

## **Relative Response of Indigenous Rice Genotypes to Low Versus Normal Planting Density for Determination of Differential Phenotypic Plasticity in Traits Related to Grain Yield**

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### **Abstract**

Increase in atmospheric CO<sub>2</sub> ([CO<sub>2</sub>]) improves the efficiency of the unsaturated photosynthetic system of C<sub>3</sub> plants, like rice, which leads to high crop productivity and increased biomass production. Planting geometry using lower than standard planting density has been shown to be an alternative pre-screening technique for phenotypic plasticity as a proxy of [CO<sub>2</sub>] responsiveness. More than 200 indigenous rice genotypes were tested for several traits, such as plant height, tiller number, panicle number, panicle dry weight, straw dry weight, total dry weight and harvest index. Their relative response for these phenotypic traits at low density planting versus normal density was determined to assess the plasticity of the genotypes. Ten genotypes were identified as significantly [CO<sub>2</sub>] responsive based on their higher panicle dry weight and panicle number. Even though it was observed that genotypes with higher days to maturity (DM) were more plastic, rice genotypes with low DM had a significantly higher relative response to harvest index, implying that rice with low DM may also be considered to be responsive to higher [CO<sub>2</sub>]. In order to associate phenotypic plasticity with the genotype, 23 SSR markers physically close to genes involved in CO<sub>2</sub> metabolism were used to amplify selected responsive and non-responsive rice. Out of 3 alleles amplified using RM17, allele A was found to be significantly associated with genotypes with higher phenotypic plasticity whereas allele C was associated with genotypes with negligible response. The identified rice genotypes and the associated marker RM17 can therefore be tested further for breeding of superior rice responsive to higher [CO<sub>2</sub>] envisaged in a changing climatic scenario of the future.

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## Introduction

Rice accounts for maximal human consumption as a staple food by three billion people and provides 20% calorie requirement of the world population (Chaturvedi et al. 2017, Jasim et al. 2018). Although the current production of rice is enough to feed the world population, only 72% of the global demand of rice can be met by 2050, assuming a continuous and steady yield enhancement rate (Ray et al. 2013, Srinivasan et al. 2017). To overcome the yield and demand gap, increasing the cultivation area is not a viable option, as the current cultivable land for rice production is already in under constant pressure of urbanization and land degradation. These leaves increasing yield per unit land area as the only suitable solution (Long 2014, Srinivasan et al. 2017). But like other grains, gain in rice productivity increase has stagnated in the last two decades (Zhang et al. 2014, Paleari et al. 2017). Use of plant dwarfism as ideal plant architecture as exemplified in the green revolution, has already reached its biological limit and has little room for improvement (Zhu et al. 2010, Srinivasan et al. 2017). Moreover, climate change also poses both abiotic and biotic threats, like drought, heat, salinity, sea level rise, new predatory insects, pesticide resistant pests as well as elevated level of greenhouse gases like CO<sub>2</sub> (Kant et al. 2012, Amin et al. 2016). The drastic change in the level of atmospheric CO<sub>2</sub> ([CO<sub>2</sub>]), primarily caused by the industrial revolution which is predicted to increase by almost 50% by mid-century due to anthropogenic changes (Shimono et al. 2014, Bishop et al. 2015). Elevated CO<sub>2</sub> (e[CO<sub>2</sub>]) is well known to have a positive effect on the C<sub>3</sub> plants, called “[CO<sub>2</sub>] fertilization effect” (Long 2014, Chaturvedi et al. 2017). Increase in atmospheric CO<sub>2</sub> ([CO<sub>2</sub>]) improves the efficiency of the unsaturated photosynthetic system of C<sub>3</sub> plants, like rice, which leads to high crop productivity and increased biomass production (Bishop et al. 2015, Tausz-Posch et al. 2015).

Beside the enhancement of reproductive and vegetative growth, elevated atmospheric CO<sub>2</sub> (e[CO<sub>2</sub>]) is found to improve water use efficiency and lower photo-respiratory losses of rice (Leakey 2009, Srinivasan et al. 2017). Several studies have been conducted to assess the extent of the positive effect of e[CO<sub>2</sub>] on C<sub>3</sub> crops including rice (Hasegawa et al. 2013, Shimono et al. 2014, Zhu et al. 2014), wheat (Ziska 2008, Tausz-Posch et al. 2015) and soybean (Bishop et al. 2015, Bunce 2015, Kumagai et al. 2015). These studies have documented tremendous diversity in e[CO<sub>2</sub>] responsiveness, as the yield increase differ among species and subspecies, even varieties (Kumagai et al. 2015, Tausz-Posch et al. 2015). Moreover, the selection procedure in breeding technology of rice varieties does not include efficient [CO<sub>2</sub>] utilization property, and as a consequence, more recently developed commercial varieties have been found to

have either same or lower responsiveness to  $e[\text{CO}_2]$  compared to commercial varieties of distant past (Ainsworth et al. 2008, Ziska et al. 2012). So, active intervention for genotypes that respond strongly to  $e[\text{CO}_2]$  will be essential for maintaining food security in future (Kumagai et al. 2015).

For directly studying the  $e[\text{CO}_2]$  utilization efficiency of rice varieties, Free Air  $\text{CO}_2$  Enrichment (FACE) system has been proven to be the best option, without any limitations of the traditional control-environment chamber systems such as reduced sunlight, warmer temperature and increased vapor pressure (Long et al. 2004). But such elaborate and sophisticated facilities are not suitable to screen the germplasms of the diverse collection of the agronomic crops, especially not for highly stratified cultivars such as rice (Shimono 2011, Shimono et al. 2014). (Shimono 2011) introduced a simple and novel idea to use planting geometry as an alternative pre-screening technique by using phenotypic plasticity as a proxy of  $[\text{CO}_2]$  responsiveness. Phenotypic plasticity is defined as the ability of changing physiological, molecular or metabolic performance of a genotype to cope up with the heterogeneous environmental variation (Price et al. 2003, Kikuchi et al. 2017). As plants grown at low density face reduced competition for solar radiation, water and soil nutrients, and as a consequence, plants with greater plasticity are able to adapt and utilize this resourceful environmental condition more efficiently (Kumagai et al. 2015, Molina-Montenegro et al. 2016). In terms of resource enrichment, the response of the phenotypic plasticity of plants in low density planting should mimic the response towards  $e[\text{CO}_2]$  (Kumagai et al. 2015, Kikuchi et al. 2017). The proposed similar behavior of phenotypic plasticity and  $e[\text{CO}_2]$  responsiveness has been applied and confirmed for rice in terms of panicle number (Shimono et al. 2014) and for soybean in terms of seed yield (Kumagai et al. 2015).

In this study, we used low planting density as a pre-screening method to assess the growth potential of local varieties in a resource rich environment. So far, no previous studies have been reported in Bangladesh which screened rice landraces for their phenotypic plasticity. Phenotypic plasticity is conferred by the ability to adapt by changing the physiochemical attributes in response to environmental change. Therefore, indigenous rice landraces are the most suitable for conducting such an experiment, because they are better adopted to our climate and soil environment. A specific set of varieties with the most and least phenotypic plasticity were further subjected to molecular characterization. A total of 23 polymorphic SSRs were used to evaluate the previously determined candidate genetic loci, which were selected based on their proximity of nucleotide distance.

## Materials and Methods

Two hundred and two Bangladeshi local varieties and 6 high yielding varieties were selected. These include BRRRI Gene Bank Accessions 1-463 from which 202 genotypes were selected based on the following criteria. The local landraces were chosen to include, 154 of T. Aman, 11 of Boro and 37 of Aus varieties. Deepwater rice and Broadcast Aman rice were excluded due to their low tillering capacity and excessive lodging. BRRRI high yielding varieties and 4 other stress resistant varieties were included in this study as control to compare the plasticity of the modern cultivars. Seed germination and seedling growth was carried out in seedbeds at the Bangladesh Rice Research Institute (BRRRI). After 3 weeks, seedlings were transplanted in fields of BRRRI by hand at two different densities - normal and low density. Normal density (ND) was 25 plants/m<sup>2</sup> with 20 cm between rows and 20 cm between plants. In low density, the density of plant per area was reduced into 12.5 plants/m<sup>2</sup> by doubling the distance between plants. To randomize the planting, ALPHA-lattice design were followed for designing the field position of transplanted seedlings (Patterson and Williams 1976). In

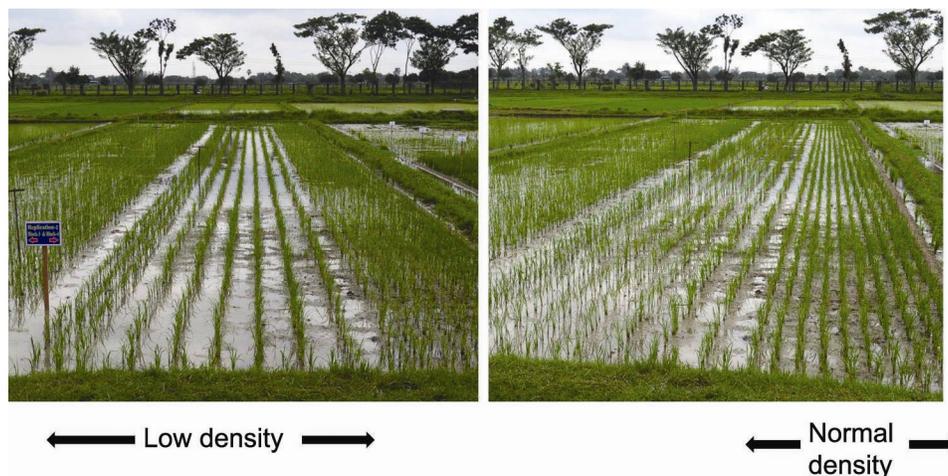


Fig. 1. The picture of the BRRRI field at the early stage of the planting. The pictures show difference between two planting densities - low density planting (left picture, center) and normal density planting (right picture, right side).

each density planting, 24 seedlings were transplanted for growing. So, for a single replicate 48 seedlings (24 plants \* 2 different density) were grown in two density, shown in Fig. 1. All the phenotypic experiment was conducted in fields at BRRRI. For assessment of the phenotypic traits of the plants such as, two replicates were used, 8 plants per replicate. Number of tillers were counted from the selected 8 plants and then the average were measured as the representative

tiller number. Plant height was measured from base to the tip of spikelet of the longest tiller in cm. After harvesting, panicles and straws were separately dried in an oven of 500 and 50°C for 5 days, respectively before measuring the weight. Sum of panicle and straw dry weights was considered as total dry weight. The ratio of panicle dry weight and total dry weight is commonly known as 'harvest index'. This particular trait represents actual yield relative to plant growth. Positive gains in yield components and biomass in low density planting in comparison with high density planting is used as a measure of phenotypic plasticity. Therefore the ratio of a specific phenotypic parameter of the low density planting and normal density planting (LD : ND) is considered as the 'relative response' of a particular genotype (Kikuchi et al. 2017). The performed analysis was conducted to evaluate the range of the relative responses of the traits as well as to compare the two different maturity group. Genotype which responded/not respond to the higher CO<sub>2</sub> were selected from this study.

Genomic DNA was isolated from (0.5 - 1.0 g) pooled leaf tissue of selected 36 accessions using the modified CTAB method (Doyle and Doyle 1987). A total of 23 genes were selected for polymorphism analysis, which were found to be highly expressed in elevated [CO<sub>2</sub>] (Fukayama et al. 2009, Fukayama et al. 2011). Moreover, three associated SNP QTLs were also included, found by (Kikuchi et al. 2017). The 23 SSR markers were selected based on their close proximity with these associated loci. The physical distance (in Mb) between the target loci and corresponding SSR shown in the Table 2. Polymerase chain reaction (PCR) was performed using 50 ng template DNA, 0.1 mM dNTPs, 0.33 μM of forward and reverse primers each, 1.6 mM MgCl<sub>2</sub>, 1 × PCR buffer, 2.6% DMSO, and 0.5 unit of recombinant Taq polymerase (Invitrogen). The mixture was then denatured at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 1 min, 1 min of annealing at 55 - 60°C (depending on primer's T<sub>m</sub>), 1 min of extension at 72°C with a final extension step at 72°C for 7 min. The 23 RM markers were obtained commercially from idt-1st base, Singapore. The amplified PCR products were resolved and visualized in non-denaturing 10% polyacrylamide gel electrophoresis. The gels were stained in EtBr and visualized with alpha imager gel documentation system. The primary assessment of polymorphism was done using the PowerMarker V3.25 software (Liu et al. 2005). All the analysis and data visualization were performed in R version 3.3.3 (Team 2014). All the plots in this study were visualized using "ggplot2" package of R (Wickham 2009).

## Results and Discussion

The effect of the planting density on the specific response of the plants and the resultant alteration in the phenotypic traits were analyzed. Low density planting reduces competition for resources like water, radiation etc., which mimics the response to elevated ( $\text{CO}_2$ ). The target of this experiment was to identify the most responsive cultivars as well as to determine the extent and type of the phenotypic differences that were generated.

The comparison of phenotypes between low density and normal planting clearly demonstrated that all the traits except plant height and harvest index increased highly significantly ( $p < 2.2 \times 10^{-16}$ ) at low density planting (Table 1). The phenotypic distribution of the two planting densities is shown in Fig. 2A. We used the ratio of phenotypic traits at low density versus normal density or the relative response of a trait as an indicator of elevated phenotypic plasticity. The average relative response of different phenotypic parameters ranged from 0.89 to 1.93 (Table 1). Among all the observed phenotypes, the largest average relative response was found for straw dry weight which was increased by approximately 2 times (1.93) with a maximum value of 3.01. This means that the plasticity of rice plants is most strongly associated with vegetative growth. The harvest index, defined as the ratio of panicle dry weight and total dry weight, is considered one of the very important agronomic traits. Although statistically insignificant, nine cultivars showed a relative increase of harvest index greater than 1.1 (10% increase at low density). The cultivar 'SHUL KUMOR' showed the highest response in harvest index (1.67). Thus plasticity of this particular phenotype may be rare, but was still found in one of the landraces. The individual range of the traits are shown in Fig. 2B.

The relative responses of these phenotypes were further analyzed for their inter-relation. For this a correlation and clustering analysis were performed in R. The result of these analyses are shown in Fig. 2C. Maximum correlation (0.92) was found between straw dry weight (SDW) and total dry weight (TDW), followed by the correlation (0.75) between total dry weight (TDW) and panicle dry weight (PDW) and correlation (0.70) between panicle dry weight (PDW) and panicle number (PN). These data suggest that relative response of these four phenotypes (PN, PDW, SDW and TDW) are strongly related with each other. Cluster analysis also gave the same result. Plant height (PH) and tiller number (TL) have lower correlation with other phenotypes, indicating presence of a separate response type (TL) or no response at all (PH).

Maturation time is the interval of time between the day of sowing the seedlings and the day of heading (the state of plant with visible panicle). Among the 208 accessions (202 landraces and 6 BRRRI high yielding varieties) maturation

date varied from 69 days to 124 days. For grouping the accessions with such a wide range of maturation time, we considered accessions which required less than 95 days to heading as early maturing cultivars and the others as late maturing cultivars. After dividing the accessions into early maturity and late maturity group, we compared the relative response of the phenotypic traits with each other. The accessions of late maturity group (LMG) showed better response in almost all the phenotypes, as indicated in Fig. 2D. Except for plant height, all the differences are statistically significant. On the other hand, early maturity group showed better performance in case of harvest index (HI). Such outcome can be attributed to interrelation of plants with respect to grain filling and leaf senescence as well as 'carbohydrate sink' hypothesis of photosynthetic acclimation (Yang and Zhang 2006).

**Table 1. Position of the target loci and their closely linked SSR markers.**

Chromosome number	Loci	Position (Mb)	SSR marker	Position (Mb)	Physical distance (kb)	
1	AK071246	19.63	RM129	19.01	626.93	
	CI333262	23.34	RM9	23.32	21.74	
	AK102220	37.08	RM11918	37.33	246.19	
	AJ308374	37.09			238.07	
2	AK072245	0.34	RM3340	0.39	44.30	
	AK121261	30.23	RM530	30.56	329.31	
	AK072292	5.65	RM545	4.92	735.12	
	AK102392	8.27	RM218	8.38	101.95	
3	qER1	9.32	RM14723	9.19	122.63	
			RM14759	9.73	408.55	
			RM232	9.75	437.57	
	AK071595	17.60	RM15245	17.63	37.78	
	CI560567	17.98			341.31	
	AK106398	25.83	RM15630	25.88	51.95	
	AU057193	26.41			529.54	
	AF058697	31.79			94.36	
	4	Os03g0830200	35.72	RM16201	35.68	42.51
		AK069195	26.94	RM17282	26.69	253.32
6	AB062676	2.92	RM204	3.17	246.83	
	qER2	5.07	RM314	4.84	222.99	
7	AK108566	15.20	RM20046	15.88	674.86	
	AK108625	1.78	RM436	2.55	774.12	
AK120691	2.49	65.22				
10	AK066625	0.77	RM24904	0.86	92.26	
11	AF017359	0.69	RM3863	0.91	213.63	
	AK064966	1.60			692.59	
12	AK073742	26.58	RM28746	26.40	176.63	
	qER3	26.58	RM17	27.02	443.83	

**Table 2. Summary statistics of the traits and relative responses of the phenotypic traits in two different density.**

Phenotypes	Low density (LD)		Normal density (ND)		Elevated traits in LD (p-value)		Relative responses <sup>a</sup>			
	Mean		Mean		Minimum	Maximum	Minimum	Maximum	Mean	Standard Deviation
Plant height (cm)	164.3		161.5		NS	0.66	1.02	0.66	1.14	0.04
Panicle number	14.27		8.41		<2.2*10 <sup>-16</sup>	0.83	2.46	0.83	1.71	0.28
Panicle dry weight (g)	31.99		20.31		<2.2*10 <sup>-16</sup>	0.82	2.56	0.82	1.59	0.30
Straw dry weight (g)	61.91		32.02		<2.2*10 <sup>-16</sup>	0.71	3.01	0.71	1.93	0.38
Total dry weight (g)	93.91		52.33		<2.2*10 <sup>-16</sup>	0.95	2.55	0.95	1.79	0.31
Harvest index	0.36		0.40		NS	0.59	1.67	0.59	0.89	0.12
Tiller number	17.91		10.01		<2.2*10 <sup>-16</sup>	1.10	2.69	1.10	1.80	0.30

<sup>a</sup>Relative response = Low density (LD)/Normal density (ND). NS = Not significant.

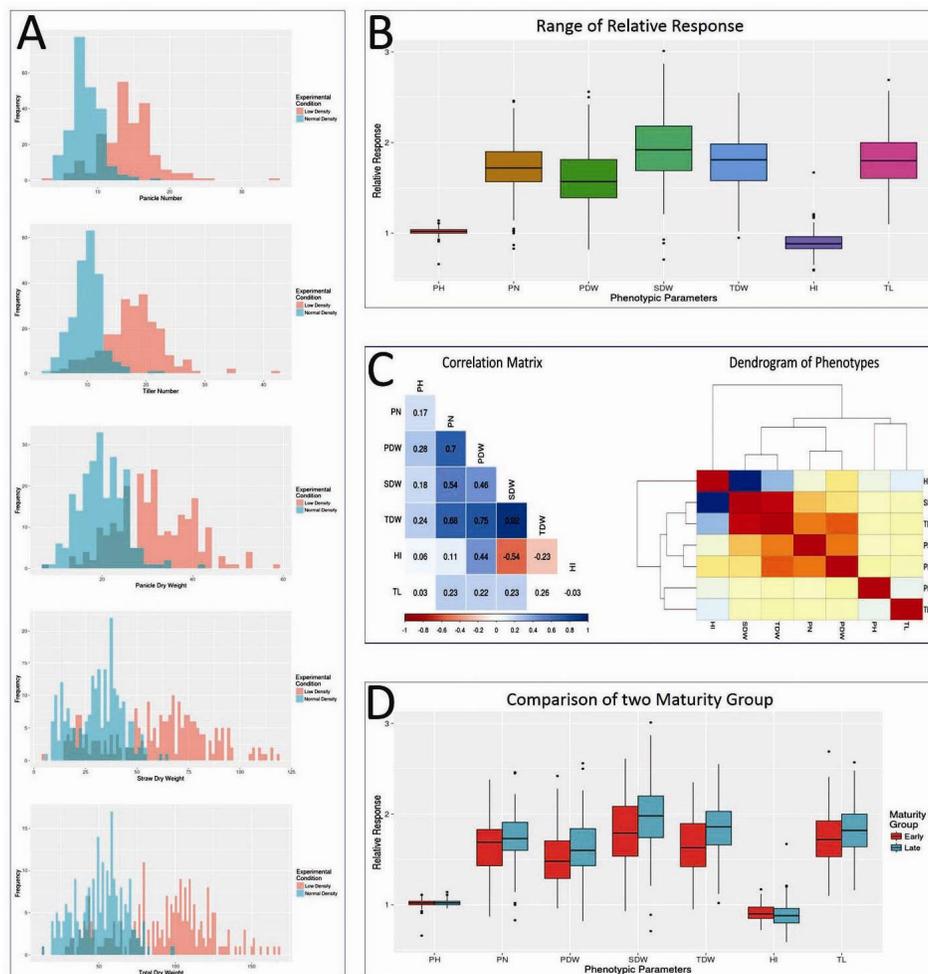


Fig. 2. **A.** Frequency of the increased phenotypic responses at low density compared to normal density for five traits including panicle number, tiller number, panicle dry weight, straw dry weight and total dry weight. In each plot, the two different colors indicate two different planting densities (light red for low density and light blue for normal density). **B.** Range of the relative responses of phenotypic plasticity of different traits (PH = Plant Height, PN = Panicle number, PDW = Panicle dry weight, SDW = Straw dry weight, TDW = Total dry weight, HI = Harvest index, TL = Tiller number). **C.** In the correlation matrix, the intensity of the color indicates the level of the correlation in addition to the number and the absence of color indicates correlation is not significant. In the dendrogram of relative response, closely linked phenotypic traits are clustered with each other on the basis of correlation. **D.** Comparison between the ranges of relative responses of different phenotypic parameters in the context of maturity group.

The cultivars which showed the highest plastic phenotypes were chosen on the basis of their relative increase in traits at low density planting. Altogether 10 accessions were selected on the basis of their superior response for multiple traits. Among the phenotypic traits, response of panicle dry weight and panicle

number was prioritized while selecting for the most plastic cultivars. Table 3 summarizes the relative response of the examined traits.

**Table 3. Relative response of the top 10 cultivars to low planting density.**

Accession number (BRR)	Accession name	Relative response				
		Panicle No.	Panicle dry wt. (g/plt)	Straw dry wt. (g/plt)	Total dry wt. (g/plt)	Tiller No.
30	HASHIKALMI-DA-26	2.38	2.42	1.90	2.14	1.53
78	KARTIK JHUL	2.46	2.56	1.99	2.14	2.48
157	CHINI SAGAR	2.15	1.94	2.84	2.48	2.31
208	KOHA BINNI	2.09	2.15	2.30	2.26	1.79
224	DEPA DHAN	2.00	2.10	2.45	2.33	2.35
237	DUDKAT	2.06	2.02	2.58	2.35	1.80
263	SHADA DUMRA	2.03	2.05	2.45	2.25	2.13
406	BUTA	2.18	2.15	2.49	2.34	2.42
419	SUNA SAIL	1.88	2.50	2.16	2.28	2.19
463	RONJAY	2.03	1.99	2.22	2.14	1.64

Simple sequence repeat (SSR) marker analysis is well established in rice due to the higher abundance and polymorphic nature of these markers. A large set of categorized SSR markers are available to analyze the diversity of the rice cultivars. However, as a complex and relatively less studied trait, no direct association of the SSR marker and rice cultivars has been studied yet for CO<sub>2</sub> responsiveness. The target loci were identified by an extensive literature mining and we selected those genes which are reported to be upregulated in rice in a CO<sub>2</sub> enriched environment (Fukayama et al. 2009, Fukayama et al. 2011). A total of 23 SSR markers were chosen to specifically analyze these target loci. All of these markers were located in a close proximity to target genes (~700 kbp). Beside this, 3 SNPs were also analyzed, which are found to be strongly associated with CO<sub>2</sub> responsiveness (Kikuchi et al. 2017). For investigating the association of the SSR markers with CO<sub>2</sub> responsiveness, 36 accessions were selected consisting of 26 and 10 cultivars which exhibited the most and least plasticity, respectively for multiple phenotypes.

All the studied markers showed polymorphism and a total of 102 alleles were found across the 36 accessions with an average of 4.43. Among the markers, a maximum of 7 alleles were found for RM14723 and RM15245 whereas a minimum of 2 alleles were detected for two markers RM16201 and RM28746. The sample picture of PAGE analysis of RM314 is shown in Fig. 3A. Marker RM9 had the maximum PIC value (0.76) and gene diversity (0.79).

To evaluate any possible correlation of SSR markers with the relative response in phenotypic plasticity, we conducted a correlation analysis among the markers and relative response in phenotypes. The correlation plot between the SSR markers and the relative response of the traits are shown in Fig. 3B. Other markers, which are located near the other 2 SNPs did not show any particular association. This may have resulted from large genetic distance (more than 3 cM) between SSR markers and the SNPs. The SSR marker RM17, which was found to have a high correlation with the selected physiological traits produced 3 alleles in our molecular analysis. The three different alleles are designated as allele A, allele B and allele C. Among the 26 cultivars with highest plasticity, 19 were found to have the allele A. All five cultivars with allele C were found to be non-plastic. The cultivars containing allele B were found to be distributed in both groups. We classified the 36 cultivars into three different groups based on the allelic distribution and performed distribution analysis of three alleles across different traits, shown in Fig. 3C. In case of panicle number and panicle dry weight, the allele A and allele C showed distinctive distribution. Allele A is more abundant in case of higher plasticity of panicle number (2.0-folds increase) and panicle dry weight (2.0-folds increase). Whereas, allele C was found with higher abundance in low response of four traits - panicle number (no increase), panicle dry weight (no increase), total dry weight (1.2-folds increase) and straw dry weight (1.5-folds increase). Allele B did not show any relation with any phenotypic response. No allelic variation was found in tiller number, most possibly due to its lower correlation with other traits.

The present study was designed to represent the diversity of the phenotypic plasticity of Bangladeshi local landraces using low density planting. We could successfully identify 10 cultivars with highest level of phenotypic plasticity, which is under investigation for further characterization. For this experiment, all the selected varieties were taken from the indigenous rice landraces. The modern high yielding varieties are genetically more uniform and thus lack phenotypic variation compared to landraces (Li et al. 2006, Ziska and Blumenthal 2007). Several studies indicated that high yielding varieties lack phenotypic plasticity and therefore show relatively lower response to  $e[CO_2]$  (Li et al. 2006, Ainsworth et al. 2008, Ziska et al. 2012). We also found similar results, where, two high yielding BRR1 varieties, which were used as control, showed lower than average plasticity towards resource enrichment.

The present study demonstrated that increased plasticity of panicle number or panicle dry weight in resource enriched low density planting is strongly associated with total dry weight (indicator of biomass), consistent with other studies (Shimono et al. 2008, Shimono and Okada 2013). The phenotypic plasticity



accessions, are currently under investigation for validation in  $[\text{CO}_2]$  enriched environment, will facilitate the selection process of plant breeders for  $e[\text{CO}_2]$  responsive varieties.

Another findings of our study is that cultivars which required more days for headings (late maturity group), showed better performance in low density planting in most of the traits we analyzed than cultivars of early maturity group. As late maturing cultivars grow for a longer time, so there is a higher chance for utilizing the assimilated carbons for reproductive and vegetative growth. Interestingly, the relative response of harvest index is higher for early maturing cultivars. This phenomenon can be attributed to the complex nature of the grain filling, which ultimately leads to panicle weight. At any given time, leaves of early maturing group cultivars are more close to senescence than late maturing group. As a consequence, there are higher chance of the early maturing groups to remobilize the assimilated carbohydrate for reproductive growth rather than vegetative growth, as growth of plant leaves are more likely to be terminated (Yang and Zhang 2006).

We also conducted a candidate gene-based analysis using simple sequence repeat (SSR) markers for identification of genes or SSR markers which are associated with the phenotypic plasticity. SSR markers were used due to their multiallelic nature, high variability and comparative abundance across the whole genome of rice. Phenotypic plasticity is the most possible causative trait of higher yield and biomass in low density planting as well as elevated  $\text{CO}_2$  ( $e[\text{CO}_2]$ ). These markers were chosen due to their close proximity to the loci of the 23 up regulated genes of rice in elevated  $[\text{CO}_2]$  (Fukayama et al. 2009, Fukayama et al. 2011) along with 3 QTLs of phenotypic plasticity (Kikuchi et al. 2017). After analyzing the marker-trait correlation we identified one particular marker, RM17, which is highly correlated with the traits. This marker is located at a close proximity to transcriptional activator HAP2 (AK073742), an up regulated gene in  $e[\text{CO}_2]$  and qER3, an associated SNP of phenotypic plasticity. In rice, the target promoter of HAP2 family transcription factor is related to starch synthesis (Morita et al. 2015). Although this correlation needs further validation, our study indicated the presence of a hotspot of phenotypic plasticity in chromosome 12 and the possibility of a link of starch synthesis regulation with enhanced phenotypic plasticity of rice.

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