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Molecular and morpho-agronomic traits for vegetative stage drought tolerance in some rainfed elite rice lines

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ABSTRACT

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Selection of drought-tolerant rice genotypes is still one of the most vital challenges in rice research and the use of molecular markers may offer a promising approach to fast track the selection and development of drought-tolerant rice. The aim of our study is to identify drought-tolerant traits, in selected rainfed elite rice lines, expressed during drought stress at the vegetative stages 20-32 DAS and 30-42 DAS. The study also validated the presence of SSR markers linked to drought tolerance. All morpho-agronomic traits examined in the study were significantly affected by drought, except for root length during 30-42 DAS of drought imposition. Drought significantly reduced the plant height, number of tillers, leaf area, and root number resulting in a significant reduction in root and shoot dry weight. On the other hand, an increase in total nodal root length was observed in all test genotypes except for AL-55, AL-97, and susceptible check PSB Rc82 under both drought conditions. All parameters examined in this study are useful traits for drought tolerance in rice, however responses might be genotype-dependent. The highest correlation was shown by root:shoot ratio (r= 0.94, r= 0.78) at 20 DAS and (r= 0.89, r=0.67) at 30 DAS under well-watered and drought conditions, respectively. Seven amplified markers were present in the test genotypes except for RM 525, RM 60, RM 201, RM 1141. These four markers were absent in the drought-susceptible check PSB Rc82, hence, these markers may be used in selecting drought-tolerant genotypes through marker-assisted selection.

Keywords: Drought tolerance, Marker-assisted Selection, SSR markers, Rainfed lowland rice

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INTRODUCTION

Drought stress constitutes the most important constraint for rice productivity in rainfed areas. The Philippines has about 1.3Mha of rainfed rice areas (Haefele and Bouman 2009). Rice usually grows in bunded fields in rainfed lowlands which are seldom flooded where the bunds serve as physical structures for water control. These can be considered as unfavorable rainfed areas where drought may occur at any time during the crop growth and can vary in space and duration. Drought tolerance in crops is a complex trait and characterized by many component responses that may interact and vary. For instance, intensity and duration of water deficit results in intricate changes in physiological, biochemical, and molecular levels (Bray 2002, Hellal et al 2017). Water deficit impairs germination of rice seedlings (Jiang and Lafitte 2007), reduces the number of tillers (Mostajeran and Rahimi-Eichi 2009, Ashfag et al 2012, Bunnag and Pongthai 2013), reduces plant height and various leaf traits such as leaf area, leaf area index and number of leaves per plant (Sokoto and Muhammad 2014, Ahmadikhah and Marufinia 2016). Usman et al (2013) reported reduced fresh shoot and root weight as well as their length, which ultimately reduce the photosynthetic rate and physiology and biochemical processes of rice. Furthermore, Sarvestani et al (2008) revealed that water stress imposed during the vegetative stage of rice results in the reduction of total biomass which is due to a decrease in the photosynthetic rate and dry matter accumulation.

Improvement of drought tolerance in rice is a major goal especially in the rainfed ecosystem where water sources could be very limited. Thus, to combat economic losses to agriculture caused by drought, continuous development for improving yield under stress conditions, through understanding its genetics and plant mechanism, will remain a priority for future research. Furthermore, the complex responses of rice plants to drought coupled with labour-intensive conventional phenotyping have made selection and breeding of drought-tolerant varieties extremely difficult (Tirado and Cotter 2010). With the advent of molecular breeding, the generation of improved rice varieties could be fast-tracked and become more accurate. Marker-assisted selection is very useful especially when dealing with large populations of inbreeding lines.

Simple sequence repeats (SSRs) or microsatellites is the marker of choice in many plant breeding programs because they are transferable, multi-allelic and co-dominant, PCR-based, easily reproducible, randomly and widely distributed along the genome and analysis may be automated (Rafalski et al 1996). Molecular markers associated with drought-related traits have been reported. Babu et al (2003) found some association of root traits measured in rice lines with the application of SSRs markers such as root thickness, root penetration index and root dry weight to be correlated with yield and yield components of rice, while Vasant (2012) found 12 SSR markers that were strongly associated with root traits. Ashfag et al (2014) reported markers, RM 315, or RM 212, RM 302, to be useful SSR markers linked to root traits and are useful in the evaluation of diverse germplasm under drought stress during the vegetative stage of rice. These results indicate that molecular markers may be utilized in screening rice for improved drought tolerance. Five tolerant lines (AL-108, AL-87, AL-97, AL-55 and AL-5) were identified based on research on early seedling vigour under polyethylene glycol-induced (PEG-induced) drought (Gaurana 2019). These elite inbreeding lines were expressing drought tolerance in terms of photosynthetic rate, relative water content, total antioxidant activity and total soluble sugar under 20-32 and 30-42 days after seeding (DAS) of stress imposition (Gaurana 2019). However, further validation is needed to confirm

the tolerance of these lines. Hence, the goal of this study was to validate the presence of selected SSR markers reportedly linked to drought tolerance in rice and to identify agronomic traits that may contribute to improved drought tolerance during the vegetative stage using elite inbreeding lines being developed for the rainfed lowland rice ecosystem.

MATERIALS AND METHODS

Planting Materials

Five tolerant rainfed rice genotypes: AL-108, AL-87, AL-97, AL-55, AL-5 selected from the PEG-induced drought research and two check varieties: NSIC Rc14 (tolerant) and PSB Rc82 (susceptible) were used. The five test genotypes were advanced inbreeding lines from the rainfed breeding program of the University of the Philippines Los Baños. The PSB Rc82 is an irrigated lowland variety and used as a susceptible check under rainfed lowland conditions in field trials conducted at the University of the Philippines, Los Baños.

Site and Experimental Design

This experiment was conducted under greenhouse conditions at the International Rice Research Institute (IRRI), Los Baños, ($14^{o}11'$N, $120^{o}15'$E, 21 meters above sea level [masl]) from January to April 2019. The soil used was obtained from an IRRI upland site, classified as clay loam (30% sand, 38% silt and 32% clay), with pH6.2, 0.26% N, 153ppm P, 5.38cmol kg^{-1} K.

Two-liter PVC pots filled with 1.2kg soil were used as experimental units. Four pre-germinated rice seeds were sown per pot at 2cm sowing depth. The experiment was laid-out in a split-plot, complete randomized design (CRD) with five replications, wherein each pot corresponded to one replicate. Water treatments were designated as main plots, with the seven genotypes as subplots. Drought stress was imposed during 20-32 and 30-42 DAS. The well-watered treatment served as the control. The mean distance between plants was 20cm. Re-randomization (pot rearrangement) was done every three days to maintain homogeneity of light absorption. The pots were filled with soil and watered at field capacity a day before sowing. This condition was maintained until drought stress was induced at 20 and 30 DAS. The control (T_0) was the well-watered condition (field capacity) that was maintained until termination of the experiment. Drought treatment was set to 75% of field capacity, while water application was withheld to impose drought at 20-32 DAS (T_1) and 30-42 DAS (T_2).

Soil moisture content (SMC) was monitored by weighing the pot early in the morning at 2-day intervals. SMC was calculated using the following formula:

$$\text{SMC} = \text{Saturated weight of each pot (weight of pot} + \text{weight of soil at field capacity)} - \text{Current pot weight}$$

In order to achieve the target SMC for all treatments, control pots were watered with the same amount of water that had been removed through transpiration and root absorption, and drought-treated pots were re-watered when field capacity reached the lower threshold of 10%. The critical soil moisture for most cereals and legumes is 8% (Suralta and Yamauchi 2008).

Cultural Management

Top soil (20cm depth) collected from the upland site of IRRI, Los Banos, was used. Weeds were removed and soil was sieved and dried. The air dried soil was placed in 2-L PVC pots with approximately 1.2kg soil in each pot. Prior to sowing, seeds from all genotypes were pre-germinated for three days in petri dishes with wet filter paper. When the roots attained 2cm in length, four germinated seeds were sown in each moist pot at 2cm depth. Thinning was done at 10 DAS. The most vigorous seedling/plant was maintained up to the end of the experiment. Complete fertilizer (14-14-14) was applied at the rate of 0.3kg per pot at sowing. Urea (46-0-0) was applied at 10 DAS at the rate of 0.3g per pot. Weeding was done by manual removal/cleaning every week. Pesticides were applied at the recommended rate whenever necessary. For proper seedling growth, pots were watered regularly until imposition of the drought treatment.

Measurements

One plant per replication was used for the measurements. The plant height and number of tillers per plant were measured at the start and end of the experiment. Plant height was measured from the stem base to the highest leaf tip. At the end of the drought treatment (20-32 and 30-42 DAS), the control and drought-stressed plants were sampled. Roots were washed with water to remove the adherent soil. Root length was measured using a ruler (cm) and the root number was counted manually. Plants were separated into shoots and roots. The fresh weight of roots and shoots were recorded. Each bulk of plant organs were oven dried at 70^{0}C for 3-4 days until constant weight to determine the root and shoot dry weight. Root to shoot ratio was obtained by dividing the root weight by the shoot weight. Leaf area was determined at 32 and 42 DAS by randomly selecting five treatment pots. The number of tillers for each sample hill in each pot was counted first, then the length and width (broadest part) of each leaf of the middle tiller in each sample hill was measured. Leaf area was computed using the formula from Yoshida (1981):

$$\text{Leaf area} = \text{Length} \times \text{Width} \times 0.75$$

Genomic DNA Extraction

High-throughput extraction of DNA was done via the modified cetyltrimethylammoniumbromide (CTAB) method (Murray and Thompson 1980). Leaf samples from stress and well-watered treatments in both 20-32 and 30-42 DAS periods were collected after drought imposition. About 0.1g of leaf samples were ground to a fine paste in approximately 1mL of pre-warmed CTAB buffer using a sterile mortar and pestle, with a small amount of PVP added while grinding. Each CTAB/plant extract mixture was transferred to a microfuge tube and incubated at 65^{0}C for 30min in a water bath. After cooling, an equal volume of chloroform: isoamyl alcohol (24:1) was added and mixed with the solution. Samples were centrifuged at 15,000rpm for 15min. at 4^{0}C. The aqueous layer was collected and 300-400µL of chloroform:isoamyl alcohol added and mixed by inverting for 15min. The crude DNA was centrifuged at 10,000rpm for 2min at 4^{0}C and the supernatant was collected. The volume was doubled by adding absolute alcohol (100%), and centrifuged at 10,000rpm for 5min at 4^{0}C. The supernatant was removed, the pellet

was washed with 70% ethanol and centrifuged at 13,000rpm for 10min at 4^0C. The pellet was air dried, after all the alcohol had evaporated 150µL of TE buffer was added and the microfuge tubes stored at 20^0C. The quality of the isolated DNA was confirmed by agarose gel electrophoresis.

PCR Primers

The polymerase chain reaction (PCR) primers used were obtained from the gramene rice genome database. Twelve primers were used in this study as listed in Table 3.

Polymerase Chain Reaction Amplification

Template DNA from plant samples were removed from storage and were thawed. The PCR reaction cocktail for each primer was prepared.

The PCR reaction protocol was carried out in a 96-well PCR plate. The 10uL reaction mixture contained 50ng of DNA template, 1uL 10x PCR buffer (containing 200mM Tris-HCL pH8.3, 500mM KCL, 15mM $MgCl_2$), 1uL of 1mM dNTP, 0.25uM each of the forward and reverse primers and 1uL of laboratory prepared Taq DNA polymerase.

The PCR amplification was performed on a Biorad Cycler (96-well). Under the following conditions: the initial denaturation temperature of 95^0C for 3min was followed by 35 cycles of 94^0C for 2min denaturation, annealing temperature depending upon the primer used (56–58^0C), 30s at 72^0C for primer elongation and ending with 7min at 72^0C for the final extension followed by incubation at 4^0C until polyacrylamide gel electrophoresis.

Polyacrylamide Gel Electrophoresis (PAGE)

Polyacrylamide gel electrophoresis (PAGE) is a high resolution technique used in studies where the amplicon size produced from PCR ranges from 50 to 200+ base pairs. Amplicons with this size range cannot be resolved using agarose gel.

The 8% polyacrylamide gel was prepared. The gel was poured into a cast plate starting from one corner until it reached the top portion of the short plate. A comb was then inserted. The gel was allowed to polymerize for 30-45min. After polymerization, the gasket was removed starting from one corner, towards the other corner of the plate. The running Tris/Borate/EDTA(TBE) buffer (1X) was added into the PAGE tank. The cast plates were placed in the tank and secured with the use of clips. Buffer was added on the top of the cast plates, and the combs were removed. The PCR products stained with 2uL of Bromothymol blue were loaded into the wells along with the 100bp DNA ladder. The gel was run for 90-120min at 100 volts depending on the size of the PCR products.

After electrophoresis, the gels were stained with SYBR Safe™ (Invitrogen) DNA gel stain solution (5uL stain per 50uL nanopure water) for 30min and were viewed for band formation.

Scoring of Bands

The amplified bands were scored for each SSR marker, generating a binary data matrix of 1 for presence and 0 for absence for each primer in each of the genotypes.

Statistical Analysis

Different parameters that were used in this experiment were analyzed following the analysis of variance (ANOVA). The Tukey's Honest Significant Difference (Tukey's HSD) test was used for treatment comparison.

RESULTS AND DISCUSSION

Agro-Morphological Response Under Drought

In rice, as well as in other crops, agronomic characteristics, such as yield, root length, root number, leaf damage and plant height are the most commonly-used criteria for measuring drought tolerance. In this study all the parameters were significantly affected by drought stress (Tables 1a and 2b). Significant differences between genotypes were observed on the number of tillers, root number and root to shoot ratio in both 20-32 and 30-42 DAS of drought imposition while differences in leaf area and root dry weight were observed at 30-42 DAS drought period. Total nodal root length was also significantly affected by drought stress during 20-32 DAS (Table 1b).

Reduction of plant growth is the most typical symptom of drought stress (Sairam and Srivastava 2001). The reduction in plant height, number of tillers, root number and leaf area led to a decrease in the total plant dry weight (root + shoot dry weight) (Figure 1 and 2). Reduction in plant height during two drought treatments had varied responses with the test genotypes. Comparing the check genotypes, susceptible Rc82 showed the highest reduction in plant height compared to the tolerant check Rc14 while, among the test genotypes, the lowest reduction was observed in AL-1 during 20-32 DAS of drought imposition and in AL-87 during the 30-42 DAS of drought imposition. Khan et al (2004) observed a decrease in plant height and leaf area in maize when exposed to increasing water stress. The observed reduction in plant height could be attributed to a decline in cell enlargement due to lower water potential and reduced turgor pressure in the cell. On the other hand, the reduction in leaf area of plants under drought might be due to the suppression of leaf expansion that is also caused by declined cell enlargement resulting in the reduction of photosynthesis (Rucker et al 1995). Leaf area expansion depends on leaf turgor, temperature, and assimilating supply of nutrients for growth. Plasticity in leaf area could be a means by which plants control water use under stress conditions (Chowdhury et al 2016).

On the other hand, total nodal root length increased under drought conditions except in susceptible check Rc82, AL-55 and AL-97 during both drought treatments (20 and 30 DAS). Furthermore, AL-108 increased under 20-30 DAS drought but reduced when exposed to 30-42 DAS drought period (Figure 2c-d). Increase in root length of nodal and lateral roots under drought was reported by Suralta et al (2008). A long root system that can extract water from a deep soil layer is a good indication of drought tolerance. While, Ingram et al (1994), Yu et al (1995), and Allah et al (2010) claimed that possession of a deep and thick root system is more important in upland conditions than rainfed lowlands due to the presence of hardpan (in lowland) which can severely restrict root growth.

In terms of root number, the lowest reduction was exhibited by AL-108 and tolerant check Rc14, these genotypes also showed the lowest reduction in the number of tillers. The results suggest that reduction in root number could be due to

reduction in tiller number in these two genotypes. On the other hand, the reduction in root number during drought might also be due to differences in supply of assimilates for root growth. Allah et al (2010) further stated that competition for assimilate supply would occur between growing tillers and the roots of the primary tiller during the active tillering stage. Drought tolerant rice genotypes may have fewer numbers of roots but higher proportions are distributed in lower soil layers (>20cm below ground).

The root:shoot ratio and shoot:root ratio are often used to estimate relative biomass allocation between roots and shoots (Poorter et al 2012). In conditions where water is limited, the root:shoot ratio of plants increases because the roots are less sensitive than shoots to growth inhibition by low water potentials (Wu and Cosgroove 2000). Furthermore, plants often re-allocate assimilates from shoot growth to root growth under drought stress conditions, thereby increasing root extension into deeper soil layers, hence this is a desirable trait (Rich and Watt 2013). Root induced signal cascades to the shoot causing stomatal closure as a result water loss through transpiration is reduced (Sharp and LeNoble 2002).

Significant increases in root:shoot ratio based on root length and shoot length were observed under the drought period. All genotypes had significantly increased root:shoot ratio except for AL-9, AL-5 and susceptible check (PSB Rc82) during 20-32 DAS of drought. However, under 30-42 DAS of drought imposition AL-5 and AL-97 had increased root:shoot ratio along with susceptible check PSB Rc82. Findings of this study suggest that test genotypes are showing tolerance in terms of their root:shoot ratio although response varied under different periods of stress. Furthermore, PSB Rc82, although considered susceptible for some parameters relative to test lines in this study, had shown high root:shoot ratio under the 30-42 DAS drought period. Therefore, this genotype might have some degree of tolerance under the 30-42 DAS stress period.

Table 1a. Table for test of significance and mean comparison of genotypes during 20 and 30 DAS of drought imposition

	20 DAS drought				30 DAS drought			
	Plant height	Number of tillers	Leaf area	Shoot dry weight	Plant height	Number of tillers	Leaf area	Shoot dry weight
Treatment	598.0**	164.6**	349.5**	36.1**	241.5**	37.8*	140.4**	68.6**
Genotypes	29.8^{ns}	25.8*	23.0^{ns}	0.5^{ns}	32.2^{ns}	39.7**	57.2**	0.8^{ns}
AL-108	54.1ab	14.9^{ab}	18.0	2.4	58.4	15.9^{ab}	23.7a	4.8
AL-5	50.9ab	14.1^{ab}	16.0	2.3	58.8	15.5ab	20.9ab	4.2
AL-55	53.6ab	14.9^{ab}	13.7	2.3	58.4	16.0ab	21.7ab	4.0
AL-87	53.1ab	16.9 ^{a}	16.5	2.5	59.8	17.4a	23.1a	4.5
AL-97	49.9b	14.9^{ab}	14.2	2.3	56.3	18.1a	20.7ab	4.1
NSIC Rc14	52.8ab	14.1^{ab}	15.3	1.9	54.8	19.1a	18.2ab	4.7
PSB Rc82	55.7a	10.9 ^{b}	13.1	1.8	60.7	12.3b	16.1b	4.1
CV	7.09	17.7	22.4	23.39	7.35	16.0	14.4	38.6

Values within drought stress treatment with the different letters are significantly different based on comparison using HSD at $p \geq 0.05$ (n=7). Legend:NSIC Rc14=tolerant check, PSB Rc82=susceptible check

Table 1b. Table for test of significance and mean comparison of genotypes during 20 and 30 DAS of drought imposition

	20 DAS drought				30 DAS drought			
	Root number	Total Nodal Root length	Root dry weight	Root shoot ratio	Root number	Total Nodal Root length	Root dry weight	Root shoot ratio
Treatment	108152.1**	3.6 *	13.8**	0.07**	118956.5**	5.34^{ns}	8.7**	0.04*
Genotypes	5052.6**	26.1^{ns}	0.2^{ns}	0.02*	8548.1**	26.03^{ns}	1.0**	0.02*
AL-108	179.4^{ab}	33.4	1.6	38.7^{a}	197.6^{b}	38.3	1.8^{ab}	38.8^{a}
AL-5	139.9^{bc}	35.4	1.6	35.7^{ab}	204.5^{b}	34.1	2.28^{a}	36.3^{a}
AL-55	132.5^{c}	34.1	1.36	34.2^{ab}	188.1^{b}	36.7	1.7^{ab}	36.7^{a}
AL-87	180.6^{ab}	36.2	1.8	36.2^{ab}	228.0^{b}	34.9	2.1^{ab}	34.9^{ab}
AL-97	156.8^{abc}	35.7	1.2	31.0^{b}	190.6^{b}	36.3	1.4^{b}	34.1^{ab}
NSIC Rc14	202.0^{a}	32.2	1.5	32.7^{ab}	282.1^{a}	33.2	2.2^{a}	33.2^{b}
PSB Rc82	181.0^{ab}	31.5	1.5	32.2^{ab}	212.1^{b}	37.2	1.5^{ab}	37.2^{a}
CV (%)	15.6	10.7	32.5	13.6	16.0	9.33	27.09	14.5

Values within drought stress treatment with the different letters are significantly different based on comparison using HSD at $p \geq 0.05$ (n=7).
Legend:NSIC Rc14=tolerant check, PSB Rc82=susceptible check

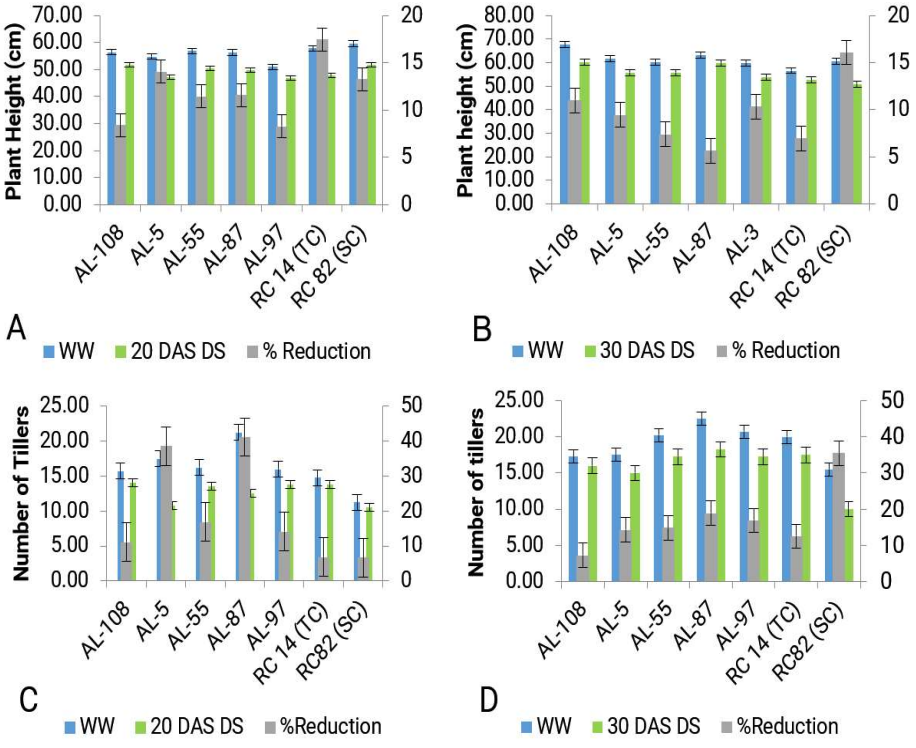
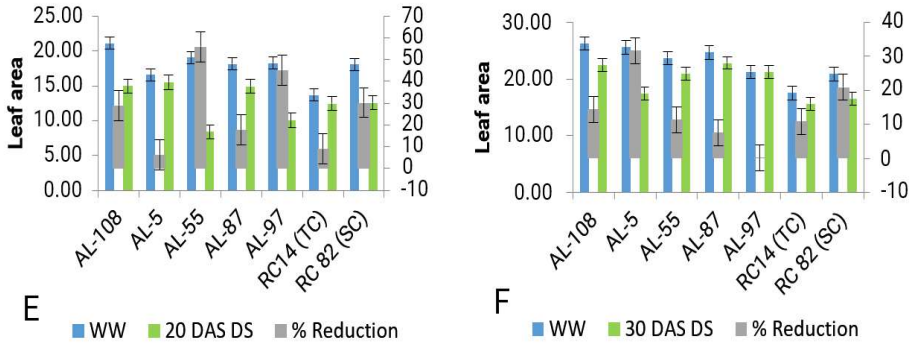


Figure 1. Effect of drought stress treatment on plant height (a-b), number of tillers (c-d) and leaf area (d-e) in rainfed rice genotypes under 20-32 and 30-42 DAS of drought. Vertical bars represent ± standard error. TC (tolerant check), SC (susceptible check), DS (Drought stress), WW (Well-watered).



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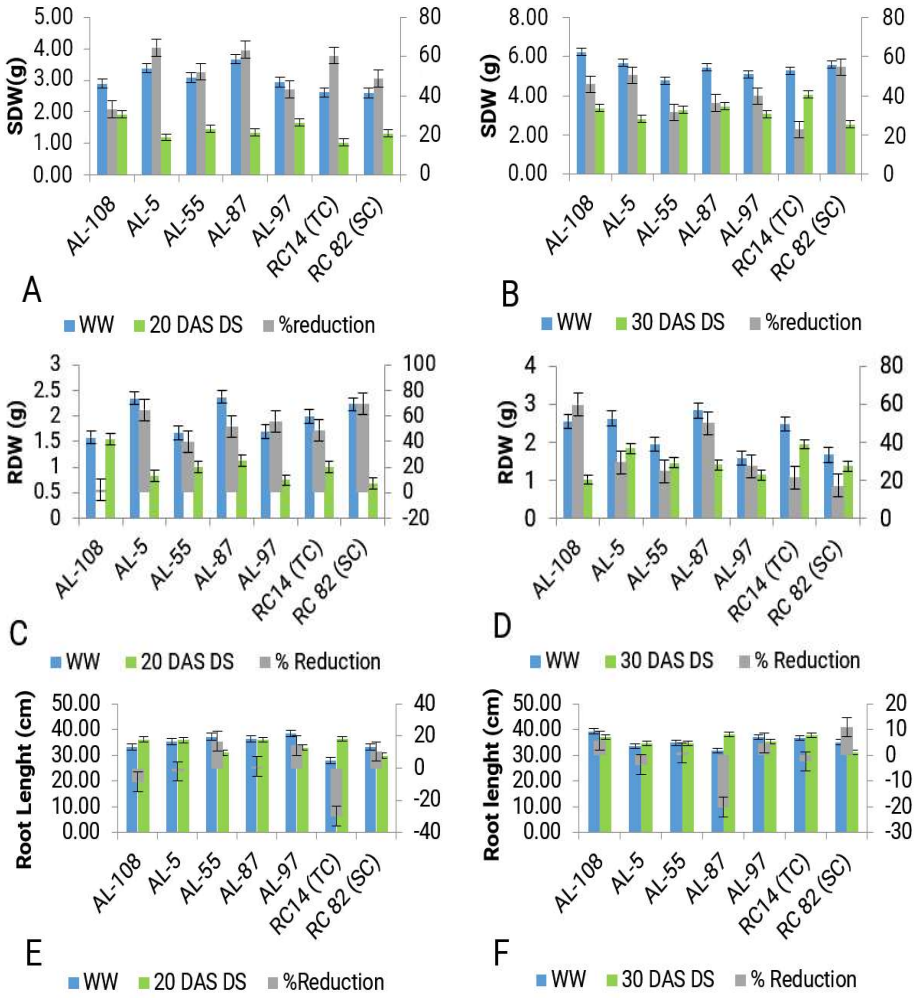


Figure 2. Effect of drought stress treatment on shoot dry weight (a-b), root dry weight (c-d) and root length (e-f) in rice genotypes under 20-32 and 30-42 DAS of drought. Vertical bars represent ± standard error. TC (tolerant check), SC (susceptible check, DS (Drought stress), WW (Well-watered).

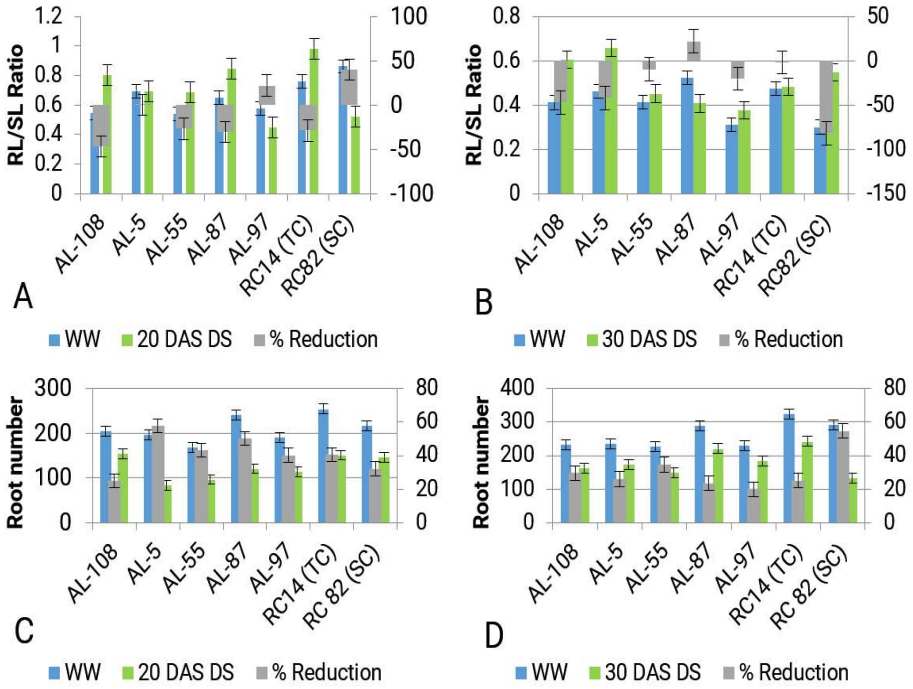


Figure 3. Effect of drought stress treatment on Root:shoot ratio (a-b) root number(c-d) in rice genotypes under 20-32 and 30-42 DAS of drought. Vertical bars represent ± standard error. TC (tolerant check), SC (susceptible check), DS (drought stress), WW (Well-watered).

Correlation Among Agronomic Traits Under Drought Stress and Well-watered Conditions

Simple correlation analysis was estimated for all ten agronomic traits under drought and well-watered conditions (Figure 5 and 6). Significant positive correlations in some agronomic traits under well-watered and drought conditions were obtained. For instance, total root length was positively correlated with root:shoot ratio (r=0.95** and r=0.78**) during the well-watered and 20-32 DAS drought period (Figure 5 A-B), respectively. The root number was positively correlated with shoot fresh weight (r=0.65*) under the 20-32 DAS drought period. The decrease in root number might influence water uptake and can therefore reduce shoot fresh weight under drought (Figure 3 C-D).

High positive correlations (r=0.89** and r=0.67**) were shown between root length and root:shoot ratio under well watered and 30-42 DAS drought period, respectively. The results of this study conformed to the findings of Zhao et al (2019), Ashfag et al (2014), and Zhang et al (2009). The high correlations indicate that development of roots and shoots were interdependent and interactive and synchronization of biomass between roots and shoots could be a key factor in the coping mechanism of rice plants under water stress conditions (Zhao et al 2019). A high root:shoot ratio is considered a desirable trait under the water-stress environment. The positive correlation between root and shoot traits in rice was also reported by Wang et al (2009) and Sandhu et al (2015). The genotypes that performed better under drought, based on these traits, could be used in future

breeding programs. This correlation shows the role of morphological adaptation leading to improved water status in the development of tolerant rice genotypes under drought conditions.

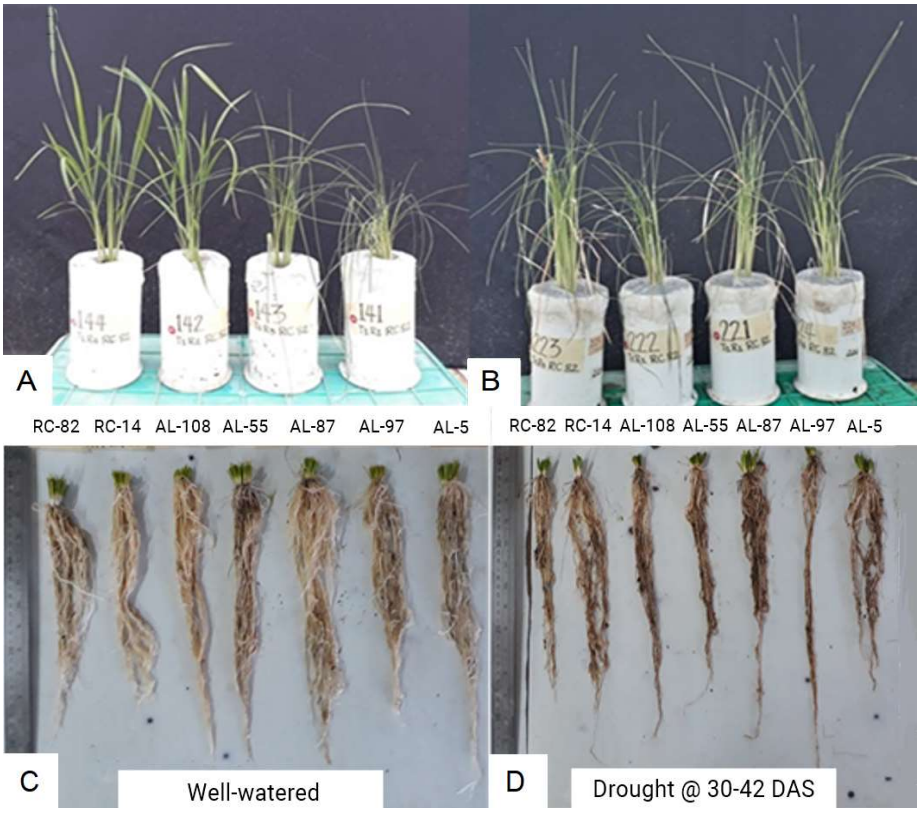
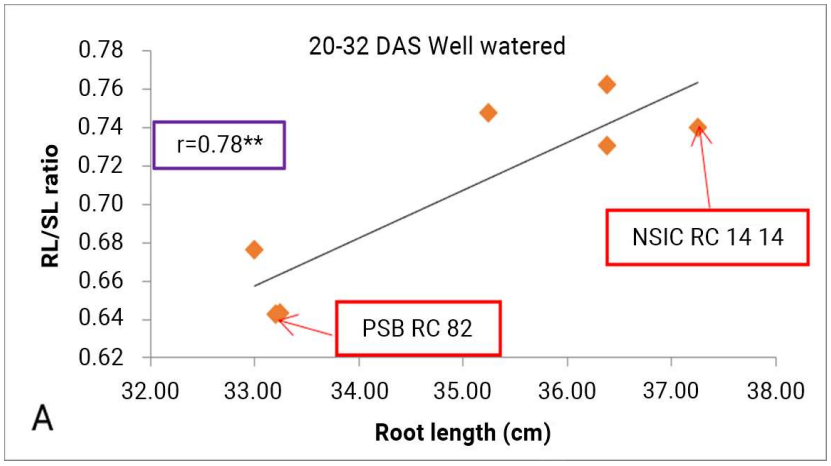
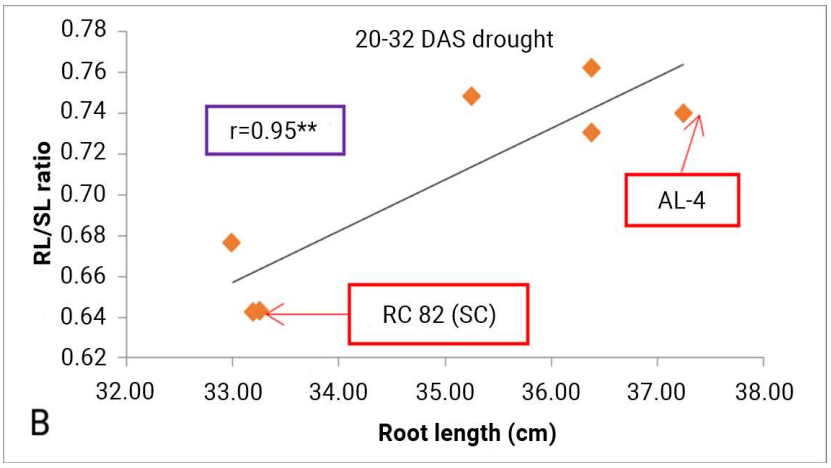


Figure 4. Effect of drought stress treatment on leaf area (A-B), total nodal root length and root number (C-D) in rice genotypes under 20-32 and 30-42 DAS respectively. TC (tolerant check), SC (susceptible check)

Molecular Evaluation of Tolerant Rainfed Lowland Rice Genotype

Molecular tools have been adopted to identify crops possessing specific traits of interest, such as tolerance to environmental stresses like drought. SSR markers have been used in molecular characterization and screening of rice and other crops because they can be transmitted accurately from generation to generation, and are not subjected to environmental influences (Afiukwa et al 2016). With this, molecular markers can fast-track the progress of breeding for drought tolerant rice. Five selected drought-tolerant lines were further validated for the presence of well-known SSR markers linked to drought tolerance in rice. Hence, the goal was to confirm if the test genotypes possess the traits based on reportedly linked markers to drought. The results will also reinforce/validate physiological, biochemical and agronomic data that phenotypically reflect the existence of drought tolerance in some rice genotypes





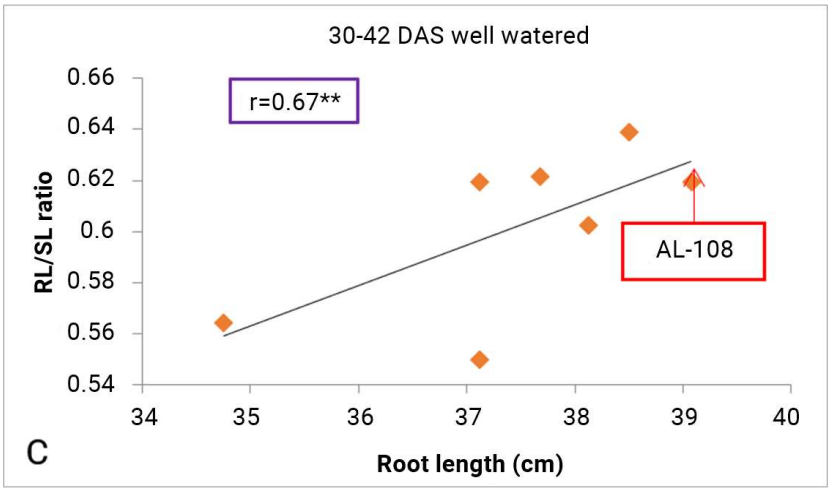


Figure 5. Correlations between root to shoot ratio and root length during 20-32 (A-B) and 30-42 (C-D) DAS of well-watered and drought imposition

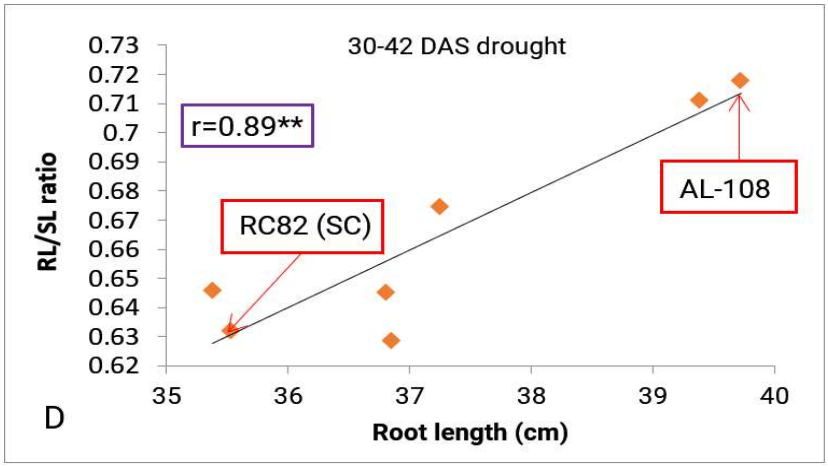


Figure 5. continued

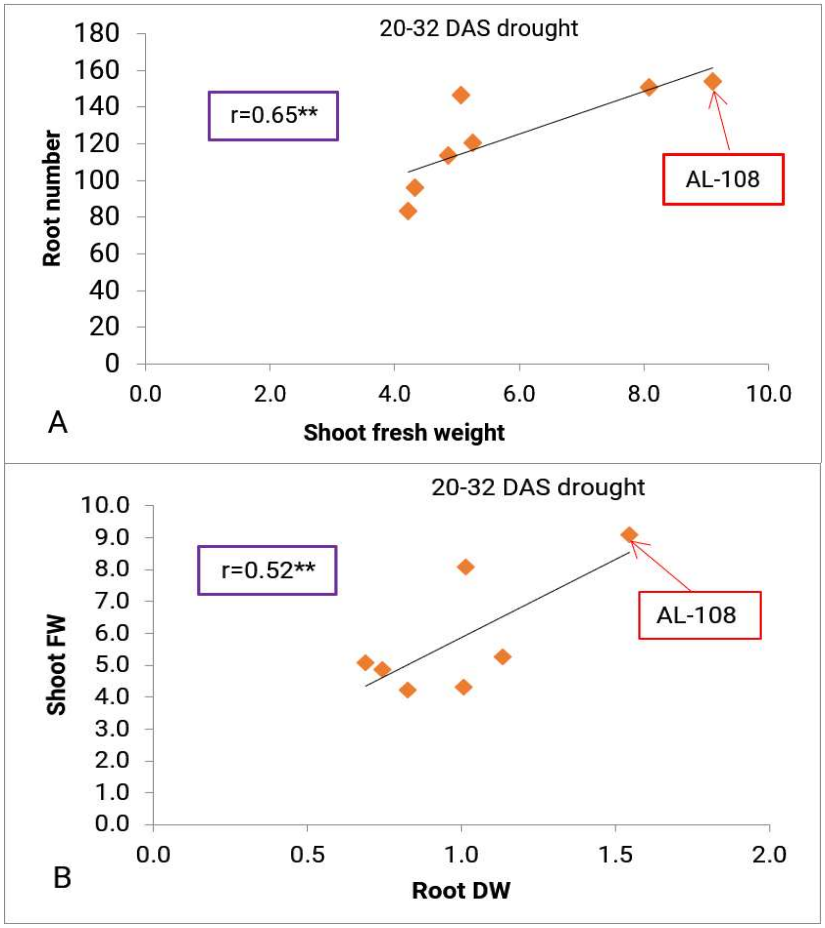


Figure 6. Correlations between root number and shoot fresh weight (A), and root dry weight and shoot fresh weight (B) under 20-32 DAS of drought imposition.

Among the 12 SSR markers (Table 3) only 7 markers provided amplified bands. Among the 7 markers, four (RM60, RM201, RM525, RM1141) further separate the tolerant from the susceptible genotypes based on the presence of bands (Figure 7). PCR results (Figure 7) revealed that DNA band at 158bp in RM201, 110bp for RM525, 100bp for RM1141, and 165bp for RM60 are present in drought tolerant genotypes, under well-watered and drought conditions at 20-32 and 30-42 DAS drought periods. These primers have been reported to be linked with several drought resistance traits (Table 3) such as deep root mass, root thickness, relative water content, osmotic adjustment, tiller number, panicle length, plant height in rice various genotypes (Afiukwa et al 2016, Ashfag et al 2014, Kanagarah et al 2010). Specific DNA bands generated from these SSR primers (RM201, RM525, RM1141, RM60), were able to differentiate NSIC Rc14 (drought tolerant) and PSB Rc82 (drought susceptible).

Marker RM201 located in chromosome 9 is associated with root traits, such as increased root length and drought tolerance and root:shoot ratio (Lang and Buu 2008, Ashfag et al 2014). The drought tolerance experiment showed significant differences in root length when seedlings are exposed to drought, though the bands showed similar patterns in both well-watered and drought conditions. This result is valid considering that SSR markers are not generally affected by environmental changes (Francia et al 2005) therefore the presence or absence of bands is consistent regardless of the treatments. On the other hand, RM24393, RM315, and RM212 were found to be present in drought susceptible genotypes. Ashfag et al (2014) reported that markers RM315 and RM212 are useful SSR markers linked to root traits, and are useful for evaluation of diverse germplasm under drought stress during the vegetative stage of rice. RM24393 is found to be present in all genotypes, this marker is linked to Dro1 QTL located at chromosome 9 which controls root growth angle and deep rooting (Uga et al 2013). This might be the reason why PSB Rc82, though found to be phenotypically susceptible under rainfed conditions, may not be totally drought susceptible under lowland conditions. Furthermore, this genotype was also found to be susceptible under PEG-induced drought; however, it may have different responses under field conditions. Future validation is needed to confirm whether NSIC Rc82 can be reliably considered susceptible to drought. The results of this study however clearly suggest that all lines selected from the PEG-drought induced set up can be considered tolerant as validated by the use of markers in this study.

Table 3. List of SSR markers used in the study and their associated traits under drought

SSR Marker	Sequence (5'-3')	Associated Traits	Sources
RM 201	F: CTCGTTTATTACCTACAGTACC R:CTACCTCCTTTCTAGACCGATA	Root number, root length, root-shoot ratio,	Ashfag et al 2014
RM 212	F: CCACTTTCAGCTACTACCAG R:CACCCATTTGTCTCTCATTATG	Root length, root number, root thickness, osmotic adjustment	Ashfag et al 2014
RM 302	F:TCATGTCATCTACCATCACAC R:ATGGAGAAGATGGAATACTTGC	Root length, root number, root thickness, RWC	Ashfag et al 2014
RM 7424	F:AGAAGCCCATCTAGCAGCAG R:TCAAGCTAGCCACACAGCTG	Deep rooting (Dro1), root growth angle, root deepening	Uga et al 2011
RM 24393	F:TAGCTGCTTAGCTTTGACTTGG R:ATGTAATCCTACGAGGAGATCG	Deep rooting (Dro1), root growth angle, root deepening	Uga et al 2011
RM 315	F:GAGGTACTTCCTCCGTTTCAC R:AGTCAGCTCACTGTGCAGTG	Root length, root number, root thickness	Ashfag et al 2014
RM 7390	F:CTGGTTAACGTGAGAGCTCG R:GCAGATCAATTGGGGAGTAC	Drought score, grain yield/plot	Afiukwa et al 2016
RM 1141	F:TGCATTGCAGAGAGCTCTTG R:CAGGGCTTTGTAAGAGGTGC	Deep root dry weight, Drought score, grain yield/plot	Afiukwa et al 2016
RM 60	F: AGTCCCATGTTCCACTTCCG R:ATGGCTACTGCCTGTACTAC	Root length, Drought score, Number of productive tillers,	Afiukwa et al 2016
RM 170	F:TCGCGCTTCTTCCTCGTCGACG R:CCCGCTTGCAGAGGAAGCAGCC	Number of productive tillers, drought score straw yield	Afiukwa et al 2016
RM 525	F:GGCCCGTCCAAGAAATATTG R:CGGTGAGACAGAATCCTTACG	Deep root dry weight, number of productive tillers, drought score	Afiukwa et al 2016
RM 36	F: CAACTATGCACCATTGTCGC R:GTACTCCACAAGACCGTACC	Deep root dry weight, drought score, root thickness	Afiukwa et al 2016

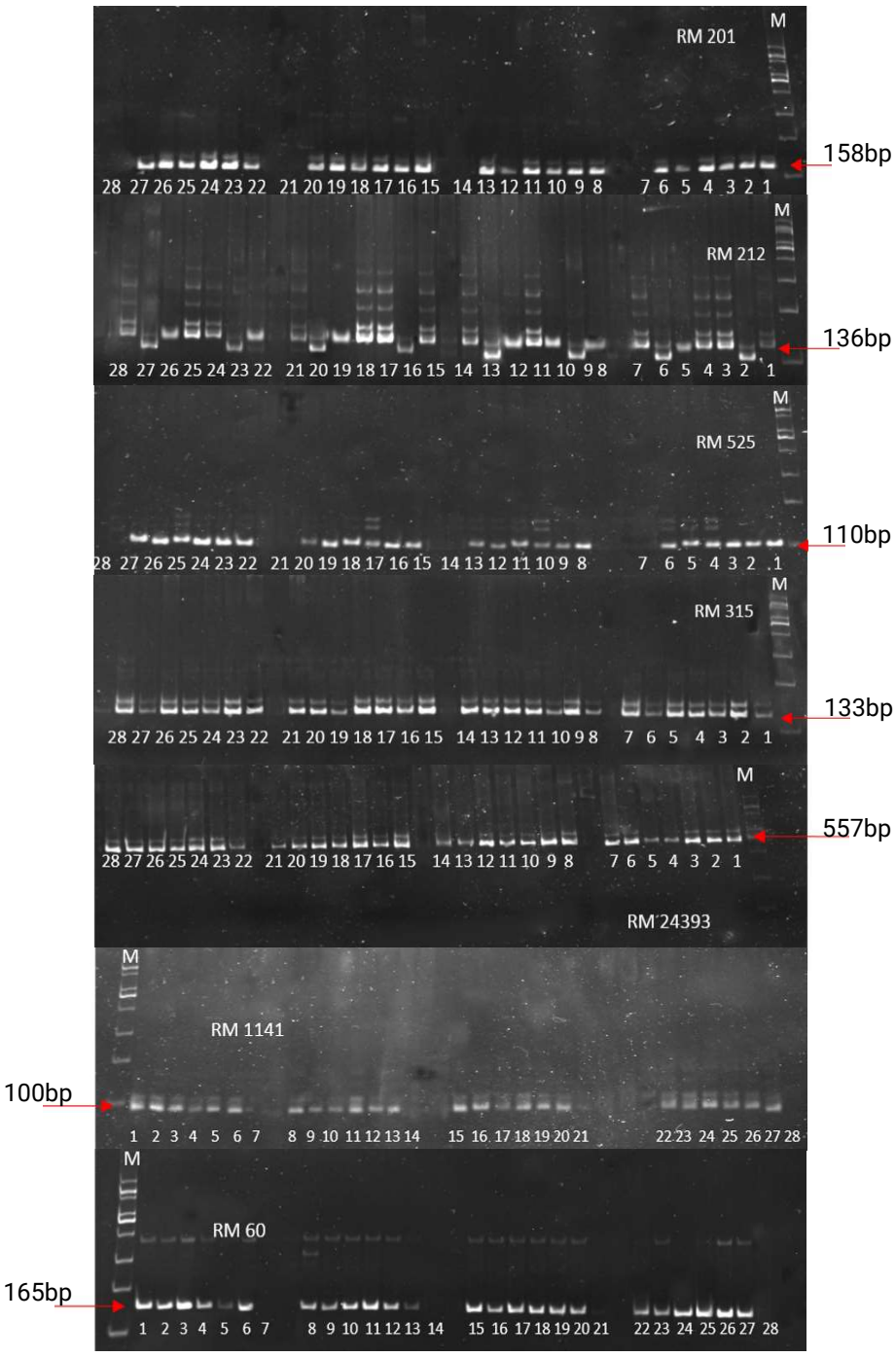


Figure 7. PCR profile of RM201, RM212, RM 525, RM315, RM24393, RM1141, RM60 showing the presence or absence of alleles in different genotypes. M represents the DNA marker, sample 1-7, 8-14 represents 7 genotypes under well-watered and drought condition respectively during 20-32 DAS stress, 15-21 and 22-28 under well-watered and drought stress during 30-42 DAS respectively. Bands in numbers 7, 14, 21 and 28 are drought susceptible check PSB Rc82

CONCLUSION AND RECOMMENDATION

The results of our study may allow for the fast and effective selection of drought tolerance genotypes. All five genotypes (AL-108, AL-5, AL-55, AL-87, and AL-97) had varied responses resulting in tolerance to drought. For instance, AL-108, AL-5 and AL-87 had high root:shoot ratio and total nodal root length under both drought conditions, while AL-5 had lowest reduction in leaf area and root length under 20-32 DAS of drought imposition. High root:shoot ratio, longer root, higher number of tillers, and root number are considered desirable traits under drought and therefore could be used in the future as indices for drought tolerance for further improvement of these genotypes. Among the 12 SSR markers used, only seven markers were amplified while only four may be considered effective in identifying drought – tolerant genotypes. Although PSB Rc82 was found to be susceptible to drought in the early stage of drought imposition it may not be absolutely susceptible based on the presence of three amplified markers linked to drought tolerance. Future validation is needed to confirm whether PSB Rc82 really should be considered susceptible to drought.

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