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Genetic inheritance of multiple traits of blast, bacteria leaf blight resistant and drought tolerant rice lines

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Abstract

Rice (Oryza sativa L.) is a cereal and staple food crop of over half of the world's population. Blast, bacteria leaf blight and drought stresses affect yield of rice drastically ranging from 1-100% loss depending on the severity of disease and water deficit condition. Resistance and tolerance high yielding varieties of blast (Putra1) and drought (MR219 IR99784-156-137-1-3) respectively and also IRBB60 (bacteria leaf blight) were used. The research considered the genetic inheritance of the new improved lines and their interactions. Pedigree breeding method was used to develop two single, double and three-way (and reciprocal) crosses through marker-assisted selection. Southern blot analysis was used to determine success of introgression of resistance/tolerance genes/QTLs and selection, also validated by phenotyped results. Agro-morphological and yield parameters of the various populations were analysed. The results indicated levels of significant differences amongst and between treatments for non-drought stress (NS) and reproductive drought stress (RS) and their interactions. There were significant variation among parents and improved lines on some traits in NS treatment, but RS significantly affected parameters of DF, FFG, YM and most especially the susceptible parent, while the improved lines were tolerant. Significant interactions was recorded (P≤0.05) between treatment and variety (Trt*Var.) on PL, T, FFG and GLW. Cluster analysis and PCA of relationship among the 9 traits in the two treatments revealed that each of single, double and three-way (and reciprocal) crosses had good lines either under NS and RS.

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Introduction

Rice, a multi-environmental (rain-fed lowland, upland and deep water) (Ou, 1985, Latif et al., 2011) cereal and staple food crop of the world, it is one of the most cultivated crops along with wheat and corn. It provides nutrient and meets the economic need of the farming populace. It is consumed by over 50% of the world's population as their major source of calorie (Luo et al., 1998). Blast and bacteria leaf blight are two major important diseases of rice that causes significant yield loss to farmers (Asghar et al., 2007, Jia et al., 2000, Zhang et al., 2015).

The management practice for the disease can be fungicide, biotechnological methods, agronomic practices and cultivation of resistant cultivars (Ribot et al., 2008). The use of the resistant variety is the most environmental and economically friendly approach to blast threat (Castano et al., 1990, Saifullah et al., 1995, Khan et al., 2001, Haq et al., 2002). The challenge is often that due to the changeful nature of the virulent races, the resistance traits may be lost in the cause of time. The most effective strategy to minimize yield losses due to blast, bacteria leaf blight and drought stress is through the development of durable, broad-spectrum resistant varieties (Jena and Mackill, 2008; Kumar et al., 2014; Sundaram et al., 2014). Sundaram et al. (2014) reported that atleast 40 genes that confer bacteria blight resistance have been identified, while many have been fine-mapped and cloned (Natrajkumar et al., 2012). 101 blast resistant (R) genes (Rajashekara et al., 2014) and 350 QTLs have been identified (Sharma et al., 2012). Closely linked markers available for many blast resistance and bacteria blight genes have been identified (Sundaram et al., 2014). Drought stress is a condition of water availability deficit, it could be water deficit condition at any of the stages of rice development which has the potential of affecting yield. Drought stress is increasingly becoming a challenge to farming communities today with a global scale, it affects over 23million hectares of farming areas in Asia (Bray et al., 2000; Kumbhar et al., 2015). The increasing human population with approximated 10billion by the year 2050 also requires 50% increase to current global rice production to meet the food projection demand (Maclean et al., 2002, Bourman et al., 2007). This places a demand for strategy to increase yield production in the phase of climate change to develop rice adapted to drought stress (Pandey and Sukla, 2015). Understanding plant diversity in relation to behaviour and adaptation of drought-prone environment is important (Alonso-Blanco et al., 2009), the ability to design an effective strategy of phenotyping requires good understanding of plant survival mechanism under drought stress.

Understanding of plant diversity is relevant to assessment of genotypic variability under different water deficit condition as an important pre-condition for a successful drought tolerance breeding programme (Sarkar et al., 2013, Abenavoli et al., 2016, Anower et al., 2017). Blast, bacteria leaf blight (BLB) and drought stress could affect rice drastically, and result showed that high incidences caused yield losses of 100% (Zhai and Zhu, 1999), 1-50% (Scardaci et al., 2003) and 100% depending on the stage of rice development and duration respectively. 50% yield loss was recorded for abiotic stress (Bray et al., 2000, Iqbal et al., 2013, Li et al., 2014).

The parameters of panicle length, effective tillers, grain length and width ratio, 100 grain weight, fully grain weight were amongst those considered important agronomic trait which has correlation with increased yield potential in rice, considered as quantitative traits which could be affected by the influence of environment (Han et al., 2004, Guo et al., 2003, Han et al., 2006, IRRI-SES, 2013, Taglea et al., 2016, Chang et al., 2016).New improved lines has been developed with three resistance/tolerant traits of blast (Magnaporthe grisea)

bacteria leaf blight (Xanthomonas oryzae) and drought tolerance (MR219) through pyramiding of marker-assisted selection. This research considered the genetic inheritance of the target genes and their interactions.

Materials and Methods

Plant material and breeding design

Plant materials used were new developed lines from three parents with resistances to blast, M. grisea anomorph P. oryzae, a popular Malaysia high yielding rice variety known as Putra1. Bacteria leaf blight resistance variety known as IRBB60, and a drought tolerant Malaysia variety referred to as MR219 (IR99784-156-137-1-3 Drought tolerance). these varieties were developed as single crosses, double cross and three-way cross with reciprocal cross as well. These new lines were developed in a glass house at Rice Research Centre and Laboratory of Climate and Smart-Food Crop Production, Universiti Putra Malaysia.

DNA extraction and nanodrop spectrophotometry

Three-four weeks old leaves samples were collected and ground using mortar and pestle in liquid nitrogen. The procedure was according to Doyle and Doyle (1987) cetyltrimethylammonium bromide CTAB method with modification following the protocol of McCouch et al (1988).

The DNA pellet was diluted in 50µl TE buffer, 2µl was pipetted on nanodrop spectrophotometry machine to measure the quantity and purity of the DNA samples. This formed the basis for the constitution of the DNA working solution for polymerized chain reaction (PCR) amplification.

Molecular markers

Simple sequence repeat (SSR) markers polymorphic and linked to the genes/QTLs for the three parental varieties were purchased. Putra1, a blast resistance variety had two polymorphic markers that were also linked to the genes of resistance (R), MR219 (IR 99784-156-137-1-3) drought tolerance variety used had three qDTY and IRBB60 had four R genes (Pinta et al. 2013, Miah et al. 2016, Kumar et al. 2014, Khan et al. 2015, Pradhan et al. 2015, Shamsudin et al. 2016, www.gramene.org).

Polymerized chain reaction and electrophoresis

The pairs of primers for the various genes/QTLs were optimized to amplify simple sequence repeat loci for polymerase chain reaction (PCR). Survey of the three parental varieties was carried out to identify SSR markers polymorphic to each of them. Total PCR reaction of 15µL which contained 70ng DNA template had 7.5µL master mix (Thermo Scientific, Waltham, MA, USA), 4.5µL nucleaus free water and 1.0 µmol L^{-1} concentration of each primer (Forward and Reverse). The PCR amplification was conducted in a thermocycler (T100TM, Bio-Rad, Hercules, CA, USA) following the touchdown protocol with the lid temperature of 105^{0}C. The initial denaturation, annealing and extension temperatures were thus; 94^{0}C for 3mins followed by 94^{0}C for 30sec, a temperature of 62^{0}C for 1min., +1^{0}C per cycle followed by 72^{0}C for 30sec., then a returned to step 2, 9× followed by 94^{0}C, 30sec., 52^{0}C, 1min, 72^{0}C, 2mins., another return to step 6, 29×., and finally 72^{0}C, 10mins. Followed by rapid cooling to 4^{0}C∞ prior to analysis.

Southern blot analysis using gel electrophoresis was carried out. 2.0% Metaphor™ agarose (Lonza) gel containing 5µL Midori green in 1× TBE buffer (0.05 mol L^{-1} Tris, 0.05 mol L^{-1} boric acid, 1 mmolL^{-1} EDTA, pH 8.0) was run with 5µL of PCR product mixed with loading dye. The gel run was at a constant voltage of 80V for 45-60 minutes. Molecular imager system (GelDoc™ XR, BioRad) was used to analyzed band pattern under UV light for amplified products.

Identification of polymorphic markers and progeny selection

The basis for a successful marker-assisted selection was the identification of the polymorphic markers for each of the parent trait. Blast resistance had RM6836 and RM8225, IRBB60 had *Xa13* prom, RM122, RG136, pTA248 and RM224 while MR219 IR99784-156-137-1-3 drought tolerance had RM511, RM1261 and RM520 polymorphic to the drought tolerance QTLs.

Two F_1s with double bands corresponding with the parent plants were selected, that was in a cross between a common recipient parent Putra 1 and donor IRBB60, Putra1 and MR219. The selected progenies formed the bases for a double cross and three-way and reciprocal crosses.

The selection of the subsequent generations of $F_1(2)$ and F_1 three-way crosses were based on southern blot analysis of the bands which coincide with the first recipient and the introgressed variety. Those were selected and advanced until a non-segregating $F_3(2)$ double, F_4 single, and F_3 three-way crosses pure-line generation was reached.

Fungal, bacteria culture and inoculation

Most virulent strains of fungus *Magnaporthe grisea* pathotype P7.2 and bacterium *Xanthomonas oryzae* pv *oryzae* MX01552 were obtained from Malaysia Agricultural Research and Development Institute (MARDI). They were sub-cultured in potato dextrose agar (PDA) for 14 days in 25^0C and nutrient agar (NA) for two days in 30^0C respectively (Suresh et al. 2013, Mahdieh et al. 2013).

The *M. grisea* mycelia culture was prepared in sterile distilled water suspension at concentration of 1.9×10^6 conidia mL^{-1}. The *Xoo* culture was also prepared in a suspension concentration of 10^9.

The fungus suspension was sprayed on young leaves of 2-3 weeks old at relative humidity >90% for 48hours for disease infection and after 7 days disease scoring was carried out according to the IRRI-SES, (2013) glass house scoring, thus; from 0-2, resistant (R), 3 considered as moderately resistant (MR) and score 4-6 as MS, while 7-9 as S. The *Xoo* pathogen was clip inoculated on 3-4 weeks old leaves at 1-2cm from the tip of the leaves. The scoring was by measuring the length of infected area with meter rule in centimeter according to IRRI-SES, 2013. Glass house scale (Banito et al. 2012) and modification according to Amante-Bordeos et al. (1992), thus; 0-5 considered as R, >5-10 MR, >10-15 MS, while >15 were considered as S.

Drought stress imposition

Reproductive drought stress was imposed on the rice lines carrying drought tolerance QTLs. According to IRRI-SES (2013) one week of water deficit stress for glass house experiment is enough to affect yield. The reproductive stage was between 70-90 days after sowing. Stress was imposed for >2weeks with leaves turned from U-shaped to 0-shaped as criterion for

scoring drought (IRRI-SES, 2013, Kadioglu and terzi, 2007), dried soil and soil moisture meter with >15cm depth measured dried.

Experimental layout and cultivation

The experiment was set out in a glass house at Rice Research Centre, Universiti Putra Malaysia with 3 plants each per bucket and label accordingly. The leaves of the parents to their progenies were collected and genotyped using southern blot analysis to determined the ideal plants for selection until line stability of the progenies as non segregating generation reached. The non-segregating (pure-lines) and parent plants were laid in rows according to their genotypes.

Data collection

Nine quantitative traits were measured and data collected from the 3 parents and their progenies (improved lines) in single trial under non drought stress (NS) and reproductive drought stress (RS) treatment in five (5) replications in around 120 days after seeds sowing. These quantitative traits included; days to 50% flowering (DF), height of plant (HP), panicle length (PL), effective tillers (ET), tillers produced (T), fully filled grain (FFG), 100-grain weight (100-GW), grain length and width ratio (GLW) and yield maturity(YM) as indicated in Table 4. for NS and Table 7. for RS treatments.

Statistical Analysis

All evaluated data were subjected to analysis of variance (ANOVA) using Statistical Analysis Software (SAS) version 9.4 in Complete Randomized Design (CRD). The expressed results were in mean, mean square, correlation coefficient (CV) and standard deviation to set the relationship among the parameters (traits). To determine the genetic variability among the 9 quantitative traits, cluster analysis was employed. The genetic relationship among the parent varieties and improved rice lines was determined in conformity with unweighted pair group method using arithmetic average (UPGMA) algorithm and sequential, agglomerative, hierarchic and non-overlapping (SAHN) method using Numerical Taxonomy Multivariate Analysis System, Exeter Software, Setauket, NY, USA software (NTSYS v2.1).

Table 1. Description of major symbols

Symbol	Description
PB	Cross between Putra1 and IRBB60
PD	Cross between Putra1 and MR219
PBD	Three-way cross between putar1 and IRBB60(F1) and MR219 Drought tolerant
PDB	Double cross(from two F_1s; P×D and P×B
DPB	Three-way reciprocal cross (between MR219 drought tolerant and F1 Putra1×IRBB60)
DF	Days to 50% flowering
HP	Height of plant measured from the base to the tip of panicle
PL	Panicle length measured in centimetre
ET	Effective tiller refers to those that had gains
T	Total number of tillers produced
FFG	Fully filled grain obtained by counting the number of grains produced per panicle
GLW	Grain length and width ratio of a grain
100GW	100grain weight is a measure of 100 grains in grams

YM	Yield maturity is the number of days from sowing to harvest.
DS	Reproductive drought stress
NS	Non-drought stress

Results and discussion

Genotypic and phenotypic selection of improved lines

Electrophoresis analysis (gel scoring) results with bands showing alignment to the recipient parents were selected as pure and non-segregating lines in the F_4 single crosses for PD and PB, F_3 three-way and reciprocal crosses (PBD, DPB) and $F_3(2)$ (double) cross (PDB) as shown in the Figures 1, 2, 3, 4 and 5 below.

Challenging the plants with the disease pathogens and subjection to water deficit stress for the improved lines and susceptible varieties was undertaken. The results showed that the improved lines were resistant and moderately resistant to blast and bacterial leaf blight and as well tolerant to drought stress according to acceptable yield percentage for tolerant rice (Miah et al. 2016, Ashkani et al. 2011, Yambao and Ingram, 1988). The susceptible varieties in each case were truly susceptible to the disease pathogens and drought stress.

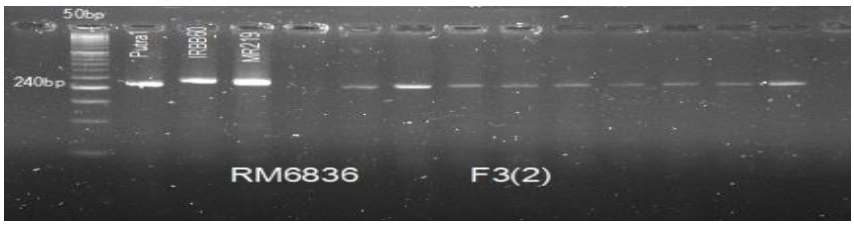

Figure 1. $F_3(2)$ cross for improved lines(PDB)

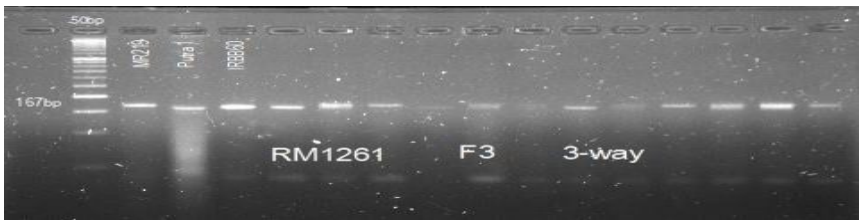

Figure 2. F_3 three-way reciprocal cross of improved lines(DPB)

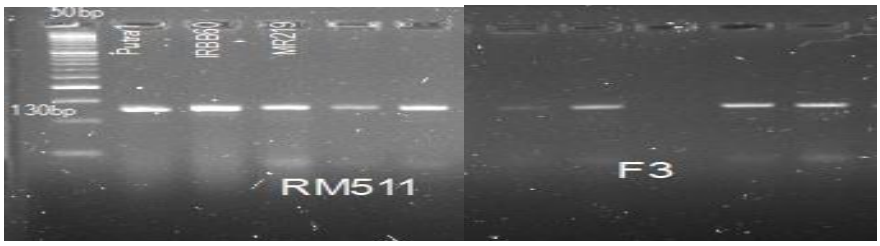

Figure 3. F_3 three-way cross of improved lines PBD

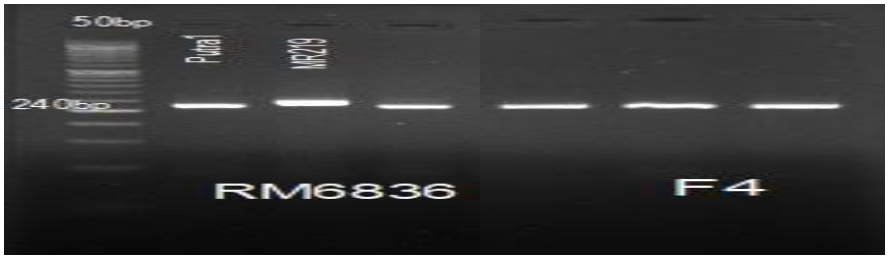

Figure 4. F_4 single cross improved lines(PD)

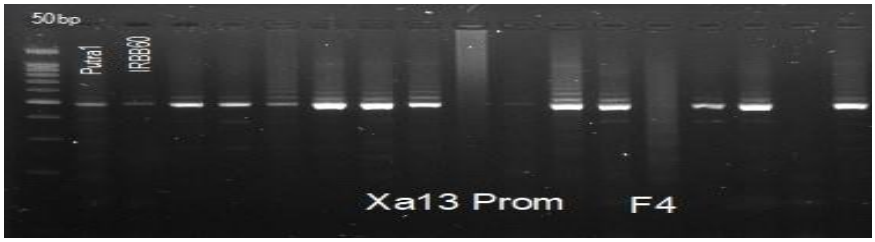

Figure 5. F_4 single cross for PB improved lines

Agronomic trait assessment of improved lines

These are also considered as agronomic traits; days to 50% flowering, height of plant, panicle length, number of tillers, effective tillers, fully filled grain, 100-grain weight, grain length and width ratio, yield maturity. The effect of blast, bacteria leaf blight and drought on any of these parameters could result in poor yield and quality. Improved lines were evaluated under two treatments condition, namely; non-drought stress (NS) and reproductive drought stress (RS) condition. Under NS the data of both parents and improved lines were analysed, whereas in RS treatment, a susceptible and improved lines were analysed. They were to further validate the results of the southern blot analysis (genotyping) and the phenotyping results that showed resistances and tolerance to blast, bacteria leaf blight (BLB) and drought.

Table 2. Polymorphic, linked/quantitative trait loci and flanking simple sequence repeat (SSR) markers for genes/QTLs used.

Variety	SSR markers	Genes/QTLs	Chromosome position	Expected base pair size	Description
Putra1					
Blast resistance	RM6836	*Piz, Pi2, Pi9*	6	240	Polymorphic/linked
	RM8225	*Piz*	6	221	Polymorphic/linked
IRBB60					
Bacteria leaf blight resistance	RM224	*Xa4*	11	157	Polymorphic/linked
	RM122	*xa5*	5	227	Polymorphic/linked
	RM153	*xa5*	5	201	Linked
	RM13	*xa5*	5	141	Polymorphic/linked
	RG136	*xa13*	8	246	Polymorphic/linked
	Xa13Prom	*xa13*	8		Polymorphic/linked
	RM21	*Xa-21*	11	157	Polymorphic/linked
	pTA248	*Xa-21*	11		Polymorphic/linked
MR219 IR99784-156-137-1-3					
Drought tolerance	RM511	$qDTY_{12.1}$	12	130	Polymorphic/linked
	RM1261	$qDTY_{12.1}$	12	167	Polymorphic/flanking marker
	RM28099	$qDTY_{12.1}$	12	120	Flanking marker
	RM28076	$qDTY_{12.1}$	12	287	Flanking marker
	RM520	$qDTY_{3.2}$	3	247	Polymorphic/linked
	RM236	$qDTY_{2.2}$	2	174	Linked
	RM276	$qDTY_{2.2,3.1}$	6	149	Flanking marker

Askani et al., 2011, Miah et al., 2016, Shamsudin et al., 2016, www.gramene.org, He et al, 2006, Khan et al., 2015, Pradhan et al., 2015

Table 3. ANOVA for the parameters showing level of significance for non-drought stress (NS)

SOURCE	DF	DF(no)	HP(cm)	PL(cm)	ET(no)	T(no)	FFG(no)	100GW(no)	GLW(cm)	YM(days)
Var	13	6.15*	73.9**	33.39*	13.73^{ns}	16.90**	336.66*	0.02**	0.61**	3.48*
Rep.	4	3.31^{ns}	50.4^{ns}	0.28^{ns}	2.13^{ns}	5.32^{ns}	239.19^{ns}	0.02^{ns}	0.07^{ns}	2.19^{ns}
Error	52	3.13^{ns}	24.6^{ns}	1.04^{ns}	5.31^{ns}	5.33^{ns}	117.92^{ns}	0.009^{ns}	0.1^{ns}	1.52^{ns}
C. TotaL	69									

∗Significant at P≤0.05, ∗∗highly significant at P≤0.01, ns: non-significant P> 0.05, DF. (degree of freedom), Var. (Variety), REP. (Replications), C. Total (Corrected total), DF (Days to 50% flowering), HP (cm) (Height of plant measured in centimetres), PL (Panicle length in centimetres), ET (Effective tillers), Tillers (total number of tillers), FFG (Fully filled grains), 100-GW (100 grain weight in grams)

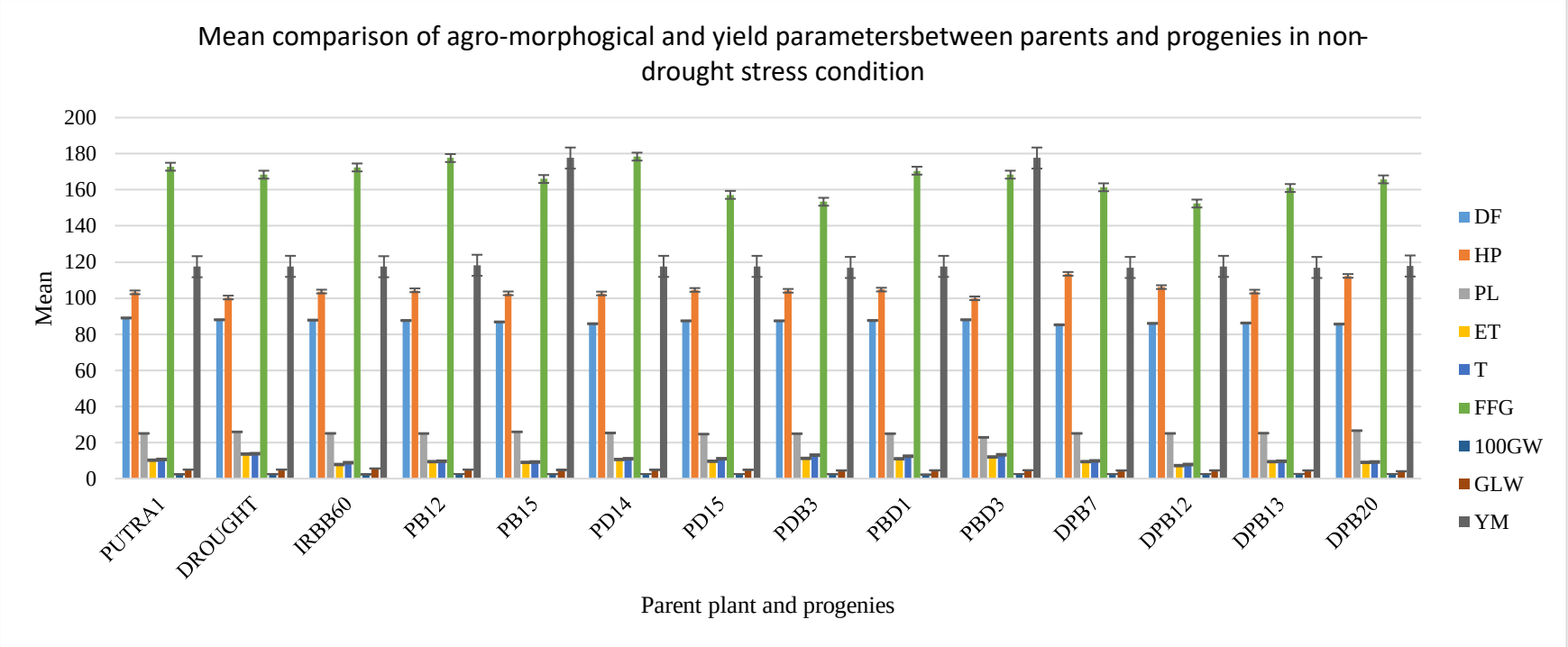

Figure 6. Comparison of agro-morphogical and yield parameters of parent plants and their progenies (improved lines) in non-drought stress condition

Table 4. Mean squares, least significant differences, coefficient of variations, standard deviation and broad sense heritability of vegetative traits and yield components of different rice varieties (genotypes) for reproductive drought stress and non drought stress treatments.

VARIETIES	DF(no)		HP(cm)		PL(cm)		ET(no)		T(no)		FFG	
	RS	NS	RS	NS	NS	RS	NS	RS	NS	RS	NS	RS
PUTRA1	89a	-	103.19c	-	25.03ab	-	10.2bcd	-	10.6bcde	-	172.8ab	-
DROUGHT	88ab	-	100.3c	-	25.9ab	-	13.6a	-	13.8a	-	168.4abc	-
IRBB60	87.8abc	96.6a	103.61c	97.52a	25.06b	20.68c	7.8de	9ab	8.8de	9.6ab	172.4ab	26.4c
PB12	87.6abc	-	104.34c	-	24.9b	-	9.4bcde	-	9.6cde	-	177.6a	-
PB15	86.8abcd	-	102.64c	-	25.9ab	-	9cde	-	9.2de	-	166abcd	-
PD14	85.8bcd	91.8d	102.56c	96.6a	25.28b	23.7ab	10.6bcd	11.4a	11abcd	11.4ab	178.4a	52.4ab
PD15	87.4abcd	92.8cd	104.54c	97.32a	24.66b	22.6ab	9.6bcde	8b	11abcd	8.4b	157.2cd	49.8ab
PDB3	87.4abcd	92.8	104.06c	100.6a	24.88b	24.1a	11.2abc	10ab	13ab	10ab	153.4d	48.2ab
PBD1	87.6abc	94bc	104.72c	100.5a	24.9b	22.1bc	11abc	11.6a	12.4abc	11.6a	170.6abc	45.4b
PBD3	88ab	94bc	99.9c	96.22a	22.86c	23.3ab	12ab	11.6ab	13.2ab	10.6ab	168.4abc	59.4a
DPB7	85.2d	91.8d	113.44a	102.1a	25.04b	24a	9.4bcde	11.8a	9.8cde	11.8a	161.4bcd	53.4ab
DPB12	86bcd	92.8cd	106.1bc	99.68a	25.04b	23.4ab	7.2e	9.2ab	7.8e	9.4ab	152.4d	52.2ab
DPB13	86.2bcd	93cd	103.6c	98.44a	25.18b	23.4ab	9.4bcde	10.2ab	9.6cde	11.4ab	161bcd	43.76b
DPB20	85.6cd	95b	112.3ab	96.3a	26.58a	23.7ab	9cde	10.2ab	9.2de	10.4ab	165.8abd	46.2b
LSD	2.25	1.56	6.29	11.82	1.3	1.74	2.92	3.33	2.93	3.07	13.78	11.83
CV	2.03	1.3	4.74	9.35	4.07	5.87	23.13	25.43	21.69	22.86	6.54	19.34
Mean	87.03	93.46	104.67	98.54	25.09	23.09	9.96	10.2	10.64	10.46	166.13	47.72
Std Dev.	1.93	1.79	5.95	8.51	1.20	1.55	2.59	2.65	2.74	2.43	12.89	11.85
h^2_B (%)	16.09	56.18	28.63	-17.54	31.13	28.40	24.03	2.18	30.24	-0.93	27.06	41.43

Heritability percentage categorised as low (0-30%), moderate (30-60%), and high (≥60%)

Table 5. Continued

VARIETIES	100GW(g) NS	RS	GLW(cm) NS	RS	YM(days) NS	RS
PUTRA1	2.41de	-	4.97bc	-	117.4ab	-
DROUGHT	2.47cde	-	4.98b	-	117.4ab	-
IRBB60	2.35e	2.43a	5.61a	4.42a	116.8b	133.2a
PB12	2.55abc	-	4.96bc	-	118.2a	-
PB15	2.49cde	-	4.81bcd	-	117.6ab	-
PD14	2.55abc	2.34a	4.86bcd	4.49a	117.4ab	128.4b
PD15	2.52abcd	2.4a	4.86bcd	4.49a	117.6ab	127.6b
PDB3	2.47cde	2.38a	4.53d	4.5a	117ab	127.8b
PBD1	2.63a	2.42a	4.59bcd	4.51a	117.6ab	127.8b
PBD3	2.52abcd	2.38a	4.63bcd	4.47a	117.6ab	129b
DPB7	2.52abcd	2.32a	4.51cd	4.57ab	117ab	127.4b
DPB12	2.52abcd	2.35a	4.57cd	4.75a	117.6ab	128.2b
DPB13	2.6ab	2.39a	4.51d	4.57ab	117ab	128.2b
DPB20	2.56abc	2.43a	4.08e	4.53	117.8ab	127.6b
LSD	0.12	0.11	0.4	0.2	1.31	1.8
CV	3.83	3.75	6.63	3.5	0.88	1.09
Mean	2.51	2.39	4.75	4.53	117.43	128.52
Std Dev.	0.11	0.09	0.44	0.17	1.02	2.05
h^2_B (%)	16.67	0.5	50.50	6.25	-7.27	56.22

Var. (variety), IRBB60 (B) (Bacteria leaf blight resistance), Drought (D) (MR219 Drought tolerance), reciprocal cross with MR219 Drought, Putra 1 and IRBB60 (DPB), Putra1, IRBB60 and MR219 Drought tolerance (PBD), Putra1, MR219 Drought tolerance and IRBB60 (PDB), Putra1 and MR219 drought tolerance (PD), Putra1 and IRBB60 (PB), LSD(Least significant difference), CV(Coefficient of variation), Mean(average), DF(Days to 50% flowering), HP(cm)(Height of plant measured in centimeters), PL(Panicle length in centimeters)ET(Effective tillers), Tillers (Total number of tillers), FFG(Fully filled grains), 100-GWT(100 grain weight in grams), YM (yield maturity counted on number of days from sowing to physiological maturity).

Agro-morphological and yield traits data for single cross (F_4), double cross (F_3(2)), and three-way cross (F_3) for non-drought stress.

Some parameters of pure-line agro-morphological and yield traits were observed and measured to ascertain the differences between the various rice lines and the parent plants. Table 3. shows an analysis of variance (ANOVA) of 9 traits, the varieties were all significant with the exception of effective tillers (ET). Days to 50% flowering (DF), panicle length (PL) and yield maturity (YM) were significant at 5% level of probability ($P \leq 0.05$), while height of plant (HP), tillers (T), fully filled grain (FFG), 100GW, grain length and width ratio (GLW) were significant at 1% level of probability ($P \leq 0.01$). No significant differences among the replications were recorded in all the morphological and yield traits under non drought stress.

Comparison of parameters of non-drought stress (NS) and reproductive drought stress (RS).

Table 4. and Table 5. compares nine (9) mean data of the two treatments of non-drought stress (NS) and reproductive drought stress (RS). The comparison was between improved lines from the three crossed methods. Even though there were 14 lines in all on NS and 10 on RS because, non drought stress had the three (3) parent plants and two (2) single cross lines PB12, PB15 which did not have drought tolerance QTLs to be subjected in to

reproductive drought stress treatment. Now, the parameters of days to 50% flowering (DF), height of plants (HP), fully filled grain (FFG), yield maturity (YM) showed that the means of reproductive drought stress (RS) was higher on all the improved lines and each compared to their non-drought stress lines. While days to 50 % flowering on NS has the highest and lowest number of days as 89 and 86 days respectively. RS had 91.8 and 96 days for lowest and highest respectively. Height of plants (HP) for NS measured 102.3-112.3 cm and RS 96.3-102.1 cm for lowest and highest respectively. The length of panicle for NS was 22.86 cm-26.5 cm and RS was 22.6 cm-24.1 cm for lowest and highest respectively. There was an exceptional difference between the line PBD3, which showed that the RS measured 23.2 cm in length whereas the NS was 22.86 cm in length. The mean number of fully filled grain on NS lines had lowest mean as 152.2 and highest 178, and RS was 43.7 and 59.4 for lowest and highest count respectively. The parameter of 100-grains weigh measurement taken indicated that the lowest weight for NS was 2.52g and the highest weight was 2.63g, for the RS it was 2.32-2.43g, lowest and highest respectively. There were slight variations between the two treatments among some lines on the parameters of grain length and weight ratio. Even though, the NS ratio was higher than RS on five (5) improved lines, four (4) of the RS and reciprocal lines of DPB7, DPB12, DPB13, DPB20 were higher than their counterpart lines. Effective tillers (ET) and number of tiller (T) didn't show any clear difference between the two treatments. It was reported that water deficit stress affect these quantitative traits which was also confirmed in this study (Latiffe et al., 2004; Atlin et al., 2006; Barnabas et al., 2008; Garrity and O'Toole, 1994). There were alternating variation among them. This was because the treatment for drought was at the reproductive stage when the tillers were matured and could not be affected by just about two weeks of water deficit stress. Record has it that drought affect tillers at vegetative stage (Cruz et al., 1986).

In consideration of non drought stress (NS) in the Table 4. and Table 5. with 14 lines (parental and improved lines), it indicated the mean days to 50% flowering of Putra1 was 89 days. This was also corroborated with the findings of Miah et al. (2015) on the same trait of the same variety (Putra 1) and significantly different from other traits, but all other traits were not significantly different from each other. DPB7 was significantly different on height of plant, while the other 8 traits were not different from each other. They tally with their parent plants, which indicated their was no variation. DPB20 and PBD3 were significantly different from each other and the other genotypes on parameter of panicle length. The other genotypes including the parent plants were not different from each other. All other parameters did not show difference between parents and progenies except the parameters of effective tillers, tillers and grain length and width ratio showing significant difference between IRBB60 and others, although with some similarities with other genotypes.

Considering the means of reproductive drought stress (RS), it has indicated that the improved lines were different from the susceptible (control) variety because, the improved lines were introgressed with drought tolerance QTLs (qDTY) which conferred on it ability to adjust to some level of water deficit stress as against the control check that had no drought tolerance qDTY. Field evaluation of MR219 carrying these three $qDTY_{2.2}$, $qDTY_{3.2}$, $qDTY_{12.1}$, confirmed tolerance to reproductive drought stress with grain yield of 756-2521 kg ha^{-1} for 2013 and 903-2523 kg ha^{-1} in the year 2014, while the susceptible variety had grain yield of 13 kg ha^{-1} in 2013 and didn't flower in 2014 (Shamsuddin et al., 2016). Venuprasad et al. (2009) and Swamy et al. (2013) reported tolerance ability of $qDTY_{3.1}$ and $qDTY_{2.2}$ respectively. Mishra et al. (2013) reported that twenty one (21) experiments conducted in eastern India

and IRRI confirmed that *qDTY*$_{12.1}$ has an effect that increases with increasing severity of water deficit stress. These confirms the reason the improved lines performed better compared with its susceptible check.

Heritability estimate on non drought stress (NS) and reproductive drought stress (RS) treatments.

Broad sense heritability is the total ratio of genetic variance to phenotypic variance. In order words, the proportion of the parental gene inherited in the progenies that are influenced by the environment and expressed in the phenotypic traits. The percentage ratio is expressed as low, medium and high represented as 0-30%, 30-60% and ≥60% respectively.

The broad sense heritability of characters studied for non drought stress (NS) and reproductive drought stress (RS) treatments (Table 4 and Table 5) were all within the range of 0 to 56.22, which was within low to moderate heritability. These indicated the magnitude of heritability that was influenced by environmental factors. Low percentage is higher environmentally influenced and moderate heritability is lesser. Climatic factors which are environmental in nature presents a great challenge to rice plant, there are various optimum temperature requirements for various growth phases and stages, outside the range it affects that phase of development (Tashiro and Wardlaw, 1989, Baker and Allen, 1993, Singh et al., 1996, http:ricepedia.org). Control experiment and field experiment often presents a variation in response to environmental factors. For instance, IRRI-SES (2013) categorised scoring and protocols for diseases and tolerance evaluation for green or glass house experiment differently from field. The low and medium heritability may not present a true genotypic content of the improved lines in respect of the treatment since heritability estimate in this case is not restrictive and does not consider experimental environment. The varieties used as recipient parents were all high yielding, resistant and tolerant. And confirmed to be so resistant and tolerant by the genotyped and phenotyped results.

The magnitude of heritability among the parameters of non drought stress (NS) showed that all characters were within the lowest percentage of heritability which suggests environmental influence, except days to 50% flowering (DF), fully filled grains (FFG) and yield maturity (YM) which were medium ranged as; 56.18%, 41.43% and 56.22% respectively. Reproductive drought stage (RS) treatment showed characters that were also low ranged with exception of traits of panicle length 31.13%, tillers (T), 30.24% and grain length and width ratio (GLW), 50.50% as medium heritability, indicated as having lesser environmental influence compared to low heritability. All ranges of heritability percentage estimates were also reported by Oladosu et al. (2014), Meena et al. (2016), Ridzuan et al. (2018). The low heritability may not present the true nature of the inherited genes, because controlled environment of research may present a variation different than when in a field environment.

Table 6. Estimates of correlation coefficients on the phenotype among 9 traits in rice lines for non drought stress

	DF(no)	HP(cm)	PL(cm)	ET(no)	T(no)	FFG(no)	100GW(g)	GLW(cm)	YM(days)
DF(N0)	1								
HP(cm)	-0.26246*	1							
PL(cm)	-0.2019	0.30623**	1						
ET(no)	0.00606	-0.07271	-0.11752	1					
T(no)	0.00196	-0.12024	-0.24529*	0.92492**	1				
FFG(no)	0.00394	-0.13601	0.06923	0.08221	-0.00771	1			
100GW(g)	-0.2765*	0.07116	0.06867	0.01494	-0.03721	0.13656	1		
GLW(cm)	0.34151**	-0.26206*	-0.11788	0.01078	0.00824	0.09208	-0.29898	1	
YM(days)	-0.01376	0.1382	0.05447	0.11179	0.04018	0.23267	0.17624	-0.12587	1

∗Significant at P≤ 0.05, ∗∗highly significant at P≤ 0.01, ns: non-significant P> 0.0

Correlation coefficient relationship for non-drought stress

The r-values and test of significance provided by proc corr in SAS program (Table 6) showed days to 50% flowering (DF) and height of plant (HP), 100-grain weight were significantly different (P≤0.05), while with grain length and width ratio showed high significant difference (P≤0.01). All the traits were low and negatively correlated. Height of plant (HP) with panicle length (PL) and grain length and width ratio (GLW) were significant at (P≤0.05) and (P≤0.01) respectively. Both were similarly low and negatively correlated. Panicle length (PL) and effective tillers (ET) were jointly significant to tillers (T) at (P≤0.05) and (P≤0.01). while the former is low and negatively correlated, the later is high and positively correlated. Notable high correlated relationship was observed between height of plant (HP), effective tillers and 100GW. The relationship with ET though both were high but it was strongly negative, while with 100GW was strong positive linear relationship. Ridzuan et al. (2019) and Oladosu et al. (2018) also used phenotypic traits to determine relationships.

Karl Pearson's correlation coefficient r-value helps to identify association that exists between two unique traits, even though it is not able to measure the magnitude (extent) of association but gives a clue as to the relationship. The interpretation of correlation coefficient was given, but Ratner (2009) provides an accepted standard guideline.

The r-value could indicate no linear relationship, positive linear relationship and negatively linear relationship represented by 0, +1, and -1 respectively. Low, moderate and strong positive linear relationships are represented with values ranging from 0-0.3, 0.3-0.7 and 0.7-1, respectively while 0 to -0.3, -0.3 to -0.7, and -0.7 to -1 would indicate low, moderate and strong negative linear relationship respectively.

Table 7. ANOVA for the parameters showing level of significance for reproductive stage drought stress (RS)

Source	DF	DF(day)	HP(cm)	PL(cm)	ET(no)	T(no)	FFG(no)	100GW(g)	GLW(cm)	YM(days)
Var	9	11.02**	21.53^{ns}	5.47**	7.47^{ns}	6.22^{ns}	384.90**	0.01^{ns}	0.04^{ns}	14.63**
Rep.	4	0.93^{ns}	74.74^{ns}	0.62^{ns}	8.65^{ns}	7.13^{ns}	86.59^{ns}	0.01^{ns}	0.02^{ns}	0.97^{ns}
Error	36	1.49^{ns}	84.94^{ns}	1.84^{ns}	6.73^{ns}	5.72^{ns}	84.83^{ns}	0.01^{ns}	0.03^{ns}	1.97^{ns}
Cor. Total	49									

∗Significant at $p \leq 0.05$, ∗∗highly significant at $p \leq 0.01$, ns: non-significant $p > 0.05$, DF. (degree of freedom), REP.(Replications), Cor. Total (Corrected total), DF(Days to 50% flowering), HP(cm)(Height of plant measured in centimetres), PL(Panicle length in centimetres), ET(Effective tillers),Tillers (total number of tillers), FFG(Fully filled grains), 100-GW(100 grain weight in grams)

Table 8. Estimates of correlation coefficients on the phenotype among 9 traits in rice varieties for reproductive drought stress

	DF(days)	HP(cm)	PL(cm)	ET(no)	T(no)	FFG(no)	100GW(g)	GLW(cm)	YM(days)
DF(days)	1								
HP(cm)	-0.11295	1							
PL(cm)	-0.34917*	0.04025	1						
ET(no)	-0.1276	0.02553	0.06464	1					
T(no)	-0.11063	0.02957	0.05282	0.98204**	1				
FFG(no)	-0.35762*	-0.06439	0.24756	0.1528	0.1108	1			
100GW(g)	0.26915	-0.0041	-0.18723	-0.13435	-0.13343	-0.11789	1		
GLW(cm)	-0.21732	0.08631	0.17222	0.07339	0.0711	0.2032	-0.00037	1	
YM(days)	0.59004**	-0.11094	-0.53245**	-0.0833	-0.06109	-0.4332*	0.13789	-0.22637	1

ANOVA for Agro-morphological and yield traits data for single cross (F_4) double cross F_3(2), and three-way cross and reciprocal (F_3) under reproductive drought stress (RS).

Phenotyping for reproductive drought tolerance was carried out to determine the level of improved lines tolerance to water deficit condition. This is very importance due to challenges of climate change and irregular rainfall for rainfed rice and also for irregular irrigation water supply. The ANOVA Table 7. showed highly significance difference ($P \leq 0.01$) among varieties (improved lines) on the parameters for DF, PL, FFG, YM, while there was non among the replicates. This clearly indicated the effect of drought on these very important parameters (quantitative traits) whose effect affect yield drastically (Taglea et al., 2016, Chang et al., 2016).

Correlation coefficient relationship for non-drought stress

In Table 8 which estimated the relationship among phenotypes of the reproductive drought stress traits indicated that days to 50% flowering (DF) had a significant relationship with panicle length (PL) and fully filled grain (FFG) at ($P \leq 0.05$) although negative and moderate but yield maturity (YM) at ($P \leq 0.01$), it was positive and moderate correlated relationship. Panicle length (PL) and yield maturity (YM), effective tillers (ET) and fully filled grain (FFG) were both significant at $P \leq 0.01$. while the former is negative and moderately correlated the later was low but positively correlated.

The strongest positively and high correlated relationship but not significant was between effective tillers (ET) and total number of tillers per plant (T). It is because most of the tillers were effective despite the drought stress. Stress was introduced at the reproductive stage when tillers were already matured and it could not affect it.

Table 9. ANOVA for the parameters showing Interaction levels of significance

SOV	DF	DF(days)	HP(cm)	PL(cm)	ET(no)	T(no)	FFG(no)	100GW(g)	GLW(cm)	YM(days)
Treatment	1	1020.17**	1091.15**	69.17**	7.04ns	0.02ns	325366.67**	0.60**	0.18ns	2733.4**
Varieties	10	6.22*	67.75ns	3.70**	10.30*	11.87**	307.53**	0.01ns	0.19**	1.09ns
Replications	4	0.89ns	89.62ns	1.89ns	10.26ns	8.51ns	165.90ns	0.01ns	0.08ns	1.92ns
Trt*Var	8	3.6ns	42.50ns	2.59*	5.43	10.66*	211.25*	0.01ns	0.17*	0.75ns
Error	76	2.52	53.68	1.13	4.33	4.40	90.70	0.01	0.06	1.25
Total	99									

∗Significant at p ≤ 0.05, ∗∗highly significant at p ≤ 0.01, ns: non-significant p > 0.05, DF. (degree of freedom), REP.(Replications), Cor.. Total (Corrected total), DF(Days to 50% flowering), HP(cm)(Height of plant measured in centimetres), PL(Panicle length in centimetres), ET (Effective tillers),Tillers (total number of tillers), FFG(Fully filled grains), 100-GW(100 grain weight in grams), YM (yield maturity counted on number of days from sowing to physiological maturity).

Table 10. Correlation coefficient of interaction of non drought stress (NS) and reproductive drought stress (RS) treatments.

	DF(days)	HP(cm)	PL(cm)	ET(no)	T(no)	FFG(no)	100GW(g)	GLW(cm)	YM(days)
DF(days)	1								
HP(cm)	-0.43695**	1							
PL(cm)	-0.5883**	0.39373**	1						
ET(no)	0.12171ns	-0.00503ns	-0.22585*	1					
T(no)	0.02853ns	-0.00643ns	-0.23369*	0.92342**	1				
FFG(no)	-0.87446**	0.37901**	0.55552**	-0.09132ns	0.0005ns	1			
100GW(g)	-0.60745**	0.30235**	0.35449**	-0.1395ns	-0.0953ns	0.68357**	1		
GLW(cm)	-0.00568ns	-0.05578ns	-0.02718ns	0.10715ns	0.11803ns	0.15551ns	0.11917ns	1	
YM(days)	0.87083**	-0.41243**	-0.56762**	0.13997ns	0.01887ns	-0.95227**	-0.64997**	-0.14577ns	1

∗Significant at p ≤ 0.05, ∗∗highly significant at p ≤ 0.01, ns: non-significant p ≤ 0.05, DF. (degree of freedom), REP.(Replications), Cor..Total (Corrected total), DF(Days to 50% flowering), HP(cm)(Height of plant measured in centimeters), PL(Panicle length in centimeters), ET(Effective tillers),Tillers (total number of tillers), FFG(Fully filled grains), 100-GW(100 grain weight in grams), YM (yield maturity counted on number of days from sowing to physiological maturity).

Interactions of reproductive drought stress (RS) and non drought stress (NS).

There were interactions between treatment and varieties (TRT*VAR.) in Table 9. Panicle length (PL), Tillers (T), Fully filled grain (FFG) and Grain length and width ratio (GLW) interacted at P≤0.05. while the other parameters were not significant. The significant interactions was because of the variation in treatments (reproductive drought stress (RS) and non drought stress (NS)) while the varieties comprised of data from the improved lines (progenies) subjected to stress and non stress conditions. Shortage of water was responsible because of its dire need for living cells functioning and turgidity (Sukhla, 2012). The parameters affected are yield borne especially fully filled grains (FFG). Juraimi et al. (2009), Sikuku et al. (2009), Masitah, (2018) reported that days to flowering, yield maturity, fully filled grain, panicle length are affected by drought stress. Variation of significance on major yield component was also attributed to biochemical, physiological, morphological and anatomical effect of water deficit condition (Serraj et al., 2009).

Reproductive stage of rice is a very sensitive stage and responded to drought stress in affecting flowering and heading as also confirmed by Davatgar et al. (2009). drought stress often results in low tissue water potential for rice (Sikuku et al., 2010). Generally, low yield underscore the importance of water (Juraimi et al., 2009).

Correlation coefficient of interaction

The interaction correlation coefficient Table 10. showed highly significant interaction between days to 50% flowering (DF) with height of plant (HP), panicle length (PL), fully filled grain (FFG), 100 grain weight (100GW) and yield maturity (YM). All correlation coefficient relationships were either negatively medium or high, with the exception of yield maturity which was both strong and positively correlated relationship. This clearly indicated the influence of days to 50% flowering to yield maturity due basically to water stress treatment which delayed maturity because of distortion of the normal physiological and biochemical processes of the rice plant (Serraj, 2009; Juraimi, et al., 2009). For height of plant (HP), there were highly significant (P≤0.01), negatively medium correlation relationship with panicle length (PL), effective tillers (ET), 100grain weight (100GW) and yield maturity (YM). the relationship between effective tillers (ET) and tillers (T) was significant P≤0.01 but with strong negative correlation. Fully filled grains (FFG) was also highly significant (P≤0.01) with 100 grain weight (100GW) and yield maturity (YM) at medium and strongly negative correlated relationship respectively. The parameter of 100 grain weight (100GW) was highly significant (P≤0.01), medium and negatively correlated.

Clustering and principal component analysis

The analysis of genetic variability is one important criterion for parental selection by the estimate of the extend of variation that existed among the genotypes. The specific information on the nature and degree of genetic variability is critical for selection of ideal parent so as to minimize the number of crosses that would have been required. (Guerra et al., 1999, Yatung et al., 2014).

Non drought stress

The study considered 14 genotypes which comprised of the 3 parents and their progenies (improved lines). They were clustered into four main groups based on their quantitative characteristics at genetic similarity of 0.122 in order of distance to the various genotypes in the population. Clustering of 39 genotypes according to morphological characteristics majorly on the size of fruit was reported by Geleta et al., 2005. The genetic distance dendrogram was constructed in accordance to the UPGMA method using NTSYS-pc version 2.1. The Figure 7. showed the clustered genotypes into four groups.

Groups III and IV comprised of two genotypes each namely DPB7, DPB20 and PB15, PBD3 respectively. Group I and II comprised of 6 and 4 genotypes respectively. While Putra 1, IRBB60, PBD1, Drought (MR219 drought tolerance), PB12,PD14 were clustered in group I, PD15, PBD3, DPB13 and DPB12 were clustered in group II. There parental plants and were clustered along with the first two crossed generations that produced the three-way and double crosses.

The PCA results are indicated in Figure 8. the farthest genotypes from the centroid were PBD3. PB15, PD14, PB12, DPB7, DPB12, while the closest to the centroid were DPB13, PD15, PDB3. three genotypes were intermediary between the farthest and closest, which included; PUTRA1, IRBB60 and PBD1.

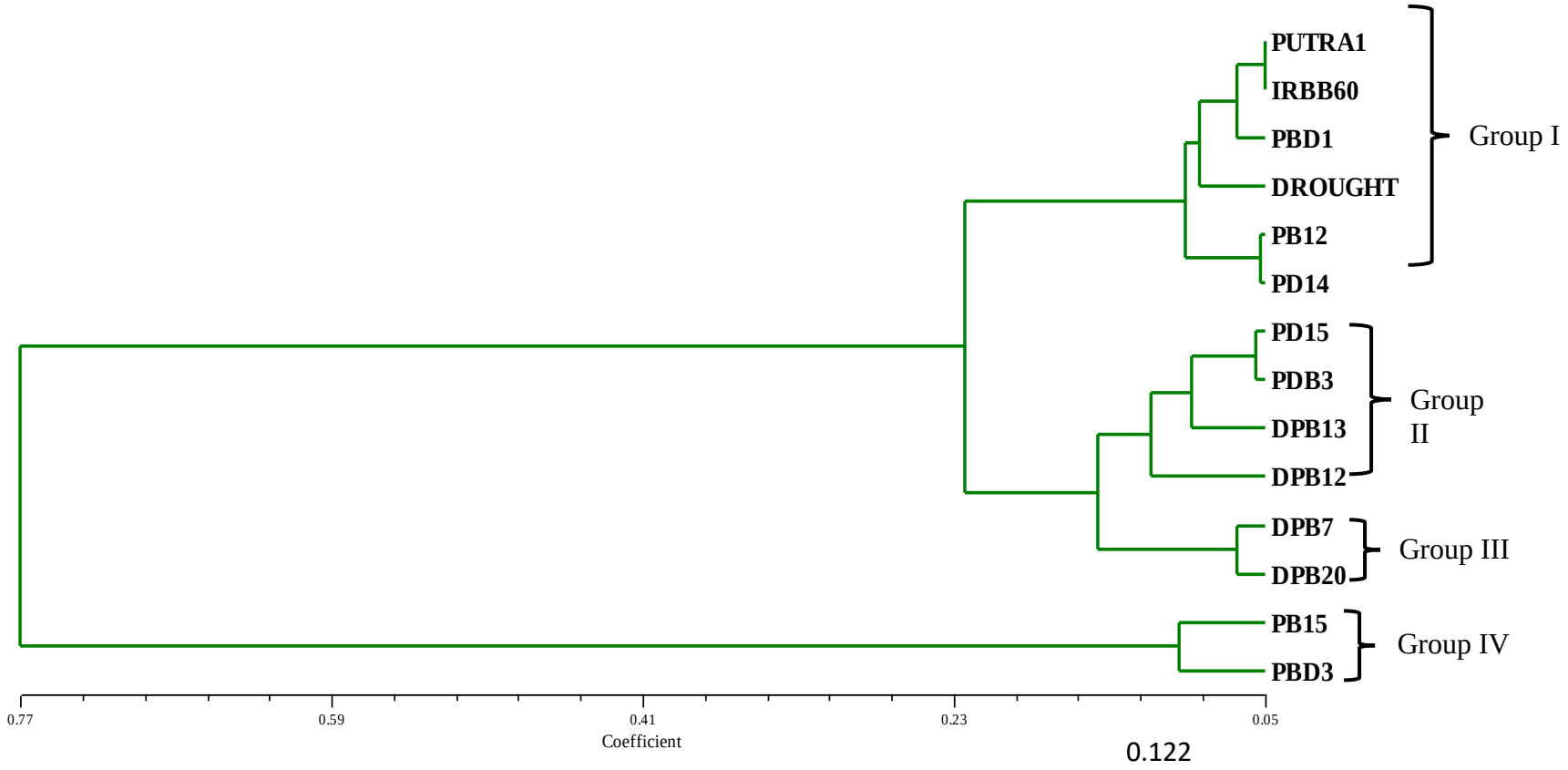

Figure 7. Clustering pattern of the agro-morphological and yield based on 14 trait at dissimilarity coefficient of 0.122

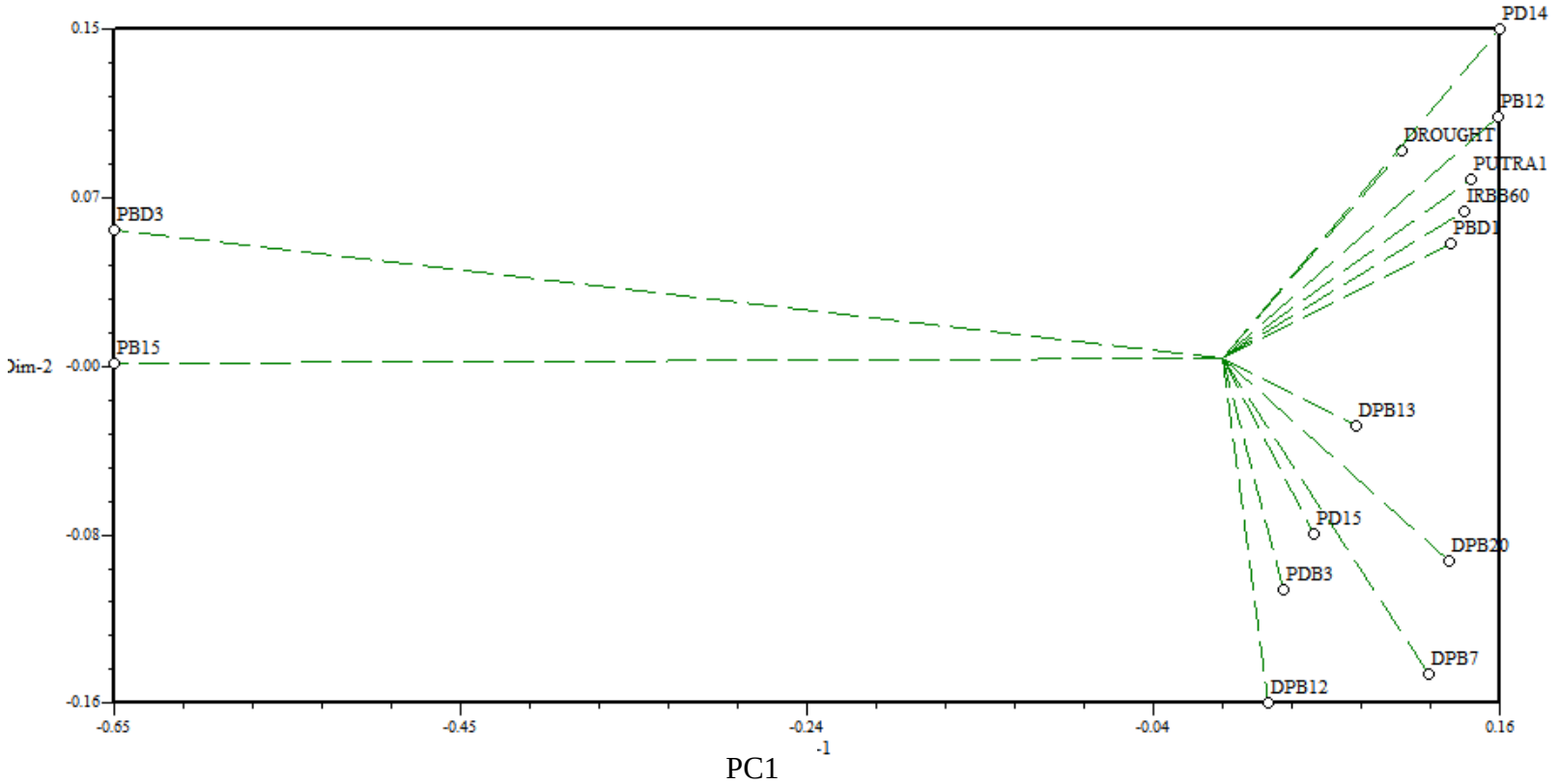

Figure 8. Principal component analysis (PCA) of relationship among fourteen (14) rice lines in a 2-dimentional graph

Reproductive drought stress

In this study, 10 genotypes (lines) were clustered together into six groups based on nine quantitative traits at the reproductive stage drought stress (RS) to separate different genotypes in the population. UPGMA method using NTSYS-pv v2.1 was similarly used to construct the genetic distance dendrogram. The genotypes here were clustered into 6-group at 0.094 genetic similarity as shown in Figure 9.

Group I, III, IV and VI comprised of only one line (genotype) each such as CONTROL, DPB7, PDB3 and PBD3 respectively. While group II and V each comprised of three genotypes; PD14, PD15, DPB12 and PBD1, DPB13, DPB20 genotypes respectively.

Figure 10. showed the result of PCA. The genotypes distanced away from the centroid in the order of distance comprised of CONTROL, PBD3, DPB7, and three genotypes were intermediary such as PBD1, DPB20, PD14, while the others were closest to the centroid, and they included PD15, DPB13, DPB12 and PDB3. Group I stood out as the lowest with yield since it was the control and was susceptible to RS.

Table 11. Grouping of 14 and 10 improved and selected lines for non drought stress (NS) and reproductive drought stress (RS) respectively for cluster analysis

Treatment	Cluster number	Number of genotypes	Genotypes
NS	I	6	PUTRA1, IRBB60, PBD1, DROUGHT, PB12, PD14
	II	4	PD15, PDB3, DPB13, DPB12
	III	2	DPB7, DPB20
	IV	2	PB15, PBD3
RS	I	1	CONTROL
	II	3	PD14, PD15, DPB12
	III	1	DPB7
	IV	1	PDB3
	V	3	PBD1, DPB13, DPB20
	VI	1	PBD3

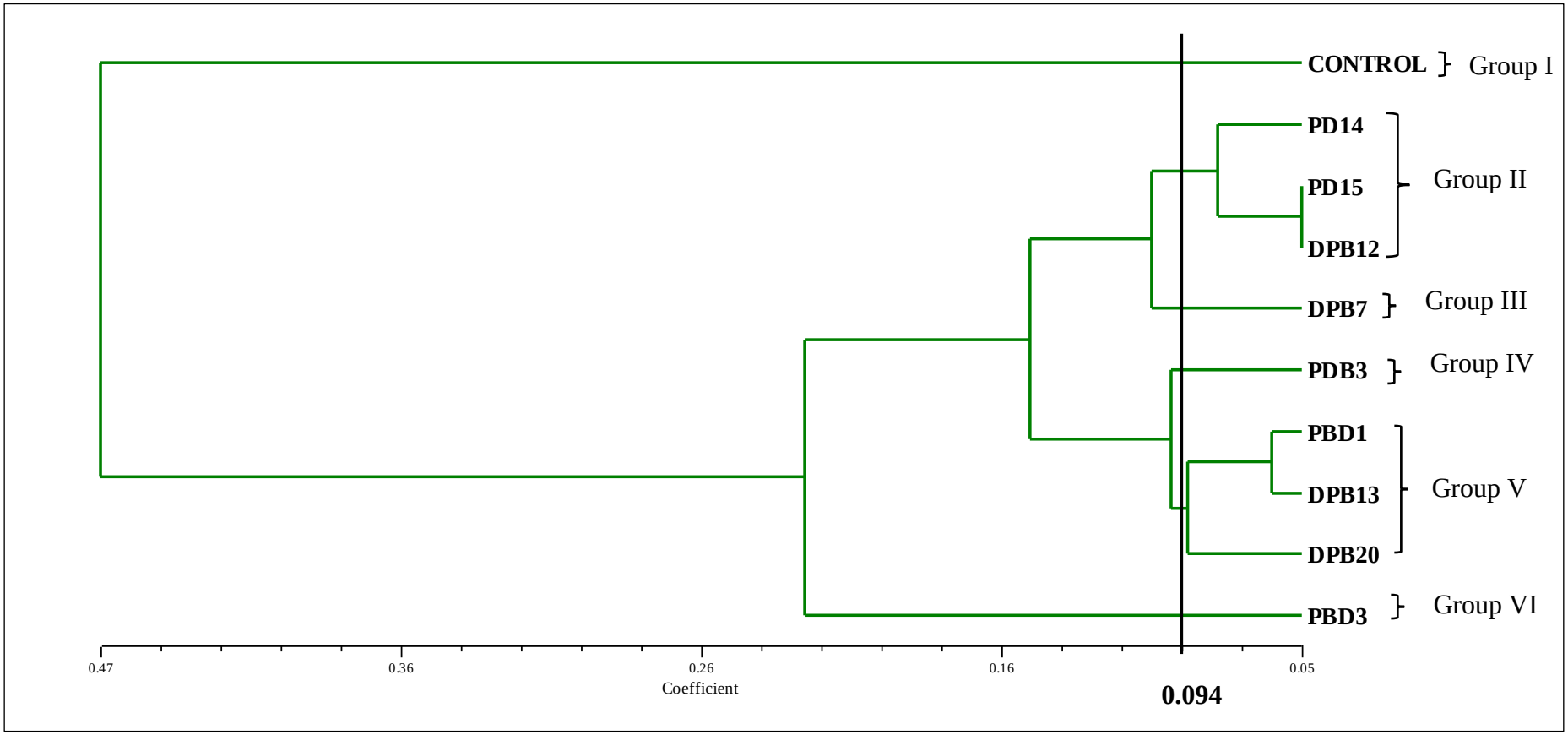

Figure 9. Relationship among the 10 developed and selected lines (genotypes) based on 9 characteristics (traits) using SAHN clustering of UPGMA method

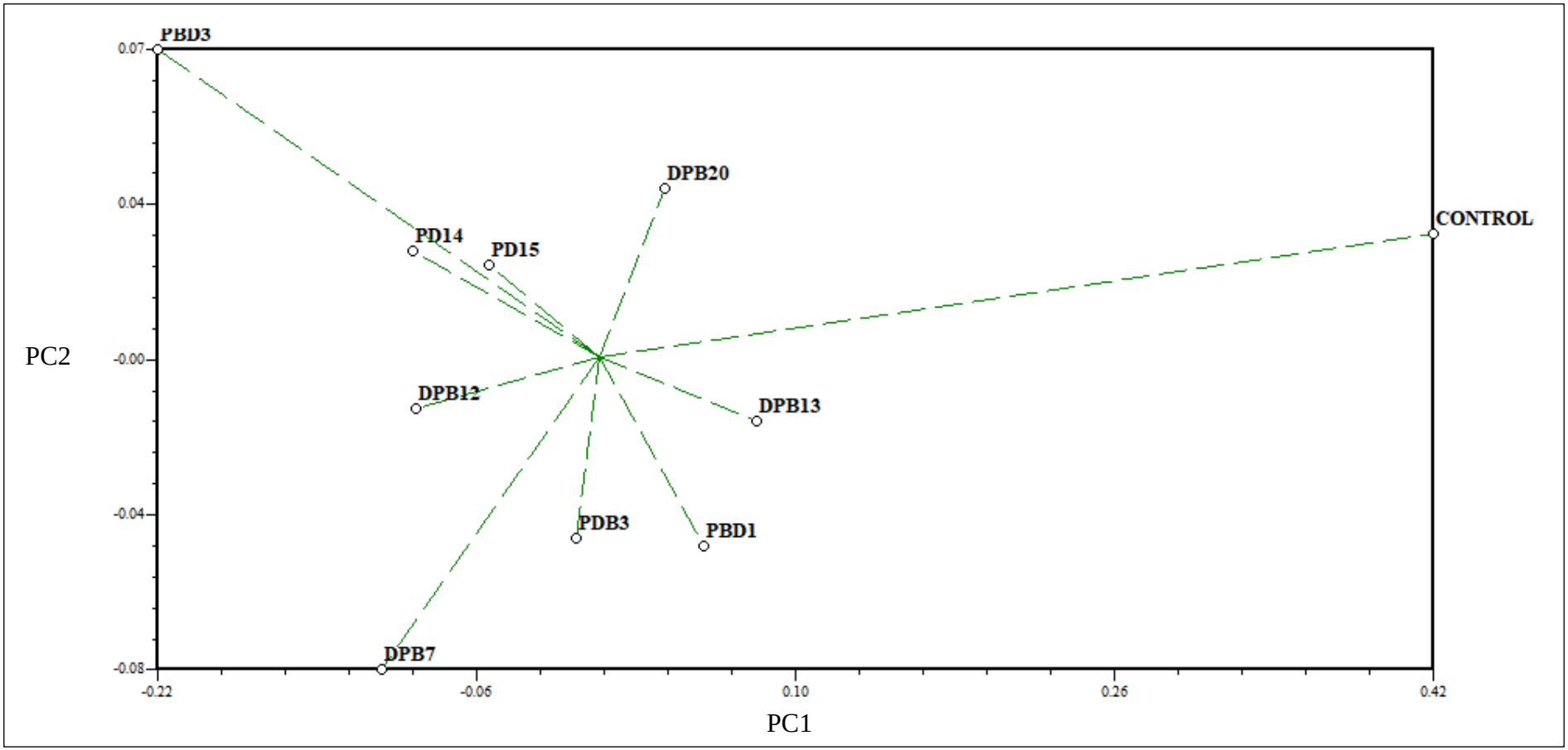

Figure 10. Principal component analysis of relationship amongst 10 improved lines and selected lines in two dimensional graph

Conclusion

The two recipient parents (Putra1 and MR219 drought tolerant) used in the different crossing methods were high yielding, resistant and tolerant to blast (*Magnaporthe grisea*) and drought respectively. And a donor IRBB60 resistant to bacteria leaf blight (*Xanthomonas oryzae*). The yield parameters showed that there was no yield loss on non-drought stress treatment on improved lines except on reproductive drought stress treatment. The interactions showed that reproductive drought stress affected days to flowering, fully filled grain and yield maturity significantly. The profitability of these parameters are often dependent on the reproductive stage, but shortage of water tempered with their due performances. Even though there was reduction in yield, but it was still within average yield performance of drought tolerant variety due to the presence of introgressed genes/QTLs compared with the susceptible variety in reproductive drought stress (RS) treatment.

Prior knowledge of the rice genotype is important in making informed decision on selection of the best cultivars for variety improvement. Genetic variation is an indication of the possibility of diverse source of origin and the result gave a clue as to the genetic/QTL variance of the rice populations.

In consideration of the variation pattern and other agro-morphological and yield performance, the improved and selected lines PB12, PBD1, and PD14 in group I, PDB3 in group II, DPB20 in group III and PB15, PBD3 in group IV could be considered as better lines on good yield under non drought stress (NS) treatment. In reproductive drought stress (RS) treatment, PD14, DPB12 in group II, DPB7 in group III and PDB3in group IV. While in groups V and VI were PBD1, DPB20 and PBD3 respectively. Generally, the yield performances of the different lines for both NS and RS were all within considerable high yielding range, with high potentials for increased yield with different environmental trials since the QTLs could be influenced by different environments, even though the recommended ones were better.

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