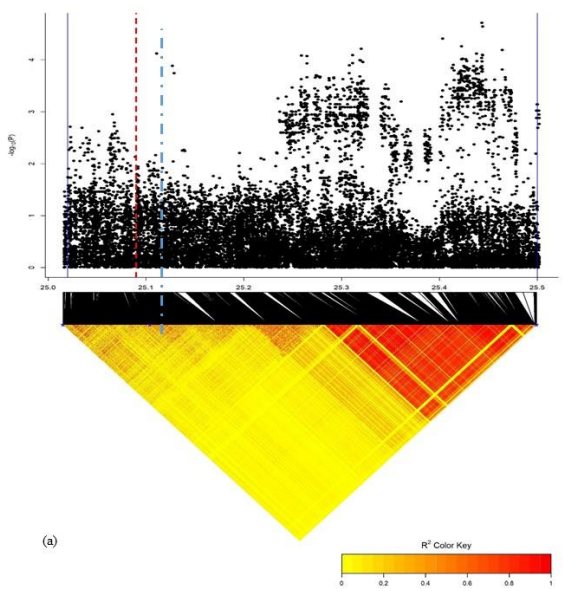
Fig.4.9 Significant association for root length on chromosome 4 at 26861688bp a) Local Manhattan plot (top) and LD heat map (bottom) of QTL on Chromosome 4 (243 kbp upstream and downstream of the peak SNP) and blue dash line represents the candidate gene *OsSAUR19* (LOC_Os04g45370); b) Boxplot and significance test representation of haplotypes for flag leaf width (c) Nonsynonymous SNPs in the candidate gene *OsSAUR19* significantly associated with root length, and amino acid variations and haplotype mean

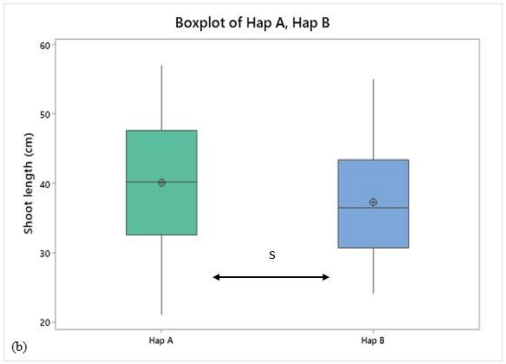


(c)

Position (bp)	14294575	14296148	14296472	14296486	14297565	Phenotype
Codon	Tca/Cca	gCt/gTt	aAg/aGg	Tct/Cct	aTg/aCg	Shoot dry weight (mg)
Amino acid	S/P	A/V	K/R	S/P	M/T	
Nipponbare	T	C	A	T	T	
Hap A	C	T	G	C	C	0.065
Hap B	T	C	A	T	C	0.052
Hap C	T	C	A	T	T	0.054

Fig.4.10 Significant association for shoot length on chromosome 6 at 25259001bp a) Local Manhattan plot (top) and LD heat map (bottom) of QTL on chromosome 6 (243 kbp upstream and downstream of the peak SNP) and blue dash line represents the candidate gene *CCR7* (LOC_Os06g41840); b) Boxplot and significance test representation of haplotypes for shoot length (c) Nonsynonymous SNPs in the candidate gene *CCR7* significantly associated with shoot length, and amino acid variations and haplotype mean





(c)

Position (bp)	25088223	Phenotype
Codon	agA/agT	Shoot length (cm)
Amino acid	R/S	
Nipponbare	A	
Hap A	A	40.10
Hap B	T	37.33

Fig.4.11 Significant association for shoot dry weight on chromosome 3 at 14212541bp a) Local Manhattan plot (top) and LD heat map (bottom) of QTL on chromosome 3 (243 kbp upstream and downstream of the peak SNP) and blue dash line represents the candidate gene *SCL28* (LOC_Os03g25040); b) Boxplot and significance test representation of haplotypes for shoot length (c) Nonsynonymous SNPs in the candidate gene *SCL28* significantly associated with shoot dry weight, and amino acid variations and haplotype mean

Table.4.19 SNP polymorphisms and haplotype group for root length, shoot

length and shoot dry weight under phosphorus deficient trial.

Root length (cm)		Shoot length (cm)		Shoot dry weight (mg)					
BAAP ID	26831650	BAAP ID	25088223	BAAP ID	14294575	14296148	14296472	14296486	14297565
NIP	GG	NIP	AA	NIP	TT	CC	AA	TT	TT
1	AA	1	AA	1	CC	TT	GG	CC	CC
5	AA	5	AA	5	CC	TT	GG	CC	CC
6	AA	7	AA	8	CC	TT	GG	CC	CC
19	AA	8	AA	14	CC	TT	GG	CC	CC
21	AA	9	AA	30	CC	TT	GG	CC	CC
23	AA	14	AA	43	CC	TT	GG	CC	CC
24	AA	15	AA	45	CC	TT	GG	CC	CC
25	AA	16	AA	47	CC	TT	GG	CC	CC
31	AA	23	AA	61	CC	TT	GG	CC	CC
33	AA	41	AA	69	CC	TT	GG	CC	CC
39	AA	46	AA	71	CC	TT	GG	CC	CC
47	AA	53	AA	92	CC	TT	GG	CC	CC
51	AA	55	AA	94	CC	TT	GG	CC	CC
55	AA	60	AA	95	CC	TT	GG	CC	CC
61	AA	65	AA	99	CC	TT	GG	CC	CC
68	AA	72	AA	102	CC	TT	G/A	C/T	CC
69	AA	73	AA	106	CC	TT	GG	CC	CC
70	AA	75	AA	114	CC	TT	GG	CC	CC
72	AA	76	AA	117	CC	TT	GG	CC	CC
75	AA	78	AA	121	CC	TT	GG	CC	CC
76	AA	81	AA	122	CC	TT	GG	CC	CC
77	AA	82	AA	123	CC	TT	GG	CC	CC
78	AA	83	AA	133	CC	TT	GG	CC	CC
81	AA	84	AA	136	CC	TT	GG	CC	CC
82	AA	86	AA	142	CC	TT	GG	CC	CC
83	AA	88	AA	155	CC	TT	GG	CC	CC
84	AA	89	AA	159	CC	TT	GG	CC	CC
86	AA	90	AA	168	CC	TT	GG	CC	CC
88	AA	91	AA	177	CC	TT	GG	CC	CC
89	AA	92	AA	180	CC	TT	GG	CC	CC
90	AA	94	AA	181	CC	TT	GG	CC	CC
91	AA	97	AA	183	CC	TT	GG	CC	CC
92	AA	99	AA	185	CC	TT	GG	CC	CC
95	AA	100	AA	187	CC	TT	GG	CC	CC
99	AA	101	AA	191	CC	TT	GG	CC	CC
101	AA	104	AA	198	CC	TT	GG	CC	CC
102	AA	107	AA	201	CC	TT	GG	CC	CC
111	AA	108	AA	215	CC	TT	GG	CC	CC
113	AA	113	AA	230	CC	TT	GG	CC	CC
114	AA	114	AA	233	CC	TT	GG	CC	CC
126	AA	117	AA	236	CC	TT	GG	CC	CC

127	AA	121	AA	238	CC	TT	GG	CC	CC
133	AA	122	AA	239	CC	TT	GG	CC	CC
149	AA	123	AA	241	CC	TT	GG	CC	CC
155	AA	127	AA	242	CC	TT	GG	CC	CC
160	AA	128	AA	245	CC	TT	GG	CC	CC
165	AA	129	AA	248	CC	TT	GG	CC	CC
167	AA	133	AA	274	CC	TT	GG	CC	CC
172	AA	136	AA	237	TT	CC	AA	TT	TT
176	AA	137	AA	284	CC	TT	GG	CC	CC
177	AA	139	AA	2	TT	CC	AA	TT	CC
178	AA	142	AA	6	TT	CC	AA	TT	CC
183	AA	150	AA	13	TT	CC	AA	TT	CC
188	AA	160	AA	19	TT	CC	AA	TT	CC
198	AA	165	AA	21	TT	CC	AA	TT	CC
201	AA	167	AA	24	TT	CC	AA	TT	CC
203	AA	168	AA	25	TT	CC	AA	TT	CC
204	AA	172	AA	33	TT	CC	AA	TT	CC
207	AA	173	AA	55	TT	CC	AA	TT	CC
208	AA	174	AA	63	TT	CC	AA	TT	CC
209	AA	175	AA	70	TT	CC	AA	TT	CC
211	AA	178	AA	72	TT	CC	AA	TT	CC
213	AA	179	AA	77	TT	CC	AA	TT	CC
215	AA	180	AA	86	TT	CC	AA	TT	CC
216	AA	181	AA	87	TT	CC	AA	TT	CC
217	AA	183	AA	88	TT	CC	AA	TT	CC
219	AA	184	AA	97	TT	CC	AA	TT	CC
223	AA	185	AA	101	TT	CC	AA	TT	CC
226	AA	188	AA	107	TT	CC	AA	TT	CC
228	AA	197	AA	108	TT	CC	AA	TT	CC
229	AA	200	AA	111	TT	CC	AA	TT	CC
230	AA	201	AA	126	TT	CC	AA	TT	CC
237	AA	202	AA	157	TT	CC	AA	TT	CC
265	AA	203	AA	175	TT	CC	AA	TT	CC
266	AA	204	AA	176	TT	CC	AA	TT	CC
270	AA	205	AA	205	TT	CC	AA	TT	CC
271	AA	208	AA	206	TT	CC	AA	TT	CC
273	AA	211	AA	207	TT	CC	AA	TT	CC
274	AA	217	AA	208	TT	CC	AA	TT	CC
284	AA	222	AA	209	TT	CC	AA	TT	CC
288	AA	223	AA	220	TT	CC	AA	TT	CC
2	GG	225	AA	221	TT	CC	AA	TT	CC
3	GG	226	AA	222	TT	CC	AA	TT	CC
4	GG	228	AA	225	TT	CC	AA	TT	CC
7	GG	230	AA	229	TT	CC	AA	TT	CC
8	GG	233	AA	265	TT	CC	AA	TT	CC
9	GG	236	AA	266	TT	CC	AA	TT	CC
13	GG	237	AA	268	TT	CC	AA	TT	CC

14	GG	238	AA	270	TT	CC	AA	TT	CC
15	GG	239	AA	271	TT	CC	AA	TT	CC
16	GG	241	AA	272	TT	CC	AA	TT	CC
30	GG	242	AA	290	TT	CC	AA	TT	CC
38	GG	245	AA	3	TT	CC	AA	TT	TT
41	GG	248	AA	4	TT	CC	AA	TT	TT
43	GG	264	AA	7	TT	CC	AA	TT	TT
45	GG	265	AA	9	TT	CC	AA	TT	TT
46	GG	266	AA	15	TT	CC	AA	TT	TT
48	GG	268	AA	16	TT	CC	AA	TT	TT
49	GG	270	AA	23	TT	CC	AA	TT	TT
53	GG	272	AA	31	TT	CC	AA	TT	TT
54	GG	274	AA	38	TT	CC	AA	TT	TT
60	GG	276	AA	39	TT	CC	AA	TT	TT
63	GG	283	AA	41	TT	CC	AA	TT	TT
64	GG	290	AA	46	TT	CC	AA	TT	TT
65	GG	2	TT	48	TT	CC	AA	TT	TT
71	GG	3	TT	49	TT	CC	AA	TT	TT
73	GG	4	TT	51	TT	CC	AA	TT	TT
87	GG	6	TT	53	TT	CC	AA	TT	TT
94	GG	13	TT	54	TT	CC	AA	TT	TT
97	GG	19	TT	60	TT	CC	AA	TT	TT
100	GG	21	TT	64	TT	CC	AA	TT	TT
103	GG	24	TT	65	TT	CC	AA	TT	TT
104	GG	25	TT	68	TT	CC	AA	TT	TT
106	GG	30	TT	73	TT	CC	AA	TT	TT
107	GG	31	TT	75	TT	CC	AA	TT	TT
108	GG	33	TT	76	TT	CC	AA	TT	TT
116	GG	38	TT	78	TT	CC	AA	TT	TT
117	GG	39	TT	81	TT	CC	AA	TT	TT
121	GG	43	TT	82	TT	CC	AA	TT	TT
122	GG	45	TT	83	TT	CC	AA	TT	TT
123	GG	47	TT	84	TT	CC	AA	TT	TT
124	GG	48	TT	89	TT	CC	AA	TT	TT
125	GG	49	TT	90	TT	CC	AA	TT	TT
128	GG	51	TT	91	TT	CC	AA	TT	TT
129	GG	54	TT	100	TT	CC	AA	TT	TT
130	GG	61	TT	103	TT	CC	AA	TT	TT
136	GG	63	TT	104	TT	CC	AA	TT	TT
137	GG	64	TT	113	TT	CC	AA	TT	TT
139	GG	68	TT	116	TT	CC	AA	TT	TT
141	GG	69	TT	124	TT	CC	AA	TT	TT
142	GG	70	TT	125	TT	CC	AA	TT	TT
143	GG	71	TT	127	TT	CC	AA	TT	TT
145	GG	77	TT	128	TT	CC	AA	TT	TT
147	GG	87	TT	129	TT	CC	G/A	TT	TT
150	GG	95	TT	130	TT	CC	AA	TT	TT

151	GG	102	TT	137	TT	CC	AA	TT	TT
153	GG	103	TT	139	TT	CC	AA	TT	TT
157	GG	106	TT	141	TT	CC	AA	TT	TT
159	GG	111	TT	143	TT	CC	AA	TT	TT
168	GG	116	TT	145	TT	CC	AA	TT	TT
173	GG	124	TT	147	TT	CC	AA	TT	TT
174	GG	125	TT	149	TT	CC	AA	TT	TT
175	GG	126	TT	150	TT	CC	AA	TT	TT
179	GG	130	TT	151	TT	CC	AA	TT	TT
180	GG	141	TT	153	TT	CC	AA	TT	TT
181	GG	143	TT	160	TT	CC	AA	TT	TT
184	GG	145	TT	165	TT	CC	AA	TT	TT
185	GG	147	TT	167	TT	CC	AA	TT	TT
187	GG	149	TT	172	TT	CC	AA	TT	TT
191	GG	151	TT	173	TT	CC	AA	TT	TT
195	GG	153	TT	174	TT	CC	AA	TT	TT
197	GG	155	TT	178	TT	CC	AA	TT	TT
200	GG	157	TT	179	TT	CC	AA	TT	TT
202	GG	159	TT	184	TT	CC	AA	TT	TT
205	GG	176	TT	188	TT	CC	AA	TT	TT
206	GG	177	TT	195	TT	CC	AA	TT	TT
214	GG	187	TT	197	TT	CC	AA	TT	TT
220	GG	191	TT	200	TT	CC	AA	TT	TT
221	GG	195	TT	202	TT	CC	AA	TT	TT
222	GG	198	TT	203	TT	CC	AA	TT	TT
225	GG	206	TT	204	TT	CC	AA	TT	TT
233	GG	207	TT	211	TT	CC	AA	TT	TT
236	GG	209	TT	213	TT	CC	AA	TT	TT
238	GG	213	TT	214	TT	CC	AA	TT	TT
239	GG	214	TT	216	TT	CC	AA	TT	TT
241	GG	215	TT	217	TT	CC	AA	TT	TT
245	GG	216	TT	219	TT	CC	AA	TT	TT
248	GG	219	TT	223	TT	CC	AA	TT	TT
255	GG	220	TT	226	TT	CC	AA	TT	TT
264	GG	221	TT	228	TT	CC	AA	TT	TT
267	GG	229	TT	255	TT	CC	AA	TT	TT
268	GG	255	TT	264	TT	CC	AA	TT	TT
272	GG	267	TT	267	TT	CC	AA	TT	TT
276	GG	271	TT	273	TT	CC	AA	TT	TT
278	GG	273	TT	276	TT	CC	AA	TT	TT
280	GG	278	TT	278	TT	CC	AA	TT	TT
283	GG	280	TT	280	TT	CC	AA	TT	TT
290	GG	284	TT	283	TT	CC	AA	TT	TT
242	GG/AA	288	TT	288	TT	CC	AA	TT	TT

4.2.2 Identification of genetic loci, superior haplotypes and superior donors associated with iron use efficiency

The variability studies and PCA results identified four traits; total root volume, average root diameter, shoot length and number of leaves with maximum contribution to the overall variability in the panel. The data collected for these traits were then subjected to GWA mapping. The clump results highlighted the peaks SNPs associated with each of these traits (Table 4.20). Shoot length had 12 QTLs, number of leaves had 13 QTLs, average root diameter had 20 QTLs and root volume 50 QTLs associated significantly. The SNP with the highest association was chosen for further analysis and identification of a putative causal gene for the associated trait. Root volume and root diameter shared a common peak on chromosome 4, while shoot length and number of leaves shared a common peak on chromosome 11. The peak SNPs associated with each of these traits, this distribution of associated SNPs and the peak is presented in the Manhattan plots in Figure 4.12.

Table 4.20 List of QTLs and their position detected in the present study under iron deficient trial

CHR	QTL	POSITIONS	SHOOT LENGTH	NUMBER OF LEAVES	AVERAGE ROOT DIAMETER	ROOT VOLUME
1	qFeSL_1.1	35.49 - 35.97	35.73			
1	qFeNL_1.1	1.01 - 1.50		1.25		
1	qFeNL_1.2	35.49 - 35.97		35.73		
1	qFeARD_1.1	7.62 - 8.10			7.86	
1	qFeARD_1.2	20.43 - 20.91			20.67	
1	qFeRV_1.1	7.62 - 8.10				7.86
1	qFeRV_1.2	20.06 - 20.54				20.30
2	qFeSL_2.1	4.81 - 5.30	5.05			
2	qFeSL_2.2	13.71 - 14.20	13.96			
2	qFeNL_2.1	0.39 - 0.88		0.64		
2	qFeNL_2.2	0.68 - 1.17		0.93		
2	qFeNL_2.3	4.81 - 5.30		5.05		
2	qFeARD_2.1	4.72 - 5.21			4.97	
2	qFeARD_2.2	16.18 - 16.67			16.42	
2	qFeRV_2.1	4.64 - 5.13				4.88
2	qFeRV_2.2	6.50 - 6.98				6.74
2	qFeRV_2.3	15.89 - 16.37				16.13
2	qFeRV_2.4	24.34 - 24.83				24.58
3	qFeSL_3.1	34.84 - 35.33	35.09			
3	qFeARD_3.1	18.86 - 19.35			19.11	
3	qFeARD_3.2	19.57 - 20.06			19.81	
3	qFeRV_3.1	4.30 - 4.78				4.54
3	qFeRV_3.2	6.54 - 7.02				6.78
3	qFeRV_3.3	14.44 - 14.93				14.68

3	qFeRV_3.4	14.76 - 15.25				15.01
3	qFeRV_3.5	18.86 - 19.35				19.11
3	qFeRV_3.6	19.57 - 20.06				19.81
3	qFeRV_3.7	20.11 - 20.59				20.35
3	qFeRV_3.8	27.82 - 28.30				28.06
4	qFeSL_4.1	4.28 - 4.77	4.53			
4	qFeSL_4.2	6.75 - 7.24	7.00			
4	qFeNL_4.1	0.87 - 1.36		1.11		
4	qFeARD_4.1	5.45 - 5.93			5.69	
4	qFeARD_4.2	31.90 - 32.39			32.14	
4	qFeRV_4.1	5.40 - 5.88				5.64
4	qFeRV_4.2	31.90 - 32.39				32.14
5	qFeSL_5.1	1.51 - 1.99	1.75			
5	qFeNL_5.1	1.51 - 1.99		1.75		
5	qFeNL_5.2	6.56 - 7.05		6.80		
5	qFeARD_5.1	1.95 - 2.44			2.19	
5	qFeARD_5.2	19.58 - 20.07			19.82	
5	qFeRV_5.1	1.94 - 2.43				2.19
5	qFeRV_5.2	5.77 - 6.26				6.01
5	qFeRV_5.3	27.45 - 27.94				27.70
5	qFeRV_5.4	28.43 - 28.91				28.67
6	qFeSL_6.1	6.62 - 7.11	6.87			
6	qFeSL_6.2	24.04 - 24.53	24.29			
6	qFeNL_6.1	6.62 - 7.11		6.87		
6	qFeNL_6.2	24.04 - 24.53		24.29		
6	qFeARD_6.1	15.71 - 16.19			15.95	
6	qFeRV_6.1	2.60 - 3.08				2.84

6	qFeRV_6.2	4.08 - 4.57				4.33
6	qFeRV_6.3	6.40 - 6.89				6.64
6	qFeRV_6.4	7.73 - 8.21				7.97
6	qFeRV_6.5	13.08 - 13.56				13.32
6	qFeRV_6.6	15.71 - 16.19				15.95
6	qFeRV_6.7	19.02 - 19.50				19.26
7	qFeARD_7.2	9.20 - 9.69			9.45	
7	qFeRV_7.1	0.17 - 0.66				0.41
7	qFeRV_7.2	1.45 - 1.93				1.69
7	qFeRV_7.3	5.09 - 5.57				5.33
7	qFeRV_7.4	9.20 - 9.69				9.45
7	qFeRV_7.5	10.89 - 11.37				11.13
7	qFeRV_7.6	18.20 - 18.69				18.44
7	qFeRV_7.7	19.27 - 19.76				19.52
7	qFeRV_7.7	21.59 - 22.08				21.84
8	qFeNL_8.1	2.09 - 2.57		2.33		
8	qFeARD_8.2	24.23 - 24.72			24.48	
8	qFeRV_8.1	1.30 - 1.79				1.54
8	qFeRV_8.2	24.23 - 24.72				24.48
8	qFeRV_8.3	27.40 - 27.88				27.64
9	qFeRV_9.1	17.85 - 18.34				18.09
9	qFeRV_9.2	22.24 - 22.73				22.49
10	qFeSL_10.1	16.39 - 16.88	16.64			
10	qFeNL_10.1	16.39 - 16.88		16.64		
10	qFeRV_10.1	1.19 - 1.68				1.44
11	qFeSL_11.1	16.12 - 16.61	16.36			
11	qFeSL_11.2	19.56 - 20.05	19.81			

11	qFeNL_11.1	16.12 - 16.61		16.36		
11	qFeARD_11.2	12.77 - 13.25			13.01	
11	qFeARD_11.2	17.41 - 17.89			17.65	
11	qFeARD_11.3	19.69 - 20.18			19.94	
11	qFeARD_11.4	20.98 - 21.47			21.22	
11	qFeRV_11.1	0.44 - 0.92				0.68
11	qFeRV_11.2	12.77 - 13.25				13.01
11	qFeRV_11.3	17.41 - 17.89				17.65
11	qFeRV_11.4	19.56 - 20.05				19.88
11	qFeRV_11.5	22.42 - 22.90				22.66
11	qFeRV_11.6	23.73 - 24.22				23.98
12	qFeARD_12.1	3.90 - 4.38			4.14	
12	qFeARD_12.2	9.11 - 9.59			9.35	
12	qFeARD_12.3	14.71 - 15.19			14.95	
12	qFeRV_12.1	9.11 - 9.59				9.35
12	qFeRV_12.2	14.71 - 15.19				14.95
12	qFeRV_12.3	26.02 - 26.50				26.26

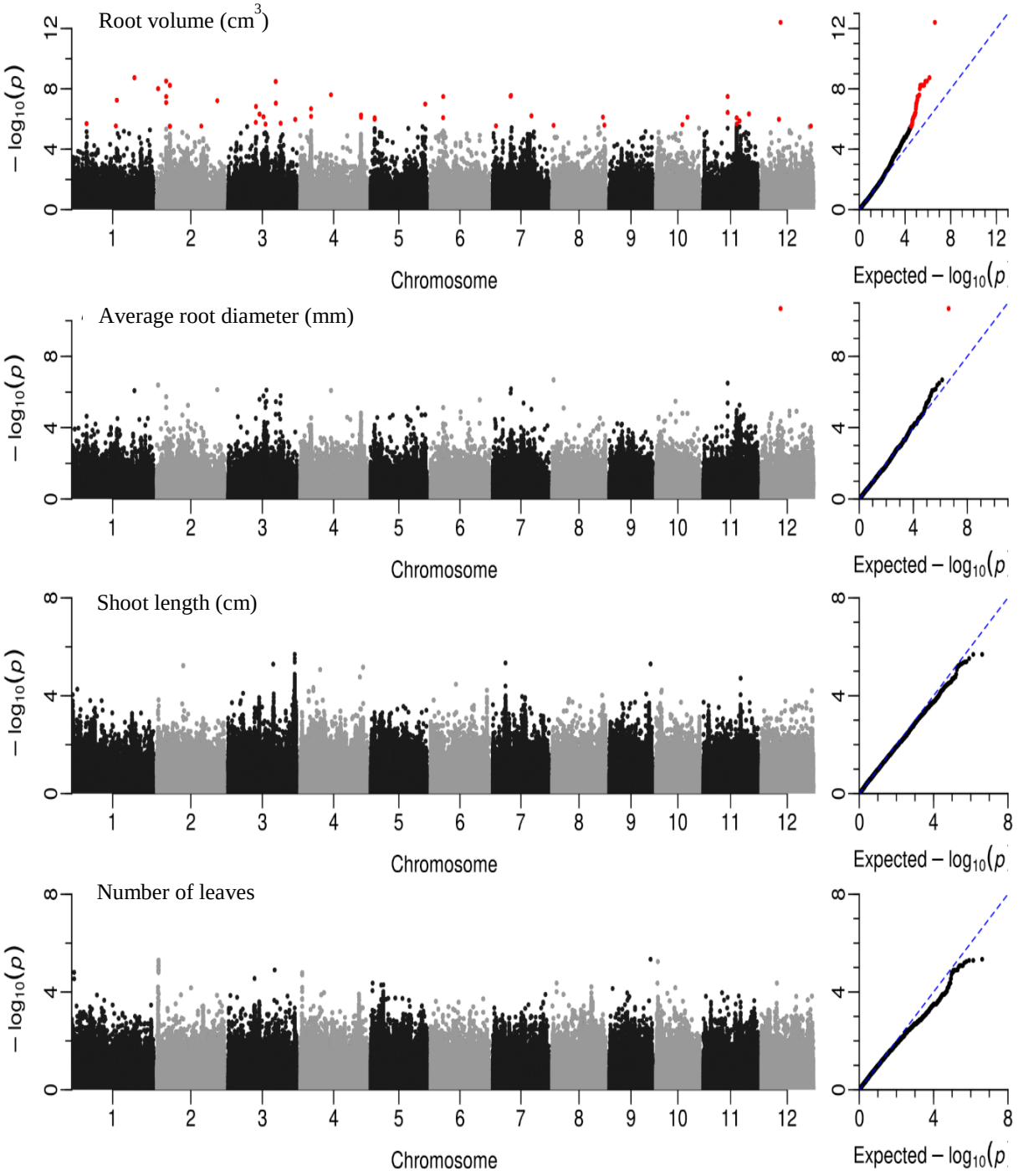
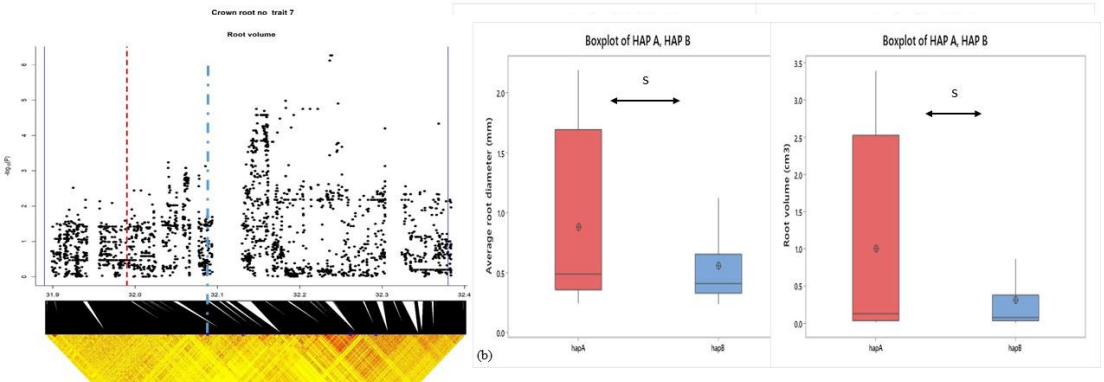


Fig.4.12 Manhattan plots from GWA mapping of total root volume, average root diameter, shoot length and number of leaves under iron deficient condition of BAAP population.

The peak SNP associated with average root diameter and total root volume was located on chromosome 4 at 32141074bp, the LD decay value highlighted a range of 243kb on either side, i.e., 31898074bp to 32384074bp on chromosome 4 (Figure 4.13). This region harboured a total of 69 genes and were annotated using the *Oryzabase* database. The gene LOC_Os04g54200, OsBIDK1 (Benzothiadiazole-inducible Diacylglycerol Kinase 1), that modulates root architecture was selected as the putative candidate gene for haplotype studies. The start position of this gene was at 32286661 bp and the end was at 32291459 bp. There were 15 SNPs in total present in this region recognised in the BAAP 2 million SNP database. Two of these SNPs were in the exon region were nonsynonymous changes localised at 31995712 bp (A/G polymorphism) and 31997883 (C/T polymorphism) which resulted in the following amino acid changes: Methionine (M) to Isoleucine (I) and Leucine (L) to Phenylalanine (F) respectively. The studied cultivars formed two haplotypes hap A (n=22) and hap B (n=138). The haplotype B was (root volume = 0.3045 cm^3 and average root diameter=0.556mm) and significantly different than superior group hap A (root volume = 1.001 cm^3 and average root diameter=0.883mm).

Shoot length and number of leaves shared a peak SNP at 16362848bp on chromosome 11 as per the clump analysis. The LD value highlighted the genomic range from 16119848bps to 6605848bps (Figure 4.14). There were a total of 32 genes that were annotated using the *Oryzabase* database. The gene LOC_Os11g28360 (16316873 – 16321329bp), encoding OsFLA (Flower and Leaf color aberrant) was selected as the putative candidate gene. There were 22 SNPs in total present in this region recognised in the BAAP 2million SNP database. Two of the 15 SNPs was in the exon region of which responsible for nonsynonymous changes. This was localised at 16319996 bp (G/A polymorphism) and at 16320009 bp (C/T polymorphism). These resulted in the following amino acid changes: Cysteine (C) to Tyrosine (Y) and Histidine (H) to Tyrosine (Y) The studied cultivars formed two haplotypes based on the abovementioned SNP; Hap A (n=22) and hap B (n=138). The haplotype B was (shoot length = 17.89cm and number of leaves = 2.48) significantly different than Hap A (shoot length = 20.67 cm and number of leaves = 2.74). Hap A was superior than Hap B.



(c)

Position (bp)	31995712	31997883	Phenotype	
Codon	atG/atA	Ctc/Ttc		
Amino acid	M/I	L/F	Root volume (cm^3)	Average root diameter (mm)
Nipponbare	G	C		
Hap A	A	C	1.001	0.883
Hap B	G	T	0.304	0.556

Fig.4.13 Significant association for root volume, average root diameter on chromosome 4 at 32141074bp a) Local Manhattan plot (top) and LD heat map (bottom) of QTL on chromosome 3 (243 kbp upstream and downstream of the peak SNP) and blue

dash line represents the candidate gene *OsBIDK1* (LOC_Os04g54200); b) Boxplot and significance test representation of haplotypes for shoot length (c) Nonsynonymous SNPs in the candidate gene *OsBIDK1* significantly associated with root volume, average root diameter and amino acid variations and haplotype mean.

Fig.4.14 Significant association for shoot length, number of leaves on chromosome 11 at 16362848bp a) Local Manhattan plot (top) and LD heat map (bottom) of QTL on chromosome 11 (243 kbp upstream and downstream of the peak SNP) and blue dash line represents the candidate gene *OsFLA* (LOC_Os11g28360); b) Boxplot and significance test representation of haplotypes for shoot length (c) Nonsynonymous SNPs in the candidate gene *OsFLA* significantly associated with shoot length, number of leaves and amino acid variations and haplotype mean

Table.4.21 SNP polymorphisms and haplotype group for root volume, average root diameter, shoot length and number of leaves under iron deficient trial.

Root volume and Average root diameter			Shoot length and Number of leaves		
BAAP	31995712	31997883	BAAP	16319996	16320009
Nip	GG	CC	Nip		
274	AA	TT	237	A G	TT
1	AA	TT	187	AA	TT
121	AA	TT	58	AA	TT
122	AA	TT	1	AA	TT
136	AA	TT	126	AA	TT
143	AA	TT	61	AA	TT
236	AA	TT	60	AA	TT
197	AA	TT	188	AA	TT
133	AA	TT	30	AA	TT
92	AA	TT	81	AA	TT
129	AA	TT	133	AA	TT
174	AA	TT	9	AA	TT
180	AA	TT	13	AA	TT
233	AA	TT	6	AA	TT
137	AA	TT	8	AA	TT
222	AA	TT	242	AA	TT
239	AA	TT	248	AA	TT
248	AA	TT	7	AA	TT
245	AA	TT	63	AA	TT
241	AA	TT	245	AA	TT
187	GG	CC	49	AA	TT
198	GG	CC	33	AA	TT
95	GG	CC	16	AA	TT
58	GG	CC	73	AA	TT
220	GG	CC	15	AA	TT
145	GG	CC	47	AA	TT
178	GG	CC	179	AA	TT

75	GG	CC	255	AA	TT
103	GG	CC	198	GG	CC
219	GG	CC	95	GG	CC
31	GG	CC	220	GG	CC
101	GG	CC	145	GG	CC
89	GG	CC	178	GG	CC
83	GG	CC	75	GG	CC
221	GG	CC	103	GG	CC
104	GG	CC	274	GG	CC
69	GG	CC	219	GG	CC
184	GG	CC	31	GG	CC
206	GG	CC	101	GG	CC
38	GG	CC	89	GG	CC
213	GG	CC	83	GG	CC
90	GG	CC	221	GG	CC
27	GG	CC	104	GG	CC
45	GG	CC	69	GG	CC
106	GG	CC	184	GG	CC
41	GG	CC	206	GG	CC
43	GG	CC	38	GG	CC
288	GG	CC	213	GG	CC
225	GG	CC	90	GG	CC
55	GG	CC	27	GG	CC
126	GG	CC	45	GG	CC
61	GG	CC	41	GG	CC
21	GG	CC	106	GG	CC
230	GG	CC	43	GG	CC
94	GG	CC	288	GG	CC
151	GG	CC	225	GG	CC
60	GG	CC	55	GG	CC
25	GG	CC	121	GG	CC
217	GG	CC	21	GG	CC
155	GG	CC	230	GG	CC
125	GG	CC	94	GG	CC
195	GG	CC	151	GG	CC
226	GG	CC	25	GG	CC
188	GG	CC	217	GG	CC
275	GG	CC	155	GG	CC
72	GG	CC	125	GG	CC
209	GG	CC	195	GG	CC
176	GG	CC	226	GG	CC
99	GG	CC	122	GG	CC

4	GG	CC	72	GG	CC
128	GG	CC	275	GG	CC
215	GG	CC	209	GG	CC
87	GG	CC	176	GG	CC
172	GG	CC	99	GG	CC
268	GG	CC	4	GG	CC
157	GG	CC	128	GG	CC
30	GG	CC	87	GG	CC
111	GG	CC	215	GG	CC
117	GG	CC	172	GG	CC
175	GG	CC	268	GG	CC
177	GG	CC	136	GG	CC
39	GG	CC	157	GG	CC
290	GG	CC	143	GG	CC
203	GG	CC	236	GG	CC
183	GG	CC	111	GG	CC
91	GG	CC	39	GG	CC
81	GG	CC	117	GG	CC
70	GG	CC	175	GG	CC
24	GG	CC	177	GG	CC
86	GG	CC	290	GG	CC
76	GG	CC	203	GG	CC
271	GG	CC	183	GG	CC
267	GG	CC	197	GG	CC
200	GG	CC	91	GG	CC
265	GG	CC	70	GG	CC
276	GG	CC	24	GG	CC
280	GG	CC	86	GG	CC
2	GG	CC	76	GG	CC
68	GG	CC	92	GG	CC
211	GG	CC	271	GG	CC
9	GG	CC	267	GG	CC
65	GG	CC	200	GG	CC
13	GG	CC	129	GG	CC
127	GG	CC	265	GG	CC
6	GG	CC	276	GG	CC
229	GG	CC	280	GG	CC
114	GG	CC	2	GG	CC
214	GG	CC	68	GG	CC
228	GG	CC	211	GG	CC
205	GG	CC	65	GG	CC
185	GG	CC	174	GG	CC

147	GG	CC	127	GG	CC
272	GG	CC	229	GG	CC
82	GG	CC	180	GG	CC
102	GG	CC	114	GG	CC
108	GG	CC	214	GG	CC
165	GG	CC	228	GG	CC
8	GG	CC	185	GG	CC
150	GG	CC	205	GG	CC
124	GG	CC	147	GG	CC
97	GG	CC	272	GG	CC
273	GG	CC	82	GG	CC
54	GG	CC	102	GG	CC
167	GG	CC	108	GG	CC
46	GG	CC	165	GG	CC
237	GG	CC	150	GG	CC
141	GG	CC	124	GG	CC
116	GG	CC	233	GG	CC
64	GG	CC	97	GG	CC
79	GG	CC	137	GG	CC
107	GG	CC	54	GG	CC
159	GG	CC	273	GG	CC
7	GG	CC	222	GG	CC
270	GG	CC	167	GG	CC
5	GG	CC	46	GG	CC
63	GG	CC	141	GG	CC
19	GG	CC	116	GG	CC
49	GG	CC	64	GG	CC
33	GG	CC	79	GG	CC
173	GG	CC	107	GG	CC
170	GG	CC	159	GG	CC
216	GG	CC	239	GG	CC
16	GG	CC	270	GG	CC
73	GG	CC	5	GG	CC
278	GG	CC	19	GG	CC
113	GG	CC	173	GG	CC
160	GG	CC	170	GG	CC
53	GG	CC	216	GG	CC
23	GG	CC	278	GG	CC
201	GG	CC	113	GG	CC
15	GG	CC	241	GG	CC
47	GG	CC	160	GG	CC
179	GG	CC	53	GG	CC
181	GG	CC	23	GG	CC
130	GG	CC	201	GG	CC
208	GG	CC	181	GG	CC

255	GG	CC	130	GG	CC
191	GG	CC	208	GG	CC
123	GG	CC	191	GG	CC
242	A G	CC	123	GG	CC

The four traits were studied together and the accessions that were common among the three were selected on the basis of root and shoot growth traits. Only 5 of the accessions were common in the superior haplotype across the four traits. Among these five lines, Rata boro had the highest root volume (3.316cm^3) and average root diameter (2.19mm) followed by Tulsi boro (root volume – 2.254cm^3 and average root diameter – 1.66mm). The shoot length was highest in Sada boro G1 (26.05cm) and the average number of leaves were 2.8.

DISCUSSION

This study aimed to highlight the genomic regions regulating plant growth under nutrient-deficient systems of nitrogen, phosphorus, and iron separately by studying the SNP polymorphisms through GWAS that can aid in developing genotypes with superior haplotypes for nutrient use efficiency traits. The results from these experiments are discussed in the following sections.

5.1 Genetic variability among rice genotypes under low levels of nitrogen, phosphorus, and iron

The BAAP accessions used in the study displayed significant variations among them in all three experiments highlighting the existence of variability among these accessions. The traits that were studied in each of the experiments were different. As evident from the reults, there was a significant amount of variability for each of these traits, however the scope of improvement for these traits depends on many other factors such as heritability, genetic advance, etc. The following sections discuss these factors focusing separately on the nitrogen, phosphorus, and iron-deficient experiments.

5.1.1 Genetic variability among rice genotypes under low levels of nitrogen

The analysis of variance indicated that the accessions used in this study exhibited significant mean sum of squares, indicating the presence of ample variability for the studied traits. Swarna, a popular rice variety in India, was one of the accessions in the panel. As an improved variety the response of Swarna towards N deficiency can be used a check to have a comparative study with other accessions in the panel. The chlorophyll index was low (i.e., below 30 for 76 accessions) which was due to the low levels (50%) of nitrogen content maintained in the field. Similar results were also reported by Swain and Sandip (2010), Yang *et al*. (2014). The reason behind this is that nitrogen is an integral part of the chloroplast and it has been reported that 80% of the leaf nitrogen is diverted to the chloroplasts, of these 50% is used in the synthesis of photosynthetic proteins (Xiong *et al*., 2015). The heritability was 0.51 and the trait can be enhanced by selection. The mean days to 50% flowering was 87 days, and more than half of the total number of accessions were earlier in flowering than the mean. Flowering is another significant trait that is influenced by different levels of nitrogen, low nitrogen status in soils accelerates early flowering, and typically higher nitrogen dose leads to a delay in flowering time and ripening. Similar results were reported by Sanagi *et al*. (2021) and Zhang *et al*. (2021). Leaf width is an important trait that affects the leaf area and ultimately the photosynthetic efficiency of plants (Rahman *et al*., 2013). In this study, the mean flag leaf width was 1.43 cm with moderate heritability amenable for improvement via selection. The mean value for all the traits in the panel except for plant height were lower than the performance of Swarna, which had

higher leaf width (1.82cm) and number of productive tillers (12.66) and maintained a SPAD value of 31.99. All the studied traits in the nitrogen-deficient trial displayed symmetric distribution in the population except for flag leaf width, flag leaf length, and panicle length which were positively skewed. An increased dose of N would mean a higher chlorophyll index and in turn enhance other yield attributing characters such as productive tillers, leaf width, etc. but identifying plants that have higher N use efficiency even under deficient soil conditions would be helpful in further deciphering the genetic regulations governing them. Under N deficiency the time taken for flowering is reduced as a result the heading commences early and yield is compromised. The decrease in yield is due to the low number of productive tillers rather than the number of tillers. The grain filling is also hampered and the number of chaffy grains is increased. This is reflected in the correlation analysis where the days to 50% flowering with a number of productive tillers and grain yield per plant but not with a number of tillers. Flag leaf width had a positive correlation with panicle length and grain yield per plant but a negative correlation with the number of tillers. This established the importance of flag leaf width (indirectly flag leaf area) with the yield. These results are in support of previous reports by Agahi *et al*. (2007), Khaliq *et al*. (2008) and Rahman *et al*. (2013). The principal component analysis highlighted the role of flag leaf width and panicle length in the first component as the major contributors to variation. The data on distribution for these traits was positively skewed explaining the variability for this trait. Based on the correlation and PCA analyses, grain yield per plant was found to be highly correlated with flag leaf width and panicle length. These traits also had major contribution to the variability in the population, thus were chosen for further GWAS studies.

5.1.2 Genetic variability among rice genotypes under low levels of phosphorus

The analysis of variance indicated that the accessions used in this study have a significant mean sum of squares, indicating the presence of ample variability for the studied traits; chlorophyll index, shoot length, root length, number of leaves, number of roots, shoot dry weight, root dry weight, and SDW : RDW ratio at 28 days of seedling stage. The mean chlorophyll index was 27.21 at 28 days of the seedling. There have been contradictory reports regarding the role of phosphorus in chlorophyll development. Studies by Peng *et al*. (1999), Wissuwa and Ae (2001), and Panda *et al*. (2021) have reported that there was no or little effect of low P on the SPAD value of the leaves. Phosphorus mainly contributes to biomass accumulation in the plants, root and shoot growth (Panda *et al*., 2021a). The mean shoot length was 38.86 cm higher than the shoot length of Dular (35.65cm), a low P tolerant cultivar (Anandan *et al*., 2022a). A total of 125 accessions had a higher shoot length than Dular. These are the accessions that have survived better in low P medium with a comparatively higher P use efficiency. The mean root length was 11.84 cm while Dular had a root length of 8.50 cm. Of the 125 accessions with higher shoot lengths, 119 were having higher shoot

length than Dular. These accessions are promising with higher biomass accumulation, showing moderate to high heritability, and selection can be effective. Apart from the root and shoot length, their dry weights are important criteria to estimate the biomass accumulated in them. The mean shoot dry weight of the panel and that of Dular was 0.062 g and 0.074 g respectively while the mean root dry weight of the panel and that of Dular was 0.02 g and 0.025 g respectively. P uptake and accumulation by the rice seedlings start as early as 2-3 days after germination, irrespective of the seed P reserves (Jia *et al*., 2011; Sun *et al*., 2012). Thus, higher root growth or preferably a large number of shallow roots is a desirable character in the initial stages of the seedling. For a plant to be efficient in P utilization, the shoot biomass must ultimately be increased, just increasing root proliferation and biomass would not suffice. Thus, the main criteria of all should have high SDW, 58 accessions had higher SDW and shoot length than Dular. These included 31 accessions that had higher RDW than Dular. There is evidence that increased root dry weight doesn't reflect a higher shoot dry weight and vice versa (Panda *et al*., 2021a), therefore we calculated an index value from the ratio of SDW and RDW to visualize the extent of root and shoot biomass accumulation in the different accession of the panel. A higher value for this ratio would indicate higher shoot biomass compared to the root and better allocation of resources to the shoot. Dular had a value of 3.02 while the panel means was 4.49. There were 49 accessions that had a higher index value than the panel means, showing better root–shoot transfer of resources as reflected in their biomass. The heritability for these traits was moderate to high and thus selection can be effective. The shoot length and SDW were symmetrical in the distribution in the panel while root length and RDW were positively skewed. The P deficient hydroponic trial highlighted a positive correlation of shoot dry weight with root dry weight, shoot length, and root length. This highlights the effect of low P levels on the growth of rice seedlings reflected in the root and shoot biomass accumulation. Number of roots didn't have any significant correlation with root length, this can be justified by the fact that P acquisition requires shallow roots rather than long deep roots (Gewin, 2010). Shoot length, shoot dry weight and root length had the highest contribution to the whole variability observed in the population.

5.1.3 Genetic variability among rice genotypes under low levels of iron

The analysis of variance indicated that the accessions used in this study have a high significant mean sum of squares, indicating the presence of ample variability for the studied traits; chlorophyll index, shoot length, root length, number of leaves, number of roots, average root diameter, root volume, number of root tips, total root length, total projected area, and total surface area at 14 days seedling stage. Under Fe deficiency, the plants start showing chlorotic symptoms (interveinal chlorosis) due to the degradation of chlorophyll (Anandan *et al*., 2021). The mean SPAD value for the panel under Fe deficiency was 7.66 on the 14th day, the highest SPAD value was 28.27. The heritability for chlorophyll Index was high and thus

can be improved further by selection. The mean shoot length was 11.42 cm and the number of leaves was 2.53, both of which had a moderate heritability. Under Fe deficient conditions, maintenance of chlorophyll, leaf number, and shoot length are critical because Fe is an integral part of the chlorophyll molecule (Kobayashi and Nishizawa, 2012). The mean root volume and average root diameter are critical for the plant under stress as they help in iron uptake, so their expression under nutrient deficiency was an important aspect of the study. The mean root volume of the panel was 0.58 cm^3, while the average root diameter was 0.62 mm. Both the number of leaves and number of root tips displayed positive kurtosis indicating that a large fraction of the accessions had extreme values and only a few hovered around the mean. The accessions from the high extreme end of the tails can be utilized for the improvement of these traits. The correlation matrix highlighted the positive correlation between chlorophyll index and number of leaves, number of leaves, and shoot length. This shows that a plant maintaining high chlorophyll index under Fe deficiency will have a higher number of leaves and ultimately a longer shoot length. Additionally, the significant positive correlation of Chlorophyll index and the number of leaves with root volume, total root projected area, and total root surface area highlight the fact that the better the root structure better is the plant's ability to maintain chlorophyll and leaf structure. The principal component analysis highlighted the contribution of the roots more than the above-ground organs. This signifies the importance of the roots and their manifestation under nutrient deficiency as compared to the shoots. When compared with the iron deficiency tolerant plant RA23, the chlorophyll index was higher in RA23 as compared to the panel mean. Similarly, number of leaves and number of roots were also high in case of RA23 when compared to the panel mean. However, the characters like the root characteristics were less developed in the tolerant line as compared to the BAAP population mean. This suggests a hypothesis that under iron deficiency the plant diverts its energy towards maintaining the shoot morphology and chlorophyll in the leaves compromising with the growth and manifestation of the roots.

5.2 Identification of genetic loci, superior haplotypes, and superior donors

The 2 million SNPs of the BAAP were distributed across the twelve chromosomes, thus can be considered as a fair representation of the whole genome. The panel had good adaptation to the target environment (with abiotic stress) and had an ideal genetic variation for the traits of interest in this study. The significant SNPs and the linkage blocks around them have been represented in the respective clump tables for N, P, and Fe separately and have been illustrated in fig.5.1. Some of the QTLs were common among traits while others were unique to the trait.

Out of these, the most significant SNPs have been selected to study the genes present in the linkage block and identified a putative candidate gene for the respective nutrient use efficiency regulating traits.

5.2.1 Identification of genetic loci, superior haplotypes, and superior donors associated with nitrogen use efficiency

The variability studies, correlation, and PCA highlighted the important contribution and relation of panicle length and leaf width with the grain yield per plant. Thus, these three traits were selected for GWAS. The most significant SNP associated with flag leaf width was located at 25266590bp on chromosome 6. The linkage block around this SNP was further studied to identify a putative candidate gene that contributes to this trait. LOC_Os06g41750 (*OsAPC3*/*OsCDC27*), Anaphase Promoting Complex3, a tetratricopeptide repeat domain-containing protein was chosen as the putative candidate gene. This gene has a role in cell division and cell proliferation (Blilou *et al*., 2002). The APC protein interacts with the cell cycle regulators like the cyclins and securins leading to their degradation via 26s proteasome by ubiquitination, as a result, the cell moves on from metaphase to anaphase and exits mitosis (Eloy *et al*., 2015). Expansion of leaves on the lateral sides, i.e., to increase the leaf width can be regulated by such a protein that controls cell division. The transgenic plant with this gene is reported to be taller with larger leaves with increased root and shoot biomass in tobacco plants (Rojas *et al*., 2009). This gene stretches from 25024626bp to 25034551bp, with 35 SNPs in the BAAP containing 2 million SNP database. The two nonsynonymous SNP at 25026948bp and 2520766bp resulted in the change in amino acid from valine (V) to alanine (A) and isoleucine (I) to leucine (L) respectively. Based on the SNP variations in the targeted candidate gene, the BAAP was grouped into three haplotypes. Among them, the haplotypes A and B were superior to the third haplotype C. The SNP at 25028000bp was distinctively different between the superior haplotype coding for isoleucine and the inferior haplotype coding for leucine. The linkage block for panicle length was centered around the SNP at 5244473bp on chromosome 4, the putative candidate gene for this trait was LOC_Os04g09900, ENT-COPALYL DIPHOSPHATE SYNTHASE 4 *OsCPS4*/ *GA1* annotated as a chloroplast precursor according to the RGAP database. This gene encodes a Gibberellic acid metabolic enzyme. Wu *et al*. (2016) have highlighted the role of GAs in rice inflorescence meristem via crosstalk with cytokinin. Grain Number per Panicle (*GNP1*) encodes rice GA20ox1 which is an important protein for the biosynthesis of *GA1*. Thus, increase in this gene ultimately leads to increased grain number and yield by regulating the panicle length (Yamaguchi, 2008; Wu *et al*., 2016). This gene stretched from 5318060bp to 5326427bp. Six nonsynonymous SNPs were identified in this stretch of a gene that grouped the whole panel into two haplotypes; Hap A (24.34cm) had a superior mean value for panicle length than Hap B (22.91cm). The Hap B had mutated SNPs leading to change in amino acids at all the six positions as compared to Hap A, which had the Nipponbare sequence. The most significant SNP for grain yield per plant was positioned at 23121131bp on chromosome 11. A thorough study of the linkage block surrounding

this SNP was done and LOC_Os11g39220, Acyl-CoA oxidase 2 (*OsACX2*) was selected as the putative candidate gene for this trait. This gene has a role in lysine and serotonin metabolism in rice endosperm and high levels of ACX transcript have been confirmed in their role in increasing lysine content in rice endosperm in developing seeds 10 days after fertilization (Yang *et al*., 2018). This gene extended from 23354149 – 23358318bp on chromosome 11, harboring two nonsynonymous mutations in comparison to the Nipponbare genome sequence at the positions; 23358183bp and 23358203bp. These mutations grouped the whole panel into three different classes (Hap A, Hap B and Hap C), of which Hap C was the inferior one which had no mutations at both loci. The Hap B had the highest mean grain yield per plant with a nonsynonymous mutation at 23358203bp inducing a change in amino acid from aspartate to glutamate while the SNP at 23358183bp was similar to the Nipponbare sequence. The superior haplotypes of the three traits mentioned above were studied together and three accessions were identified; DA24, DM56, and AUS210 which had higher expression of flag leaf width, panicle length, and grain yield per plant as compared to the population mean. The high grain yield of these accessions was taken into account while selecting them as superior donors. Of these three, DA 24 had the highest grain yield per plant along with a high SPAD value and a comparatively high number with 88 days to 50% flowering. When compared to Swarna, DA24 outstands the improved variety in terms of chlorophyll index, flag leaf width and grain yield. However, the number of productive tillers were 10.66 in DA24 as compared to 12.66 in Swarna. The reason behind this can be the chaffy grains that develop under low nitrogen conditions. These trends suggest that a tolerant donor is the one that can maintain its chlorophyll content and have a sufficient number of days in vegetative growth before flowering can be expected to yield more. Under nitrogen-deficient conditions the higher number of productive tillers need not always be related to high yield because of chaffy grains in the panicle. N uptake during the grain filling stage plays an important role in regulating the yield by meeting the nutrient demand in the panicles, this is achieved through development of new roots at the later part of the reproductive phase (Panda *et al*., 2021b). Thus, plants that can maintain the uptake of N in N limited soil and efficiently reallocate resources from the vegetative organs to the inflorescence at the grain filling stage can be high yielding.

5.2.2 Identification of genetic loci, superior haplotypes, and superior donors associated with phosphorus use efficiency

The correlation results and PCA highlighted the importance of root length, shoot length and shoot dry weight when the BAAP accessions were subjected to low P conditions. Under P deficiency conditions, shoot biomass accumulation can be regarded as one of the important aspects of low P tolerant plant, thus the traits that correlated positively with shoot dry weight were taken into consideration. Additionally, they

also contributed the maximum to the total variability in the panel. These three traits were then processed for GWAS analysis to identify putative candidate genes responsible for these traits. The peak SNP associated significantly with root length was at 26861688bp on chromosome 4. After a thorough study of the region in LD with this SNP, LOC_Os04g45370 or SMALL AUXIN-UP RNA 19 (*OsSAUR19*) member of the Auxin responsive SAUR gene family. The role of auxin in rice root growth has already been reported extensively (Ni *et al*., 2011; Zhu *et al*., 2012; Sun *et al*., 2018). The SAUR genes are reported to be involved in cell expansion and including increasing the hypocotyl size (Spartz *et al*., 2012). It was also reported that the miRNA targeting the SAUR189-24 subfamily produced plants with reduced hypocotyls. Studies on Arabidopsis and tomato plants have suggested that *AtSAUR19* inhibits PP2C.D phosphatase activity, and then activates PM H+-ATPases to promote auxin-mediated cell expansion (Spartz *et al*., 2017). This gene harboured a nonsynonymous mutation at the SNP level at 26831650bp (G/T polymorphism) leading to the change in amino acid from methionine to arginine. This SNP grouped the panel into two classes, Hap B (12.17cm) was superior with the unmutated SNP coding for methionine while the inferior group was with the mutated SNP in comparison to the Nipponbare sequence. The peak SNP for shoot length was located at 25259001bp on chromosome 6. Studying the genes in this region after accounting for the LD, a putative candidate gene LOC_Os06g41840, Cinnamoyl-CoA reductase 7 (*OsCCR7*) was chosen. This gene has a role in maintaining the cell wall via lignin biosynthesis, a major component of the secondary cell walls is a heterogeneous tridimensional phenolic polymer formed as a result of the oxidative polymerization of monolignols (Takayoshi, 1985). Under low P conditions, there is an increase in ROS (mainly H2O2) accumulation (Zhang *et al*., 2019b). The monoignols catalyzed by CCR are transferred into the cell wall and polymerized with H_2O_2 leading to the biosynthesis of lignins (Kawasaki *et al*., 2006). The gene *OsCCR7* stretched from 25016001 to 2502001bps and harboured one nonsynonymous SNP at 25088223bp (A/T polymorphism) leading to amino acid change from arginine to serine. This SNP classified the whole panel into two groups, Hap A and Hap B. Hap A was superior to the unmutated SNP while the inferior group Hap B had a lower expression of shoot length and had the mutated SNP in comparison to the Nipponbare sequence. The peak SNP highlighted for shoot dry weight was at 12412541bp on chromosome 3. The putative candidate gene identified from the region in LD with the peak SNP was LOC_Os03g25040, *SCL28*, a gene involved in both cell proliferation and cell expansion of the shoot by activating SIAMESE-RELATED cyclin-dependent kinase inhibitors (Goldy *et al*., 2021a) by regulating mitotic division by directing the cell progression through G2/M and modulates the selection of cell division planes (Goldy *et al*., 2021b). Additionally, along with shoot biomass, this gene is also reported to be regulating root growth (Goldy *et al*., 2021b), thus being an ideal candidate gene for the accumulation of root and shoot biomass under P deficiency. This gene extended from 14294507bp to 14297898bp on chromosome 3. Five nonsynonymous SNPs were identified in this region at 14294575bp, 14296148bp, 14296472bp,

14296486bp, and 14297565bp and grouped the whole panel into three haplotypes. Among them, Hap A was superior and had mutated SNPs at all five positions compared to the Nipponbare sequence. The superior haplotype for the three traits discussed above was compared together and three accessions; Shada boro, Kele (AUS), and Kada 68-1 were selected as the superior donors based on their biomass accumulation in the shoot, shoot length, and root length. Of the three, Kada 68-1 had the highest shoot biomass accumulation along with more than the average root length, shoot length, and crown root number. The shoot biomass accumulation and shoot length of Kada 68-1 was higher than Dular. Under low P conditions, a plant with higher number of shallow root developed at the early stage of seedling ultimately increase shoot biomass can be an ideal way to select for high P use efficiency genotypes.

5.2.3 Identification of genetic loci, superior haplotypes, and superior donors associated with iron use efficiency

The GWAS studies under iron deficiency were done for four traits that represented both the shoot and roots. The root traits were root volume and average root diameter while the shoot traits were shoot length and number of leaves. The peak SNP for root volume and average root diameter was common and located at 32141074bp on chromosome 4. The region in LD with the peak SNP was searched extensively and LOC_Os04g54200, Benzothiadiazole-inducible Diacylglycerol Kinase 1 (*OsBIDK1*) was chosen as the putative candidate regulating average root diameter and root volume. This gene is reported to be involved in root growth and development as it phosphorylates diacylglycerol (DAG) to produce phosphatidic acid (PA). DAG promotes lateral root (LR) formation and suppresses surface root (SR) growth whereas PA suppresses LR number and promotes SR thickness (Yuan *et al*., 2019). The gene stretches from 32286661bp to 32291459bp and harbours two nonsynonymous at 31995712bp (A/G polymorphism) and 31997883bp (A/G polymorphism) resulting in a change in amino acids from Methionine (M) to Isoleucine (I) and Leucine (L) to Phenylalanine (F) respectively. This grouped the whole panel into two groups, Hap A was superior to Hap B in both root volume and average root diameter. The superior Haplotype A has mutated loci at 31995712bp. The peak SNP associated with shoot length and number of leaves were located on chromosome 11 and had a common SNP at 16362848bp. The region in LD with this peak SNP had a total of 32 genes, of which LOC_Os11g28360, Flower and Leaf color Aberrant (*OsFLA*) was selected as the putative candidate gene. This gene encodes a homolog of ubiquitin-specific protease. The mutant *fla* plants are reported to be exhibiting a variety of developmental defects including leaf growth and chlorophyll accumulation (Ma *et al*., 2019).

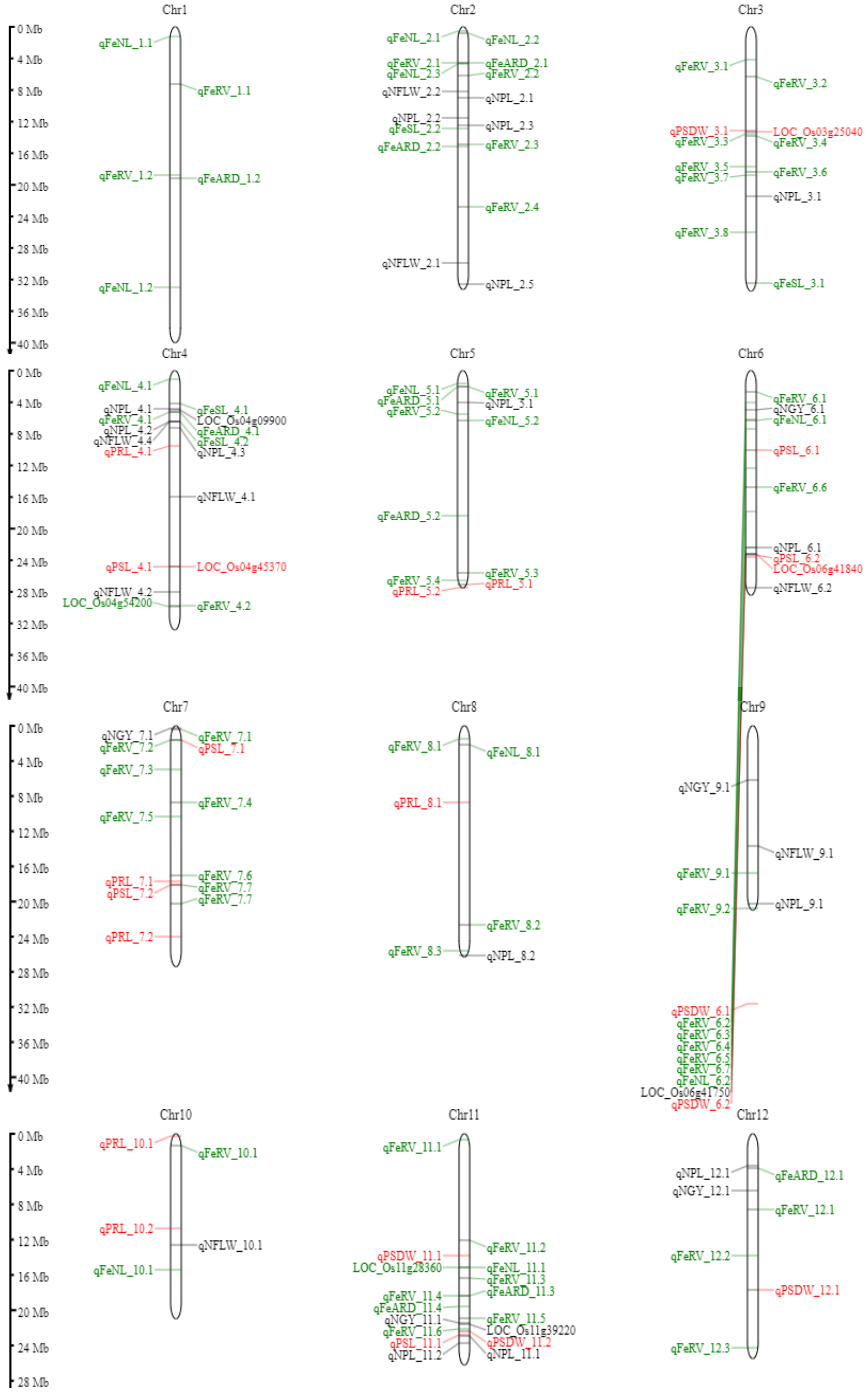


Fig.5.1 QTLs associated with various traits under nutrient deficient conditions detected across 12 chromosomes in the GWAS studies; qNPL (panicle length) , qNFLW (flag leaf width) and qNGY (grain yield per plant) under N deficient trial, qPRL (root length), qPSL (shoot length) and qPSDW (shoot dry weight) under P deficeint trial, qFeNL (number of leaves), qFeSL (shoot length), qFeARD (average root diameter) and qFeRV (root volume) under Fe deficient trial.

As the shoot length here was calculated by measuring from the base of the shoot to the tip of the longest leaf, this gene was ideal. Additionally, by the second week, most of the plants had dead leaves due to degraded chlorophyll under iron deficiency conditions, as genes such as this can participate and maintain chlorophyll levels. There were two nonsynonymous mutations at the SNP level located at 1631996bp (G/A polymorphism) and 16320009 bp (C/T polymorphism) resulting in the change in amino acid from Cysteine (C) to Tyrosine (Y) and Histidine (H) to Tyrosine (Y) respectively. These mutations grouped the whole panel into two classes; Hap A was superior to Hap B in both traits. The Hap A had the mutated SNPs at both the above-mentioned loci which indicates that the mutations were favorable for the plant under Fe deficiency. Interestingly, the superior haplotype; Hap A group also had a higher SPAD value as compared to the Hap B. The superior donors were selected by studying all the better performing haplotypes together to find common accessions. A plant with a higher shoot length, above mean SPAD value, a high number of leaves, and a well-developed root system were decided to be better in Fe use efficiency. There were five accessions that were common in all the haplotypes and none of them had higher expression in all the traits taken together. The shoot length was high in Sada boro G1 (26.05cm) with a 2.8 average number of leaves. Rata boro had the highest root volume (3.316cm^3) and average root diameter (2.19mm). Both of these accessions had the above mean SPAD value. The superior donors had better morphological characters when compared to the tolerant check RA23.

SUMMARY AND CONCLUSION

The following section briefly summarises and presents conclusions from the investigation titled, "Assessment of useful genetic variation for nutrient use efficiency in *aus* rice (Oryza sativa L.) using genome wide association studies". The nutrient use efficiency was studied as a function of nutrient utilization efficiency i.e., how well a plant maintains its growth and development under a nutrient deficient environment.

- The accessions included in the BAAP population differed significantly for all the traits under low levels of N, P and K and had varying levels of expression via morphological traits observed in each of the respective nutrient deficient mediums.
- There was significant positive correlation of grain yield per plant with panicle length and flag leaf width under N deficient condition, shoot dry weight with shoot length and root length in P deficient conditions and chlorophyll index with number of leaves and root volume in Fe deficient conditions.
- The GWAS studies under N deficient trial was done for three traits; panicle length, flag leaf width and grain yield per plant directed by the correlation and PCA results. A total of 30 QTLs were identified in association with these three traits.
- The putative candidate gene for flag leaf width; LOC_Os6g41750 (25024626 – 25034551 bp) was identified in LD with the peak SNP at 25266590bp on chromosome 6. The nonsynonymous SNPs in the gene grouped the whole panel into three haplotypes, of which Hap A (1.52cm) and Hap C (1.53cm) were found to be significantly superior. The putative candidate gene for panicle length; LOC_Os04g09900 (5318060 – 5326427bps) was in LD with the peak SNP 5244473bp on chromosome 4. There were six nonsynonymous SNPs which grouped the panel into two haplotypes and Hap A (24.34cm) was found to be superior. The putative candidate gene for grain yield per plant was LOC_Os11g39220 (23354149 – 23358318bp) in LD with the peak SNP at 23121131bp on chromosome 11. Three accessions were identified as superior donors from the better performing haplotype groups of the above mentioned traits; DA24, DM56, and AUS210.
- The GWAS study for the P deficient trial was done for three traits; root length, shoot length and shoot dry weight based on the results from correlation analysis and PCA. A total of 20 QTLs were identified with significant association levels with these traits.
- The putative candidate gene for root length; LOC_Os04g45370 (26831005 to 26831867bp) was identified in LD with the peak SNP at 26861688bp on chromosome 4. The nonsynonymous SNPs in the gene grouped the whole panel into two haplotypes, of which Hap B (11.17cm) was found to be

significantly superior. The putative candidate gene for shoot length; LOC_Os06g41840 (25016001 to 2502001bp) was found in the LD region with the peak SNP 25259001bp on chromosome 6. One nonsynonymous SNP in this region groped the panel into two haplotypes of which Hap A (40.10cm) was identified as the superior haplotype. The shoot dry weight had peak SNP at 14212541bps on chromosome 3, the putative candidate gene for this trait was identified as LOC_Os03g25040 (14294507bp - 14297898bp). This gene harboured five nonsynonymous SNPs, resulting in three different haplotypes. Hap A (0.065mg) was significantly different and superior. Twenty accessions were common for the superior haplotypes among the three traits. Of which, Shada boro, Kele (AUS) and Kada 68-1 were the selected as superior donors for the selected traits.

- The GWAS study for the Fe deficient trial was done for three traits; root volume, average root diameter, shoot length and number of leaves based on the results from PCA. A total of 78 QTLs were identified with significant association levels with these traits.
- The peak SNP for root volume and average root diameter was located at 32141074bp on chromosome 4 and the putative candidate gene; LOC_Os04g54200 (32286661bp to 32291459bp) was selected and harboured two nonsynonymous SNPs grouping the whole panel into two haplotypes. Hap B (root volume = 0.3045 cm^3 and average root diameter=0.556mm) was significantly superior with respect to Hap A. The peak SNP for shoot length and number of leaves shared a peak at 16362848bp on chromosome 11 and the putative candidate gene was identified as LOC_Os11g28360 (16316873 – 16321329bp) harbouring two nonsynonymous SNPs. The panel was grouped into two haplotypes based on these SNPs, of which Hap A (shoot length = 20.67 cm and number of leaves = 2.74) was significantly superior. Only 5 of the accessions were common in the superior haplotype across the four traits. Among these five lines, Rata boro had the highest root volume (3.316cm^3) and average root diameter (2.19mm) followed by Tulsi boro (root volume – 2.254cm^3 and average root diameter – 1.66mm). The shoot length was highest in Sada boro G1 (26.05cm) and the average number of leaves were 2.8.

CONCLUSION

The present study has exploited the knowledge of sequence information and gene annotations available in rice genome to develop SNP haplotypes for various nutrient use efficiency regulating traits under low levels of N, P and K using genome wide association mapping. The results from the study have highlighted the important traits that can be improved to regulate nutrient use efficiency; panicle length and leaf width under N deficiency, shoot length, root length and shoot dry weight in P deficiency, and shoot length, number of leaves, average root diameter and root volume in case of Fe deficiency. The haplotypes

constructed for various traits have highlighted the mutations/variations (nonsynonymous) at the SNP levels leading to changes in amino acids were highlighted for different nutrient deficient environments which can be further validated by expression studies. This information generated at the SNP level can enable further studies on the genomic variability of rice crop. The superior SNP haplotypes identified, can be used as markers in haplotype assisted breeding programmes to develop the respective nutrient use efficient traits. The accessions identified as superior donors; DA24, DM56, and AUS210 with high nitrogen use efficiency, Shada boro, Kele (AUS) and Kada 68-1 with high phosphorus use efficiency and Rata boro, Tulsi boro and Sada boro G1 with high iron use efficiency can be utilized as donors in crop improvement schemes through backcross. The genes identified as putative candidate genes need to be further validated and expression studies can be followed to identify their nature of function in the cell. This study has also focused on the root growth aspects under phosphorus and iron deficient nutrient medium. This aspect of the study has shed light on the important root traits that can be regulated in order to enhance nutrient use efficiency.

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Multiple trait contribution towards phosphorus deficiency tolerance at species level in early vegetative stage of rice

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Abstract

Phosphorus deficiency is more pronounced in rice at the early vegetative stages. Compensating rice cultivars with Phosphorus UseEfficiency traits would require identifying the component traits involved and the level of dependence between these traits. This study evaluated four diverse groups of rice, comprising the accessions of improved cultivars, landraces of *Oryza sativa*, representatives of *Oryza nivara* and *Oryza rufipogon* to understand the P uptake and its allocation between shoot and root traits. The results highlighted that traits regulated in a low P tolerant line such as root volume, total root surface area, total dry weight, SPAD value, number of root tips and leaf width. Landraces were found to have higher root volume (3.62cm), shoot dry weight (0.81g), root dry weight (0.25g) and shoot-root P ratio (2.51) as reflected in the group mean. The study also identified accession AC100219 of *O. rufipogon* group which had overall higher shoot P content, root volume, total root length and number of roots tips and may serve as a potential donor.

Keywords: *Oryza* spp., phosphorus use efficiency, rice, SPAD value.

Introduction

Phosphorus (P) is one of the key elements and a pivotal component in the structure of nucleotides, energy transferring inorganic phosphates, involved in cell growth and development, root growth, tillering, flowering and ripening. The element is highly mobile in plant systems and available in the shallow layers of the soil. But even when abundantly present in the soil, its availability to the rootsremains scarce as it becomes immobile and inaccessible forming complex with Ca, Fe, and Al in the rhizosphere (Abel et al. 2002) especially in acidic soils where the pH is low (< 6) (Penn and Camberato 2019). Estimates show that around 60% of the rice grown in rainfed conditions in Asia face P deficiency (Gamuyao et al. 2012). Especially in India,where 90% of its P demand is met from imports making it costly (Swamy et al. 2019). The practice of external P fertilizer application is reliable only up to a certain extent as the rockphosphate reserves are non-renewable and with the current rate of consumption it is set to be exhausted in near future (Anandan et al. 2021a). This calls for an alternative strategy, wherein the external application is controlled and the plant is supplemented with better P use efficiency traits (Pandit et al. 2018; Reddy et al. 2020). However, the hindrances the absence of P efficient traits in most of the modern cultivars of rice (Mahender et al. 2017; Nirubana et al. 2020). For instance, in the sequencing of *Pup1* locus in the Aus variety, Kasalath has revealed that the characteristic 90kb transposon rich indel is missing from the Nipponbare reference genome (Gamuyao et al. 2012). Thus, mining out alleles conferring tolerance in the genotypes expressing them must be the primary effort. Rice is being grown in diverse ecological systems: this has led to diverse adaptive features, with higher degrees of variability between genotypes. Such variations can be exploited especially when we try to improve a trait like abiotic stress tolerance.

Landraces have always been the source of abiotic stress tolerance, may it be the low P tolerance, (Gamuyao

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et al. 2012), submergence (Bailey-Serres et al. 2010), or heatstress (Prasanth et al. 2017). They have been traditionally cultivated and adapted to tolerate stress and give a stable yield even under a low input environment. Abiotic stresstolerant accessions for soil moisture deficit have been identified in the *O. nivara* L. and *O. rufipogon* L. (Kaur et al. 2018; Luo et al. 2019). A study comprising of 182 accessions of *O. rufipogon* was undertaken for highlighting the P use efficiency traits and the accession IRGC 106506 performed better with respect to root weight and P content in comparison to the positive control, Vandana (Neelam et al.2017). Screening diverse lines for adaptation to low P traits would require focusing not only on the shoot but also onthe root traits. Therefore, various root features along with the shoot morphological traits that would best define a low P tolerant plant have been considered in the present study.This information would improve the basic understanding of the overall response of *Oryza* spp., towards low P stress in the soil during the early vegetative stages. The inclusion of *O. nivara* and *O. rufipogon* in addition to the landraces, improved cultivars would allow us to have a comparative analysis between the four distinct groups and highlight thespecific traits regulated under low P stress.

Materials and methods

Plant materials, experimental design and crop growth conditions

The population for the study included 155 accessions (Supplementary Table S1); 77 improved varieties, 37 landraces, and 41 wild species (22 *O. rufipogon* and 19 *O. nivara*) originating from eight different states of India. Seeds for the experiment were collected from ICAR-National Rice Research Institute (NRRI), Cuttack, Odisha, and Regional Research & Technology Transfer Station (RRTTS), Coastal zone, Bhubaneswar, Orissa University of Agriculture and Technology (OUAT).

Phosphorus stress phenotyping in the microplot

The low P stress for the experiment was created in a microplot facility at NRRI, Cuttack (20°27'09" N, 85°55'57" E, 26 masl). The genotypes were direct seeded in the plots with a spacing of 20cm X 15cm in three replications during June 2019. Soil testing was performed to determine the pH in 1:25 soil-water suspension, available P was determined following the Bray and Kurtz no.1 method, alkaline-KMnO4 extractable nitrogen using a Kelplus-Elite Ex distillation unit (Pelican Equipments, Chennai, India) and 1 N NH4OAc extracted available potassium using a flame photometer (Flame photometer-128, Systronics Limited, India) were measured in the soil samples (Chatterjee et al. 2021). The micro plots were characterised by acidic pH (4.9), low levels of P (<3kg/ha), while nitrogen (295 kg/ha) and potassium (174 kg/ha) levels were in the medium range which was sufficient to grow a crop for 45 days. However, basal recommendation dose (80:0:40 Kg) of N and K were given without any external P fertilizer. The seeds were heat-treated at 50°C for 45 h in a hot air oven to break seed dormancy ifpresent. The average day/night temperature was 33.6/26°Cand the relative humidity was 85.9% in bright sunlight of the experiment period. Irrigation was given every alternate day and thinning was done to maintain the population at two seedlings per hill. The chlorophyll content was measured on the 44th day for three plants of each accession with a SPAD meter (SPAD-502, Konica Minolta). Three plants from each genotype were uprooted the next day (45th day) to observe the morphological traits such as shoot length (cm), number of tillers plant^{-1}, number of leaves plant^{-1}, 3rd leaf length (cm) and width (cm), stem thickness (mm), maximum root length (cm) and root shoot length ratio. Other root traitssuch as total root length (cm), projected root area (cm^2), root surface area (cm^2), average root diameter (mm), root volume (cm^3), and the number of root tips were recorded for each genotype per replication by analyzing them with WinRHIZOPro 2013e (LA 2400, Regent Instruments INC.) root scanner. The dry weight of shoot and root were recorded in grams after drying the samples in a hot air oven (5-6 days, 60°C). The geometric trait; top view area (mm^2) was calculated utilising Image J software as described by Anandan et al.(2020) and Bhatta et al. (2021). The P quantification was done following the phospho molybdo vanadate colorimetricmethod with dry powdered 300 mg of the shoot and 90 mgof root sample. The concentration of P in these samples wasdetermined using Systronics, UV Spectrophotometer at 420 nm. The total shoot and root P contents were determined on mgg^{-1} dry weight basis. In order to derive an index value for shoot and root P content, shoot-root P ratio was calculated for ease of categorising genotypes. Additionally, mycorrhiza colonization was also examined following Trypan blue staining method (Koske and Gemma 1989) withminor modifications. The final slides were prepared with atleast 10 root pieces to study under a stereomicroscope and the colonization in the roots were calculated using the formula described by McGonigle et al. (1990). Percentage of colonization = (number of root segments colonized /totalnumber of root segments) x 100.

Statistical analysis

The variation in the sample population due to the P factor was determined using analysis of variance and descriptive statistics in Windostat 7.5 for the 23 traits mentioned above. Principle Component Analysis (PCA) was performed usingFactoMine R package (Lê et al. 2008) in R on a matrix of 23 morphometric and geometric traits. Correlation between the traits under low P environment was executed with the corrplot function from the corrplot package in R (version
3.6.3) (Wei and Simko 2016). The K means clustering approach was carried out separately for each group of

genotypes to identify genotypes having similar phenotypic expression and traits that are similar under the P deficiency condition. Similarly, a cluster heat map function (x, scale = "none", dual Scale = FALSE, method = "ward.D2") of the R package made4 (Culhane et al. 2005; Bhatta et al. 2021) was performed for each category of genotypes using 23 traits.

Results

Principal component analysis

The analysis of variance revealed that significant variation ($P < 0.001$) was observed among the genotypes for all 23 traits studied (Supplementary Table S2). Data of 155 genotypes were subjected to principal component analysis (PCA) (Fig. 1). It reduced the data dimensionality and highlighted the traits that contribute the most to the total variance. The analysis revealed six main PCA axes, each having eigenvalues more than one which ensured that each one of these components explained variance more than that of the original variables accounted in the standardized data. This aided in reducing the number of PCs involved in the study. PC1 explained 38.6% of the total variability between the sample genotypes and individual traits. Root volume was the most significant character for PC1 followed by total root surface area, total root projected area, and total dry weight. The genotypes on the higher end of PC1 were the ones that were affected by the low P in soil but still maintained high values for all the traits except for root P, shoot P, root/shoot length, and root/shoot dry weight which were uncorrelated or negatively correlated with rest of the traits. PC2 explained 15.8% of the rest of the variance where SPAD value, number of root tips, and leaf width had a major contribution. The genotypes on the higher end of PC2 maintained high root P content but were not reflected in the shoot P (uncorrelated). The rest four of the PCs had eigenvalues of more than one but their contribution in the variance was below ten percent. Interestingly, the mycorrhizal colony had a positive correlation with PC of 5 and 6. Overall the PCA revealed the heterogeneity between the samples, whereas samples within the group more or less followed a similar trend. This calls for treating the four groups of rice and wild relatives separately.

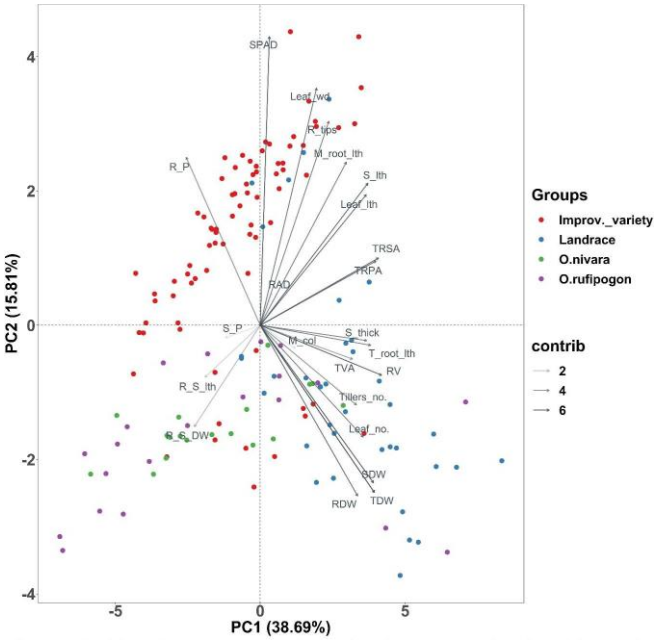

Fig. 1. PCA Biplot graph represents genolypes (Improved, landrace, *O. nivara* and *O. rufipogan*) in two main principal components for traits measured under P deprived condition. The two components explained 38.6% and 15.8% of the variance, respectively. The direction and length of the vector indicate the traits' contribution to the first two components in the PCA. The transparency of the trait vectors represents the contribution to the variance in the dataset, ranging from 2% (lightest) to 6% (darkest)

Descriptive statistics and correlation

The comparative results and spread of the variation (data) of individual group have been represented by the violin graphs in Figs. 2 and 3. The mean value for root volume (3.62 cm^3) was highest in the case of landraces with a CV of 39% (Supplementary Tables S3 – S6). Both shoot dry weight and root dry weight were higher in the landraces with values 0.81 g and 0.25 g respectively (Supplementary Table S3). However, the values for root dry weight were skewed (1.48) and the kurtosis peaked at 2.24. This indicates that few cultivars within the landraces have out-performed the others. Interestingly, the root-shoot dry weight ratio was high in the case of *O. rufipogon* (1.07) (Supplementary Table S4), while the lowest value for this trait was observed in the case of landraces (0.44). The total root length (904.12 cm) and shoot length (26.1 cm) were higher in the case of landraces, however, the root-shoot length ratio was found to be proportionately higher in the case of *O. rufipogon* with a CV of 78% with a minor skewness of 1.1. This shows that

O. rufipogon has physiologically balanced the proportionate values for both root-shoot dry weight and root-shoot length comparatively. The total P content in root was higher in the case of improved varieties (1.25 mg/g) (Supplementary Table S5), while the highest P content in the shoot was found in *O. rufipogon* (1.36 mg/g). But, the shoot-root P ratio revealed a different picture. The landraces had a value of 2.51, the highest among the four groups indicating its efficiency in uptake and distribution of a major part of its P to the shoots. The four different groups manifested different correlations (Figs. 4a-d) among the traits except a few that were similar in more than one of the groups. Each of the groups' shoot length had a high positive correlation with leaf length. Leaf width was positively correlated with maximum root length in improved varieties and *O. rufipogon* with values

0.70 and 0.66, respectively. Shoot length was negatively correlated to the root-shoot dry weight ratio in all the groups except improved varieties. Total root length was positively correlated with the number of root tips (ranging from 0.55 in

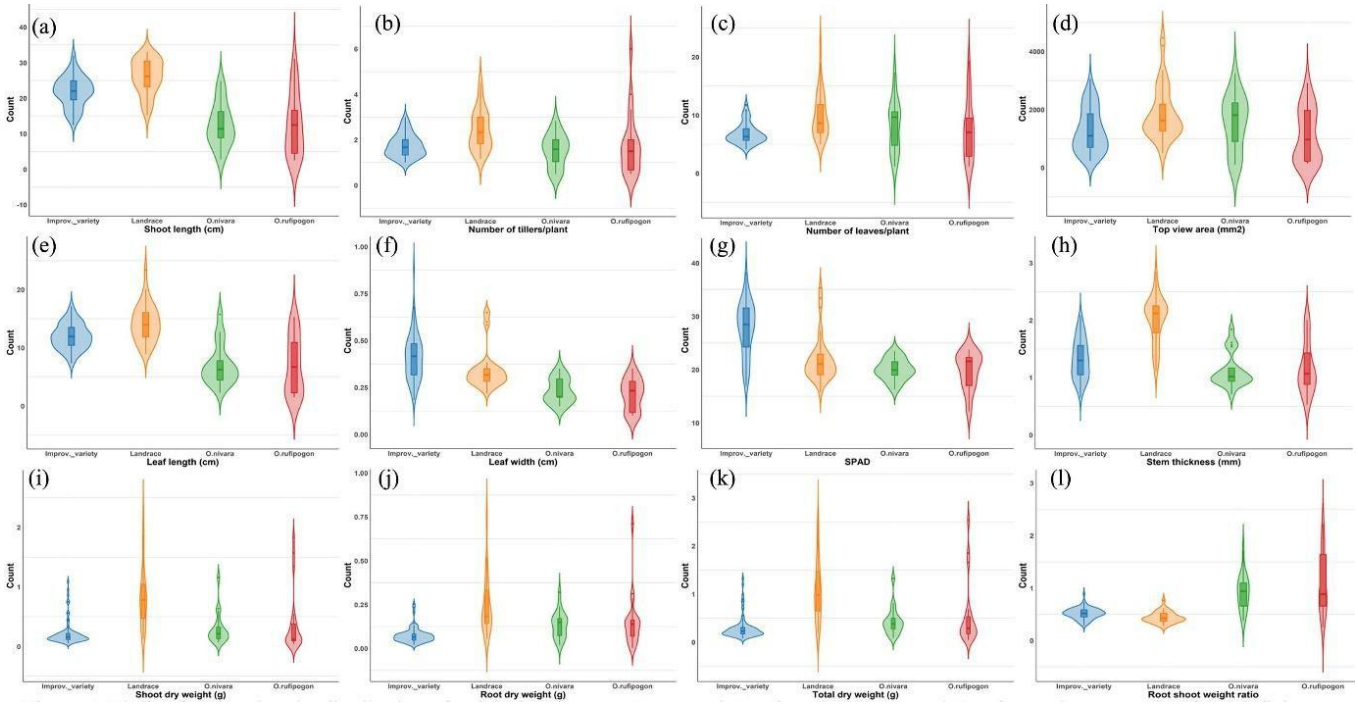

Fig. 2. Violin plot showing the distribution of traits of each group (Improved, landrace, *O. nivara* and *O. rufipogan*) measured under P deficiency.

(a) Shoot length, (b) Number of tillers-1, (c) Number of leaves-1, (d) Top view area, (e) Leaf length) (f) Leaf width, (g) SPAD, (h) Stem thickness, (i) Shoot dry weight, (j) Root dry weight, (k) total dry weight and (l) root shoot weight ratio. The upper, median and lower quartiles of boxes (inside violin plot) represent the 75th, 50th and 25th percentiles of the population, respectively. The line extendedfrom both side of the inside box represents the maximum and minimum value of the trait. Dots after the extended line represents outliers. Width of the plot represents frequency

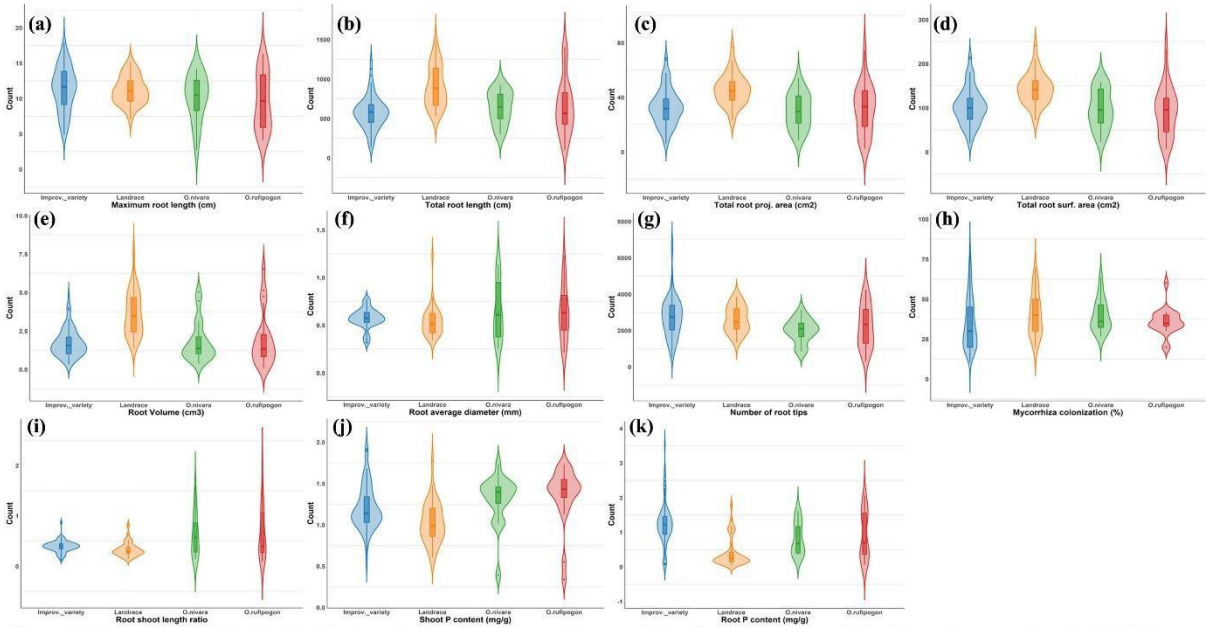

Fig. 3. Violin plot showing the distribution of traits of each group (Improved, Landrace, *O. nivara* and *O. rufipogan*) measured under P deficiency.

(a) Max. root length, (b) Total root length, (c) Total root projected area, (d) Total root surface area, (e) Root volume, (f) Root avaragediameter, (g) Number of root tips, (h) *Mycorrhiza* colonization, (i) Root shoot length ratio, (j) Shoot P content and (k) Root P content.The upper, median and lower quartiles of boxes (inside violin plot) represent the 75th, 50th and 25th percentiles of the population, respectively. The line extended from both side of the inside box represents the maximum and minimum value of the trait. Dots after the extended line represents outliers. Width of the plot represents frequency

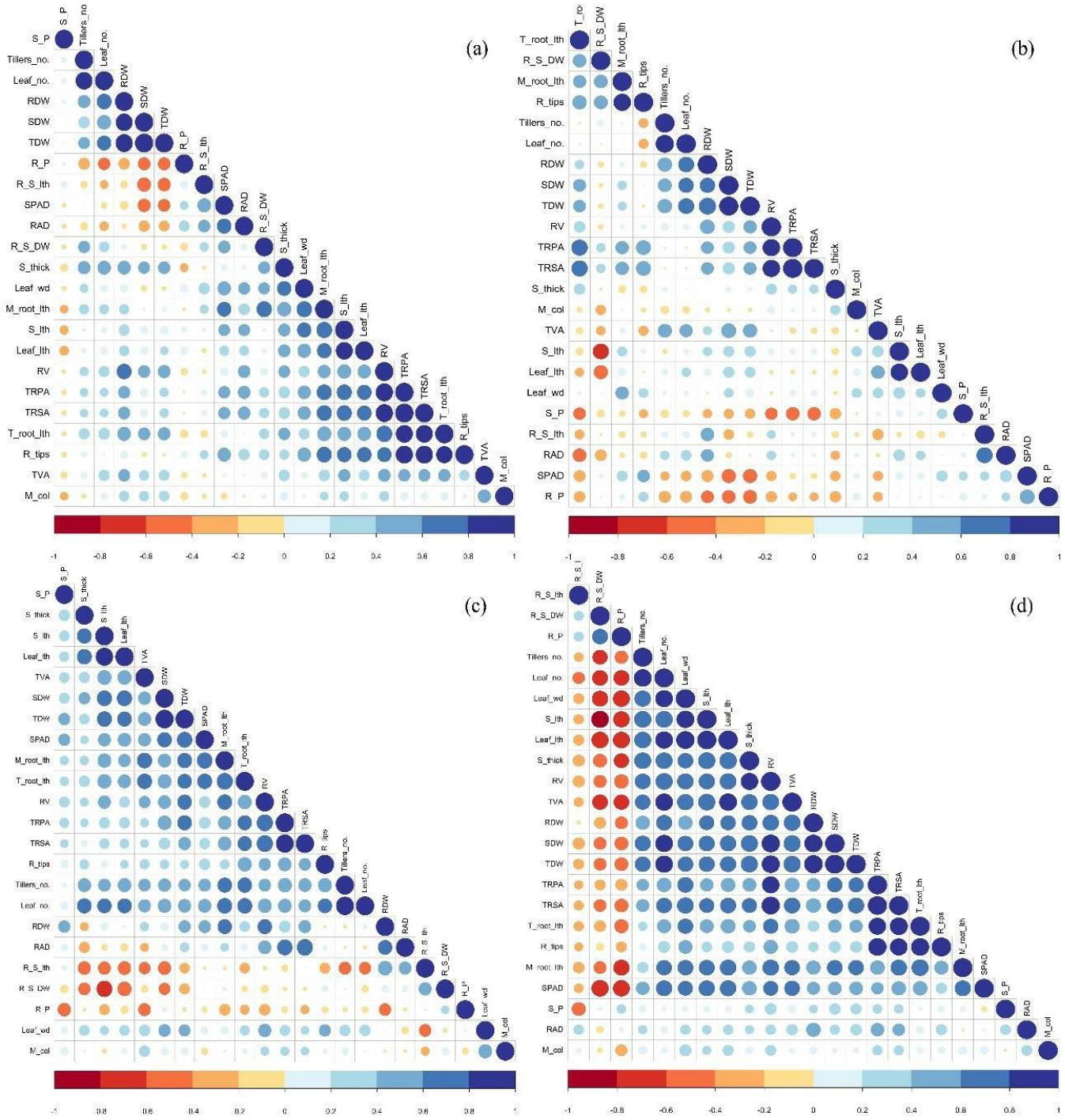

Fig. 4. Pearson correlation matrix among the 23 traits measured under P deficiency, (a) improved genotypes, (b) landrace, (c) *O. nivara* and (d) *O. rufipogan*. The colour denotes the sort of correlation, where 1 represents complete positive correlation (dark blue) and -1 represents complete negative correlation (dark red) between two traits. Large circle denotes strong and small circle denotes weaker correlation

landraces to 0.87 in *O. rufipogon*). The P content in root and shoot had no significant relation except in *O. nivara*, where they were negatively correlated (-0.50).

K means cluster analysis

The sample cultivars were subjected to K means cluster analysis to highlight the underlying trend and the subgroups

Improved varieties had the highest number of samples that were grouped into four clusters based on their respective trait expressions. Cluster 2 with 17 genotypes had higher mean values for most of the traits, comparatively. However, the shoot P content was higher in cluster 3, a desirable trait for low P tolerance. Cluster 1 had higher expression in the case of dry matter accumulation. The heat map for improved varieties also highlighted the representatives of Cluster 2with the maximum amount of correlation for multiple of the traits (Supplementary Fig. S1.a). The heatmap identified few genotypes like Bhanja, Keshari, and Improved Lalat that had a high expression for all traits except for shoot P and root P accumulation. However, Gajapati had a better accumulation of shoot and root P but lacked superiority in other traits. Landraces were categorized into three clusters based on their trait expressions. Cluster 1 and 3 had high mean values for a different set of traits followed by Cluster 2 under the low P environment. Cluster 2 had a high mean characteristically for root traits. The hierarchical heat map conformed with k-mean cluster analysis (Supplementary Fig. S1.b). *O. nivara* was grouped into two clusters where all the morphological trait means were high for cluster 2 except for root P content which was high in cluster 1. *O. nivara* displayed higher trait correlation with shoot P for most of its representative cultivars, especially AC100142, AC100123, AC100117 and AC100285 (Supplementary Fig. S2.a). Especially, AC100142 stood out and had higher correlation with most of the traits as revealed by the heatmap. *O. rufipogon* was grouped into three clusters and clearly, the mean value for cluster 2 was the highest. This cluster had three genotypes that also had higher intensity for most of the traits in the K mean and heat map (Supplementary Fig.S2.b). Accessions like AC100062, AC100219, and AC100219(A) were having high trait correlation for most of the traits shoot P content as inferred from the heat map.

Discussion

To expedite understanding of the heterogeneous population, the sampled cultivars were studied in four different groups. This made the study focus on the diversity within and between groups rather than just highlighting a few tolerant lines from all the groups together. The P deficiency symptoms, in general, are so pronounced that studying morphological characters can itself sketch a clear picture like the type of root, root length, root volume, SPAD, leaf width, shoot P, and root P content can be regarded as indicative traits (Anandan et al. 2021b) that can determine the adaptability of a genotype.

The descriptive statistics have highlighted landraces to be the best group that survived a low P environment. The mean performance (for traits like shoot length, tiller numbers, root volume, total root surface area, root dry weight, total root length, and shoot-root P) of the group is suggestive of the fact that most of the landraces were well adapted to face the low levels of P in their respective places of origin. It is known that more than 90% of India falls into the category of low to medium levels of P in the soil (Tiwari 2001) and landraces are adapted more to manures than synthetic fertilizers. Only from the mid of 21st century, farmers were driven towards high-input agriculture and ideally designed improved varieties. Thus, it is expected from the landraces to perform better in a low P stress environment comparatively. For other traits like leaf width, shoot P, and dry weight accumulation, landraces performed at par or higher than the other three groups.

Narrowing of the leaf is a sign of P deficiency in rice plants (Chen et al. 2014) and leaf width had the highest mean in the case of improved varieties. The correlation analysis indicated a positive correlation between the leaf width and root length and this is in agreement with Anandan et al. (2021b). In a P deficient soil, higher root development helps in mining greater amount of P, ensuring unhindered supply to the shoot and leaves (Panda et al. 2021). The total root length and number of root tips had a positive correlation between all four sample groups. This shows that the plants under low P have developed newer roots (for mining shallow depth) for maximum P acquisition (Vejchasarn et al. 2016). The measure of root and shoot P in the samples revealed an interesting fact. High P content in the roots does not always correspond to low P tolerance. There are examples from this study where the root P is high but the same is not reflected in the shoot. The lack of association between root and shoot P is also highlighted in the correlation analysis, showing an absence of correlation (or even negative in case *O. nivara*). Thus, it was decided to go for a simple ratio of shoot P to that of root P, creating an index for comparing P levels both above and below the ground. This index using group means was as high as 2.51 in case of landraces, the lowest was in improved varieties (0.97).

The clusters formed using K means analysis has helped in revealing the subgroups within each sample population expressing specific traits with higher magnitude in response to low levels of P in the soil. It is appropriate to mention here that we were able to appreciate the differences between the clusters formed by k means and the hierarchical grouping of the heat map. The former clustered genotypes having similar magnitude of trait expression into one whereas the latter considered the correlation of individual traits with the respective genotypes and then grouped them in different branches. The hierarchical heat map (Supplementary Figs. S1 and S2) was found to be more versatile in representing multiple traits and genotypes in a single illustration.

A few genotypes were sorted out that had the combination of some of the desirable traits for a low P tolerant plant, these were scattered across the four different groups (Fig. 5). The most important trait in such a study is

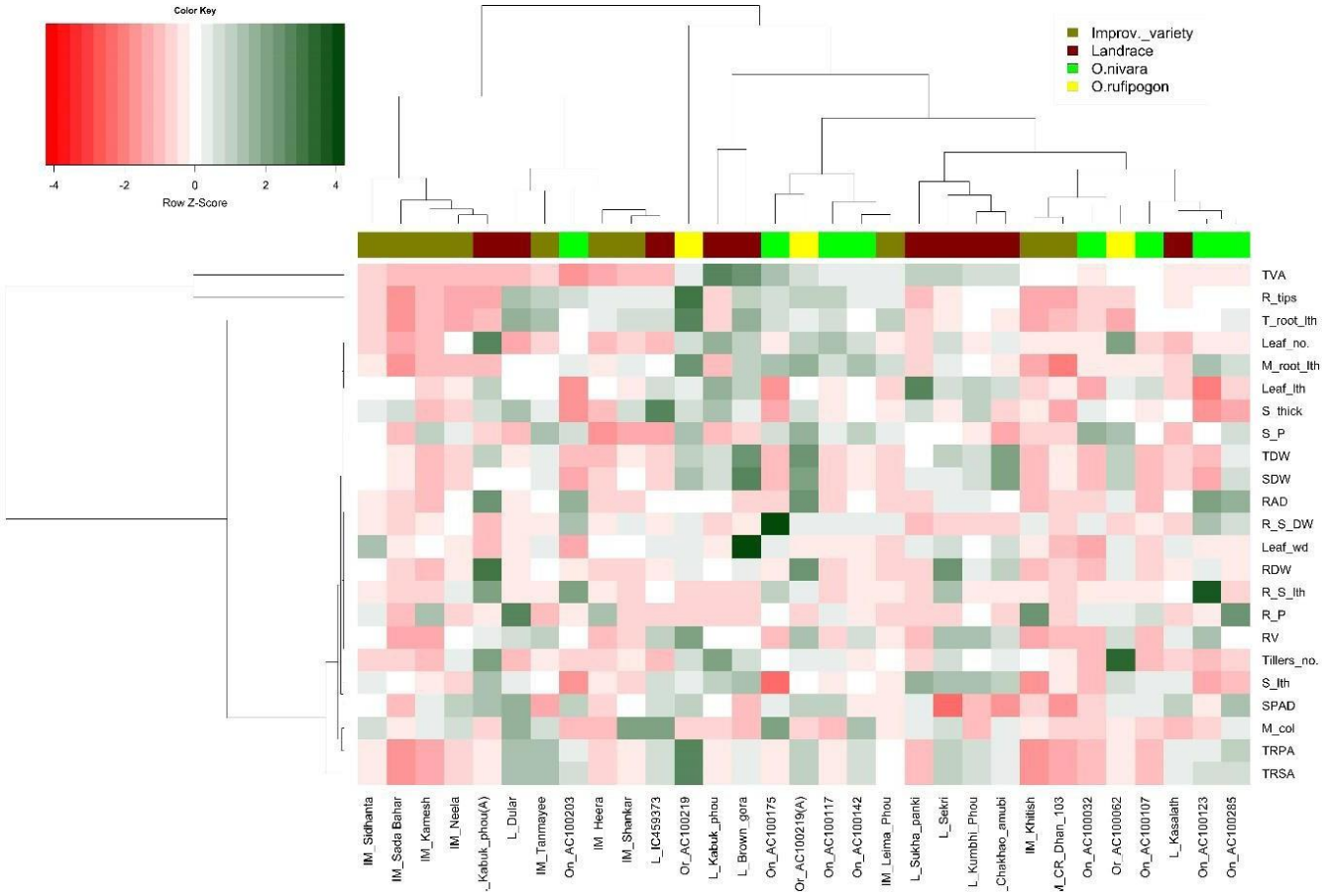

Fig. 5. Hierarchical clustering and heat map of selected rice genotypes and low P tolerance related traits. Each column represents an accession and each row represents a trait. Genotypes divide into two main clusters. Both upper and lower clusters are divided into two sub- clusters. The colour spectrum (legend) illustrates the Z-score across the range of –4 to +4. A Z-score of '0' indicates that the trait valueis identical to the mean, and values greater than the mean are categorized into +4 (green) and *vice versa* (red)

the ultimate accumulation of P in the shoot and roots. It was observed that, on an average shoot P was high in *O.rufipogon* group (1.36 mg/g) but looking at the individual cultivars, we found the highest shoot P content was in themembers of improved varieties (Bhatta et al. 2021) such asSuphala (1.37 mg/g) and Sarathi (1.45 mg/g). Interestingly, inthe improved varieties, the corresponding root P was also high (1.25 mg/g) (Fig. 1; Supplementary Table S5) suggesting that the nutrient allocation from the root to the shoot was not efficient. On the contrary, the members with relatively high shoot P content in *O. rufipogon* displayed proper P allocation between the root and shoot. The shoot and root biomass accumulation as reflected by their dry weight was highest in landraces while the cultivars from the twogroups of wild species did not fare well in this regard. The morphology of the root is another important yardstick to measure the tolerance to a low P environment (Bhatta et al. 2021). Root volume and total root length were high in the case of landraces with respect to the group mean. Moreover, AC100219 (*O. rufipogon*) had one of the highest figures for root volume and total root length apart from few superiorrepresentatives of the landraces. As P in soil is limited to the shallow layers, deeper roots are not desirable, thus it is apt to look for a cultivar with a higher value for bothroot volume and number of root tips. Besides, the positive correlation between the total root length and number ofroot tips supports the notion that plants under P stress donot need longer roots rather a higher number of shallow roots/lateral roots (Panda et al. 2021).The highest expressions for each of these traits were seen in AC100219 of *O.rufipogon*.Each group (*O. nivara*) had displayed a positive correlation between root dry weight and shoot dry weight indicating a synergistic relationship between the underground and above the ground traits. This finding supports the argument that screening for low P condition in rice suggest equal emphasis should be given for the shoot as well as the root morphological features. The highlighted tolerant genotypes can be further studied to dissect the genetic basis of their tolerant trait manifestation and the effect on yield under a low P environment through molecular approaches.

Although, genetic improvement with regards to PUE is highly challenging one due to several reasons including incomplete understanding of control of P uptake, appropriate phenotyping, variable soil properties across

the cultivable land, gene interactions under variable environments and conduct of large scale trials (Bovill et al. 2013). There are several reports of the traits result in substantial improvement in P nutrition under controlled conditions like pot study in laboratories, screening under hydroponics fail to show similar advantages in field soil. To understand the genetics of PUE, relatively a few studies haveidentified QTLs in the crop species. Generally, the process has been to measure traits of interest such as biomass production and shoot P concentration under both limiting and non-limiting P conditions (Bovill et al. 2013; Bhatta et al. 2021) and the QTLs have been detected for these traits. However, it has been also noticed that QTLs for biomass and yield often co-locate with QTLs for P uptake and/or P utilisation efficiency, for example, in wheat (Su et al. 2009) and rice (Wissuwa et al. 1998). They further argued that it is because of the correlation between biomass production and shoot P uptake is often extremely high, however, it would be difficult to improve both the traits simultaneously (Bovill et al. 2013). A few authors have attempted to overcome this issue by assessing relative yield, and detecting QTLs for relative yield. Yang et al. (2010), in a study assessing the relationship between QTLs for root traits and P uptake in Brassica napus, found that QTLs for P uptake and biomass production were linked. The present study has identified lines that can be used in identification of QTLs responsible for low P tolerance and also serve as a source of breeding material for developing low P tolerant rice cultivars through classical breeding approach. It is also suggested that the advances made in molecular and genomic tools combining traditional science of plant breeding may facilitate to study the genetic differences in PUE, bringing P-efficient crops.

Authors' contribution

Conceptualization of research (AA, SP); Designing of the experiments (AA); Contribution of experimental materials (BCP, DB); Execution of field/lab experiments and data collection (SP, BB); Analysis of data and interpretation (AA,SP); Preparation of the manuscript (SP, AA).

Supplementary materials

Six Supplementary tables and two figures are supplied.

Declaration

The authors declare no conflict of interest.

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Assessing genetic variability of Bengal and Assam Aus panel rice lines under low nitrogen soil status

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Abstract
Nitrogen deficiency is more pronounced in rice especially in the upland aerobic soils. Compensating rice cultivars with Nitrogen Use Efficiency traits would require identifying the component traits involved and the level of dependence between these traits, in the present study 204 Bengal Assam Aus Panel rice lines were used for studying their response under low nitrogen soil status. It was found that the traits like chlorophyll index and days to 50% flowering were the most influenced trait under low nitrogen. There was high correlation of grain yield per plant with flag leaf width, panicle number and days to 50% flowering. The highest contribution was from the trait flag leaf width and panicle length.

Keywords: Assam Aus panel rice lines, low nitrogen soil, *Oryza sativa*

Introduction
Rice (*Oryza sativa* L.), the second most cultivated crop in the world, nurtures billions of lives including humans and animals, and is of prime importance in developing nations. Especially inAsia where it is the sole source of energy (carbohydrate) for a majority of the population. The nutritional content of rice grain is 80% carbohydrates, 7–8% protein, 3% fat, and 3% fibre (Chaudhari *et al*., 2018) [4]. India's total rice production was 127.93 million metric tons in the year 2021-2022 (www.indiastat.com). As an energy-giving food, the consumption of rice is as high as 103.5 million metric tons in India and 509.87 million metric tons (estimated) worldwide in the crop year 2020-21 (www.fao.org/worldfoodsituation). notable increase in the grain yield of rice in the 1960s but eventually the growth rate declined and was almost stagnant by the mid-1980s (Dobermann *et al*., 2004) [5]. This decline can be attributed to multiple reasons. The most significant being the incessant application of fertiliser and the reduced nutrient use efficiency of the newly developed varieties. This was the case despite the development of varieties with higher yield potential. This motivated farmers retorted to high blanket application of fertilisers (especially nitrogen) in order to suffice the shortcomings of yield leading to deterioration of soil (physically and chemically), declining water table andalso damaging the soil fauna (Srivastava *et al*., 2020) [12]. the total nitrogenous fertiliserapplied, the nitrogen recovery efficiency remains less than 30% in the rainfed areas while it ranges between 20-30% under irrigated field conditions (Roberts, 2008) [10]. Rice is being grown in diverse ecological systems: this has led to diverse adaptive features, with higher degrees of variability between genotypes (Panda, Bhatt, *et al*. 2021) [7]. This paper studies the variability and correlation of Bengal and Assam Aus Panel under 50% nitrogen status of soil inorder to understand the effect of low nitrogen on the panel and highlight the traits influencedby it. The peculiarity of the aus rice lines is that they harbour many benefical novel genes absent in other indica or japonica cultivars, such as *Pup1*, *Sub-1*, *Xa5* etc. This would mainly cater to the needs of resource poor upland rice cultivation systems where nutrient use is an important factor deciding the farmer's income (A Anandan *et al*. 2021.) [2].

Materials and Methods
Experimental site
The nitrogen deficient trial was undertaken in field condition at ICAR-National Rice Research Institute, Cuttack during Rabi 2021 in transplanted system of rice cultivation. The soil was sandy clay loam with 0.53% organic carbon, 240 kg/ha of N, 26.3 kg/ha of P, 164.05 K kg/ha, 51.6% sand, 18% silt, 30.4% clay with bulk density of 1.41 g/cm3. In order to maintain a nitrogen deficient condition only 50% of the total recommended dose of N was applied.

The recommended dose was 80:40:40:N:P2O:K2O, while the applied fertilizer in the trial was 40:40:40::N:P2O:K2O. A total of 203 BAAP lines were used for sowing. Transplanting was done at 28 days after germination in a single row of each genotype in four replications. The planting followed randomized block design in four replications. The spacingwas maintained at 20 cm between rows and 15 cm between plants. After each row of entry one row of Naveen variety was grown to ensure uniform competition in the field. Standard agronomic practices were followed with respect to irrigation schedule and controlling weed in the field. Six plants were taken from an entry in each replication to record the observations and the mean was calculated using the values recorded from the six plants in each replication for further statistics. The traits studied were chlorophyll index of the flag leaf, days to 50% flowering, number of tillers, number of productive tillers, flag leaf length, flag leaf width, panicle length and grain yield per plant.

Results

All the traits studied displayed significant differences among the genotypes and there was no significant difference among the replications for these genotypes. The ANOVA for the above-mentioned traits in the nitrogen deficient trial is presented in table 1 and the data distribution is depicted in Fig.1.

The chlorophyll Index was measured using SPAD. The SPADvalues ranged from 20.33 in the accession Kele Bari to 42.01 in AUS453. The mean value for chlorophyll index in thepanel was 30.95 with a standard deviation of 4.66. The PCV (14.7) was higher than GCV (10.46) with moderate heritability (0.51). The data here shows a skewness of 0.17 and kurtosis of 0.34. The days to 50% flowering was in the range from 80 (Assam4) to 96.33 (Raj mundo) days. The mean was 87days, the CV (4.97%) was low compared to other traits. The PCV (4.05) was higher than the GCV (3.32) and the heritability was 0.67. The data distribution here shows a skewness of 0.36 and kurtosis of 0.4. A considerable amount of variation was seen, as plant height ranged from 81.29cm (Zhenshan 97 B) to 165.02cm (AUS321). The mean plant height was 118.95cm with a standard deviation of 12.23. The PCV (14.14) was higher than the GCV (11.48) and the heritability was 0.67 with a genetic advance value of 19.23. The data distribution here shows almost symmetrical skewness of -0.4. Flag leaf length as a trait displayed variationamong the genotypes with the lowest at 22.97cm (Zhenshan 97B) and the highest was 43.87cm (AUS362) with a mean of 31.16cm. The heritability (0.37) was low with a higher PCV value of 17.47 and lower GCV value of 10.56. The data distribution here shows a positive skewness of 0.51. There was a wide variation among the genotypes for flag leaf width ranging from 0.80cm in (AUS paddy white) to 2.98cm (Chhola boro 2). The mean flag leaf width was 1.43cm with a standard deviation of 0.25. There was moderate heritability with 0.47 and genetic advance of 15.58. The data distribution here shows a positive skewness of 0.57. Panicle length rangedfrom 17.58cm (AUS paddy white) to 31.10cm (AUS99). The mean was 24.08cm with a cv of 11.13%. The heritability(0.49) was moderate. The distribution of data for this trait in the panel is positively skewed (0.72). Number of tillers for the panel ranged from a minimum of 7.06 (AUS440) to a maximum of 21.20 (Shada boro). The mean number of tillers per plant was 13.05 with a CV of 19.28%. The heritability was 0.44 and the genetic advance was 25.81. The data distribution here shows symmetrical distribution with a skewness of 0.39. Number of productive tillers ranged from

3.61 in LI-JIANG-XIN-TUAN-HEI-GU to 15 in KALASU. The mean number of productive tillers per plant was 8.59 and the CV was 18.28%. The heritability was moderate with 0.41. The data distribution here shows an almost symmetrical skewness of 0.28. Grain yield per plant showed a wide range of values with the lowest being 2gms (AUS paddy white) to as high as 20.36gms (ARD11600). The mean grain yield per plant in the panel was 11.45gms with a CV of 17.98%. The heritability (0.35) was low and genetic advance of 24.90. The data distribution here shows an almost symmetrical skewness of 0.39.

Correlation analysis and Principal component analysis

A considerable positive link between grain production perplant and flag leaf width (0.43), panicle length (0.30), and the number of productive tillers was underlined by the Pearson's correlation of the aforementioned parameters under the nitrogen deficiency condition (0.37). Days to 50% flowering significantly positively correlated with both the number of tillers (0.22) and the average grain yield per plant (0.15). Number of tillers and flag leaf width revealed a significant negative correlation (-0.21). The results of the correlation analysis for the BAAP lines under nitrogen deficient trial are presented in Table 2 and Figure 2.The PCA results further highlighted the amount variation contributed by each trait and the nature of relationship the traits studied (Table 4.5). Only three PCs had eigenvalues greater than one, leading to the consideration of three primary axes for additional deductions. Between the genotypes in the panel and specific phenotypes, PC1 accounted 28.5% of the variation while PC2 explained 20.3%. The most variable factor in PC1 was panicle length (25.62), followed by flag lead width (22.03). The most significant variations in PC2 and PC3 were caused by the number of productive tillers (31.93) and chlorophyll index (38.46), respectively. The PCA biplot for yield and yield attributing traits under nitrogen deficient trial is presented in Fig 3.

Table 1: ANOVA of BAAP lines for yield and yield attributingtraits under nitrogen deficient trial

	Mean Sum of Squares		
Traits	**Genotype**	**Replication**	**Error**
Chlorophyll Index (SPAD)	51.14**	30.08	10.2
Days to 50% flowering	37.41**	14.23	4.06
Plant height (cm)	843.41**	432.28	96.28
Flag Leaf length (cm)	62.09**	10.46	8.81
Flag Leaf width (cm)	0.64**	0.19	0.1
Panicle length (cm)	17.03**	9.56	3.46
Number of tillers	32.38**	22.9	7.9
Number of productive tillers	15.96**	7.63	4.28
Grain yield per plant (g)	32.33**	20.4	10.31

Treatment degrees of freedom = 202, Replication degrees of freedom = 3

*, ** Significant at 0.05 and 0.01 levels, respectively

Table 2: Estimates of variability of BAAP lines for yield and yield attributing traits under nitrogen deficient trial

Traits	RANGE	MEAN	SD	CD (5%)	CV (%)	GCV	PCV	h^2_{bs}	GA	SKEWNESS	KURTOSIS
Chlorophyll Index	20.33 - 42.01	30.95	4.6	4.44	14.97	10.46	14.7	0.51	15.34	0.17	0.34
Days to 50% flowering	80.00 – 96.33	87.00	3.53	2.79	4.05	3.32	4.05	0.67	5.61	0.36	0.4
Plant height (cm)	81.29 – 165.02	118.95	12.23	13.62	10.31	11.48	14.14	0.66	19.23	-0.04	0.71
Flag Leaf length (cm)	22.97 - 43.87	31.16	5.55	6.05	17.85	10.56	17.47	0.37	13.14	0.51	0.42
Flag Leaf width (cm)	0.80 - 2.98	1.43	0.25	18	17.46	11.07	16.19	0.47	15.58	0.57	1.94
Panicle length (cm)	17.58 - 31.10	24.08	2.68	2.58	11.13	7.65	10.88	0.49	11.08	0.72	1.05
Number of tillers	7.06 - 21.20	13.05	2.89	3.9	19.28	18.96	20.7	0.44	25.81	0.39	0.2
Number of Productive tillers	3.61-15.00	8.59	1.82	2.87	18.28	19.88	21.23	0.41	26.05	0.28	-0.33
Grain yield per plant (g)	2.00 - 20.36	11.45	2.67	4.46	17.98	20.49	34.78	0.35	24.9	0.39	0.03

Table 3: Estimates of correlation among yield and yield attributing traits under nitrogen deficient trial

	Chlorophyll Index	Days to 50% flowering	Plant height	Flag Leaf length	Flag Leaf width	Panicle length	Number of tillers	Number of productive tillers	Grain yield per plant
Chlorophyll Index	1								
Days to 50% flowering	0.125	1							
Plant height	0.012	-0.042	1						
Flag Leaf length	0.029	-0.044	0.558**	1					
Flag Leaf width	0.071	-0.013	0.053	0.119	1				
Panicle length	0.016	-0.073	0.149*	0.161*	0.585**	1			
Number of tillers	0.020	0.074	0.116	-0.077	-0.211**	-0.039	1		
Number of productive tillers	0.087	0.222**	-0.007	-0.043	-0.136	0.113	0.247**	1	
Grain yield per plant	0.030	0.153*	0.057	0.082	0.436**	0.307**	0.079	0.377**	1

*, ** Significant at 0.05 and 0.01 levels, respectively

Table 4: Estimates of eigen values and contribution of yield and yield attributing traits towards the major principal components under nitrogendeficient trial

Traits	PC1	PC2	PC3
Eigen values	2.27	1.77	1.14
Chlorophyll Index	0.47	4.51	38.46
Days to 50% flowering	0.56	2.26	31.24
Plant height (cm)	16.85	8.51	8.72
Flag Leaf length (cm)	16.56	5.36	1.16
Flag Leaf width (cm)	22.03	0.61	10.75
Panicle length (cm)	25.62	0.14	0.003
Number of tillers	5.91	30.31	7.57
Number of Productive tillers	2.4	31.93	0.07
Grain yield per plant (g)	0.12	16.33	1.99

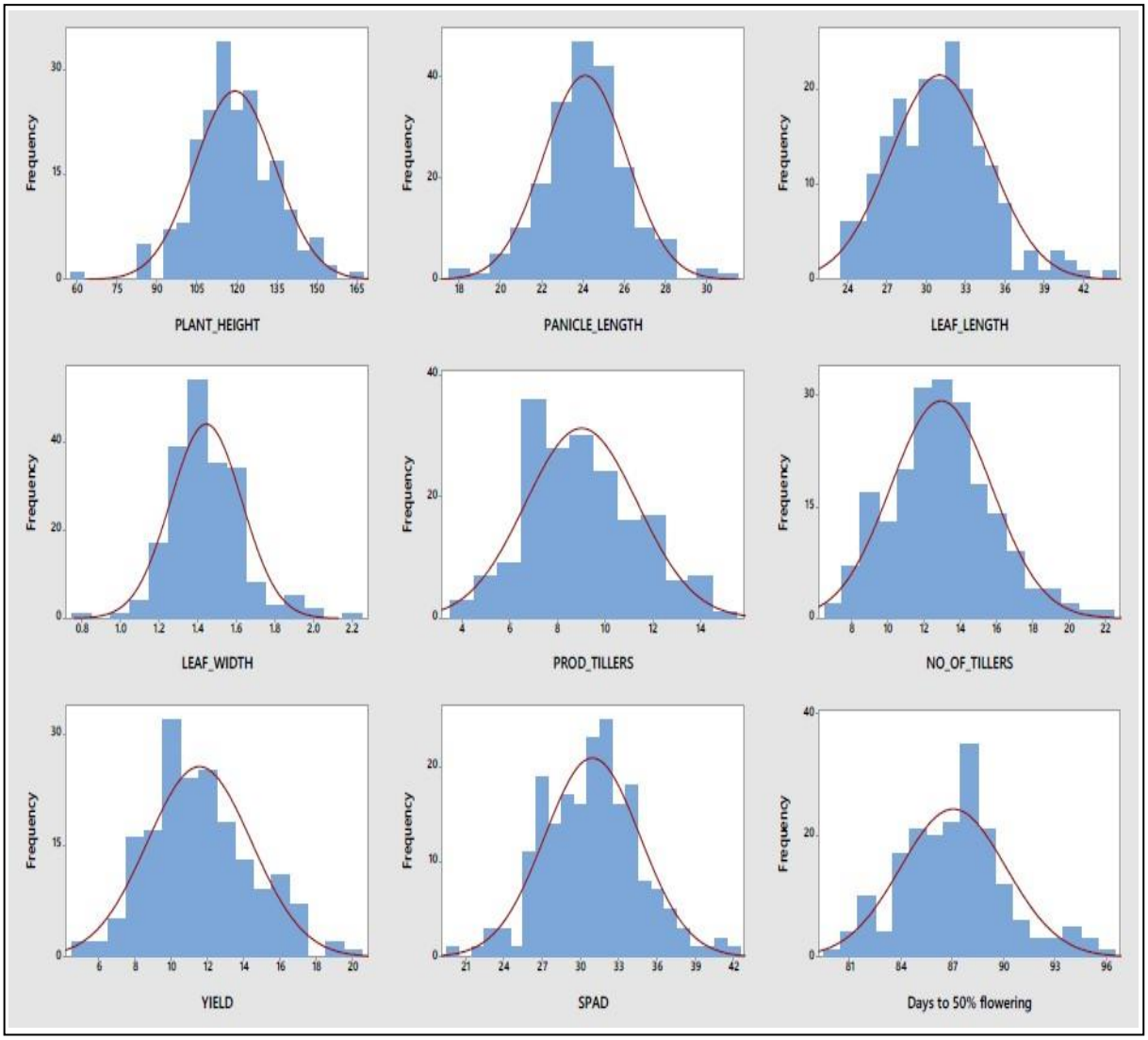

Fig. 1: Morphological data distribution of the BAAP lines for yield and yield attributing traits under nitrogen deficient trial

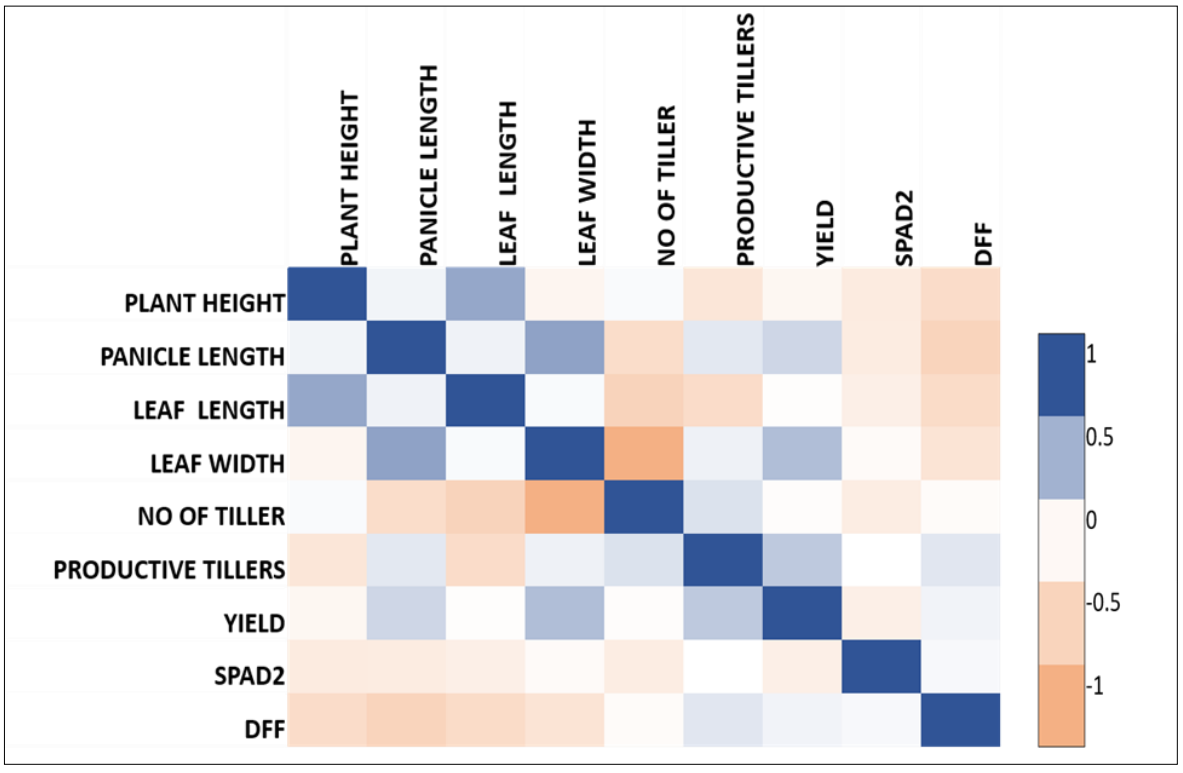

Fig 2: Correlation heat map for yield and yield attributing traits under nitrogen deficient trial

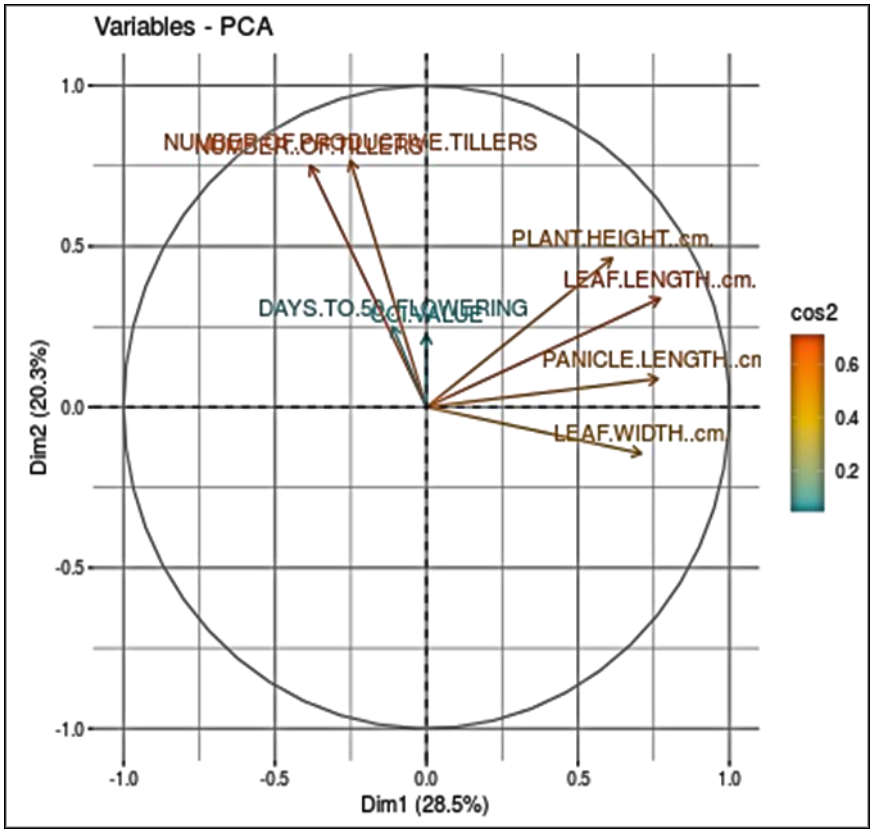

Fig 3: PCA biplot for yield and yield attributing traits under nitrogen deficient trial

Discussion

The accessions employed in this study showed considerable mean sum of squares, according to the analysis of variance, indicating that the features under examination had a lot of variability. One of the accessions on the panel was the popular indica rice variety; Swarna. Swarna's reaction to N deficit can be utilised as a benchmark for other accessions in the panel when comparing it as an enhanced variety. The low levels (i.e., below 30 for 76 accessions) of nitrogen content maintained in the field were the cause of the low chlorophyll index. Similar results were also reported by Swain and Sandip(2010) [13], Yang *et al.* (2014) [15]. This is due to the fact that nitrogen is a necessary component of chloroplasts, with reports stating that 80% of leaf nitrogen is diverted there, with 50% of that nitrogen going toward the production ofphotosynthetic proteins (Xiong *et al*., 2015) [14]. The trait can be improved by selection, and the heritability was 0.51. More than half of all accessions were flowering sooner than the average, with the mean days to 50% flowering being 87 days. Another important feature that is affected by nitrogen status in the soil is flowering. Early flowering is accelerated by low nitrogen status, and normally higher nitrogen doses cause delayed flowering and ripening. Similar results were reported by Sanagi *et al*. (2021) [11] and Zhang *et al*. (2021) [16]. Leaf width is a crucial characteristic that influences leaf area and, in turn, the effectiveness of a plant's photosynthetic process. (Rahman *et al*., 2013) [9]. The mean flag leaf width in this study was 1.43 cm, with a moderate heritability that might be enhanced through selection. With the exception of plant height, all of the panel's features had mean values that were lower than Swarna's performance, which had higher leaf width (1.82 cm) and more prolific tillers (12.66), while still maintaining a SPAD score of 31.99. Except for flag leaf width, flag leaf length, and panicle length, which were positively skewed, all the examined attributes in the nitrogen-deficient trial showed symmetric distribution in the population. A greater chlorophyll index would result from a larger dose of N, which would also improve other yield- attributing traits like productive tillers, leaf width, etc. However, identifying plants that have superior N usage efficiency even under deficient soil conditions would be helpful in further deciphering the genetic regulations governing them. These results are in support of previous reports by Agahi *et al*. (2007) [1], Khaliq *et al*. (2008) [6] and Rahman *et al*. (2013) [9]. Flag leaf width and panicle length in the first component were identified by the principal component analysis as the main sources of variance. The distributional data for these traits were positively skewed, which explains the trait's variability. Grain yield per plant was discovered to be strongly linked with flag leaf width and panicle length based on the correlation and PCA analyses. This work can further be studied for genome wide association studies in the BAAP panel (Annamalai Anandan *et al*. 2022) [3]. Apart from the above mentioned traits a focused study on root morphological traits is also needed for a future model nutrient use efficient crop (Panda, Majhi, *et al*. 2021) [8].

Conclusion

The present study has identified BAAP lines that can be used in identification of QTLs responsible for low N tolerance and also serve as a source of breeding material for developing low N tolerant rice cultivars through classical breeding approach. It is also suggested that the advances made in molecular and genomic tools combining traditional science of plant breeding may facilitate to study the genetic differences in NUE, bringing N-efficient crops.

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