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Development of New Drought Tolerant Breeding Lines for Vietnam Using Marker-Assisted Backcrossing

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Abstract. Development of drought tolerant high-yielding varieties is essential because increased areas are subject to drought in the Mekong delta, Vietnam, during 2015-2016. The purpose of this experiment was developed using IR75499-73-1-B as drought tolerant donor and OMCS2000 as a recipient parent basis of a phenotypic and molecular marker for BC_2F_2 generation. Seven markers (RM219, RM201 RM105, RM23602, RM23877, RM24103 and RM328) were used for an identification to drought tolerant. Primer RM23877 detected the highest number of lines as homozygous donor alleles (11 lines), followed by RM105 and RM201 (9 lines). The drought gene was introgressed into the new breeding lines. The plant height, number of tillers, and filled grain had positive correlation with yield/hill under drought stress. The lines BC_2F_2-45 and BC_2F_2-54 developed as drought tolerant, and gave high yield. This is an opportunity to improve breeding for high yield and drought tolerant rice varieties in Vietnam.

1. Introduction

Drought stress is one of the major constraints to rice production and yield stability in rainfed upland ecology and estimates indicate that 70% of the yield losses can be attributed to abiotic stresses, especially drought [1]. Developing drought resistant cultivars will help to increase production of rice from climate change. Molecular markers help in identification of quantitative trait loci (QTLs) associated with drought resistance traits and their use in breeding high yield rice varieties suitable for drought-prone areas through marker-assisted breeding, thus reducing the need for extensive field testing over space and time. Development of new lines with tolerance to drought, a multipart trait, is a major challenge and a thorough understanding of the physiological and molecular mechanisms that direct the yield of rice under drought stress condition is a necessity.

Recent advances in plant breeding with developments in agricultural technology have provided breeders with numerous tools to enhance phenotypic screening, ranging from marker assisted selection (MAS) of key traits to molecular breeding (MB) and genetic engineering [2]. Current research is using these tools to develop enhanced drought tolerance in rice cultivars in China, India and Thailand [3]. In this context, conventional breeding techniques have been combined with MAS, and several drought tolerant rice varieties were released for commercial cultivation in these countries. Bernier et al. [4] detected a QTL on chromosome 12 in a large population from the cross of Vandana/Way Rarem that accounted for about 50% of the genetic variance, and was expressed consistently over 2 years. A chromosome 3 QTL had a large effect on drought tolerance in the cross between the tolerant variety Apo and the widely grown susceptible variety Swarna [5]. Lang et al. [6] reported fine mapping of the drought gene on chromosome 9.

Conventional and modern methods using molecular markers have been used for many breeding applications in rice. Breeding for drought tolerance could be accelerated by marker-assisted selection. The drought tolerant varieties can survive for 4 weeks or more without water, while most of the susceptible varieties died within a week. The most tolerant varieties (WAB, IR i) are from IRRI and their tolerance is controlled at the drought locus on chromosome 9 [6]. The variety IR74371-70-1-1 has been developed through conventional breeding and is disseminated to farmers in drought-prone areas. These varieties perform well even during favorable years, and they provide about 1 t/ha yield advantage under severe drought stress [7]. In this context, conventional breeding techniques have been combined with MAS, and several drought tolerant rice varieties were released for commercial cultivation in these countries.

Drought periods are also inevitable in the current status of climate change in the Mekong delta, Vietnam. Development of new lines with tolerance to drought, a multipart trait, is a major challenge and a thorough understanding of the physiological and molecular mechanisms that direct the yield of rice under drought stress condition is a necessity. Therefore, the objective of this study was to investigate the effect of drought stress at seedling and reproductive stages in the development of drought tolerance. Then, this new approach will be applied when developing more drought tolerant cultivars which are acceptable to farmers.

2. Materials and Methods

2.1 Rice materials

Backcross (BC) population was developed from OMCS2000/IR75499-73-1-B to map the genes for drought tolerance in IR75499-73-1-B. IR75499-73-1-B has always been the breeders' choice as donor for drought tolerance in their hybridization programs. OMCS2000 is an improved variety developed at CLRRI (Vietnam), but it is drought sensitive. The following were the steps in generating the BC_2F_2 population.

2.2 Development of the backross population

About 100 BC1F1 seeds were produced for the cross OMCS2000/IR75499-73-1-B and planted in a protected field to produce F_2 seeds. In the BC_2F_1 generation, background selection was carried out from phenotypical selection plants. Plant height and flowering dates were recorded in the BC_2F_2 generation. Any segregating lines, based on plant height and flowering were discarded and 200 were harvested as recombinant inbred lines. Each harvest consisted of a single plant from every line and a bulk of controlled-drought tolerance screening and field evaluation. Single plant seeds were used in final screening and DNA isolation for SSR analysis.

The population used consisted of 200 BC_2F_2 and conditions at CLRRI. A total of 30 lines were advanced and evaluated for yield and yield components using OMCS2000 and IR75499-73-1-B as the check.

2.3 DNA extraction

The 50 lines/varieties were grown in pots. Maximum protection was employed to ensure healthy and disease-free seedlings. The leaves were collected 2-3 weeks after planting for DNA extraction. Standard molecular grade chemicals and general techniques for preparing stock solutions, buffers and reagents were prepared following Sambrook et al. [8].

Molecular work was conducted at the Genetics and Plant Breeding Department of the Cuu Long Delta Rice Research Institute, Cantho, Vietnam from 2015.

DNA extraction was prepared according to a method described by McCouch et al. [9], and conducted at the Genetics and Plant Breeding Department of Cuu Long Rice Research Institute, Cantho, Vietnam. A piece of young rice leaf (2 cm) was collected and placed in a 1.5 ml centrifuge tube in ice. The leaf was ground using a polished glass rod in a well of a Porcelain Spot Test Plate (Thomas Scientific) after being added 400 µl of extraction buffer. Grinding was done until the buffer turned into green, an indication of cell breakage and releasing of chloroplasts and other cell contents. Another quantity of 400 µl of extraction buffer was added into the wells. An aliquot of 400 µl of the

lysate was transferred to a new tube. The lysate was deproteinized using 400 µl chloroform. The aqueous supernatant was transferred to a new 1.5 ml tube and the DNA was then precipitated using absolute ethanol. Afterward, it was air-dried and re-suspended in 50 µl of 0.1 mM TE buffer.

DNA quality was checked using 1% agarose (melting 3 g agarose in 300 ml TAE buffer). The mixture was heated in a microwave oven for 5-6 minutes and then cooled to around 55-60^{0}C. This was then poured on a prepared electrophoresis box with combs. Gels were ready and the combs were removed after about 45 min. Seven microliliters of DNA sample and 3 µl loading buffer (Tris 1M pH = 8.0, glycerol, EDTA 0.5M pH = 8.0, xylene cyanol 0.2%, bromphenol blue 0.2% and distilled water) was mixed and placed in the wells. The electrophoresis program was run at 70-80v, 60mA for 45 min or until loading buffer dye moved far from the wells. Gel was then taken out and stained with ethidium bromide. The gel image was visualized under UV light.

2.4 Molecular marker analysis

The whole microsatellite analysis included PCR assay, polyacrylamide gel electrophoresis, and band detection and scoring. Microsatellite primers were used to survey polymorphism of the samples. These were randomly selected from the 7 microsatellite primer pairs currently available for rice such as RM201; RM105; RM219, RM105, RM23602, RM23877, RM24103 and RM328 (Table 1) [10].

The PCR reaction was overlaid with mineral oil and processed in a programmable thermal controller set for 35 cycles of 1 min at 94 °C, 1 min at 55 °C, and 2 min at 72 °C, with a final extension at 75 °C for 5 min. After amplification, 10 µl of stop solution was added to the PCR product, which was then denatured at 94 °C for 2 min. Eight microliters of each reaction were run on polyacrylamide gel.

Table 1. Table of information molecular markers used in diagnosis of drought on chromosome 9.

Name of markers	Notation	Sequence	Repeating sequence
RM201	F	ctcgtttattacctacagtacc	CT)17
	R	ctacctcctttctagaccgata	
RM105	F	gtcgtcgacccatcggagccac	(CCT)6
	R	tggtcgaggtggggatcgggtc	
RM219	F	cgtcggatgatgtaaagcct	(CT)17
	R	catatcggcattcgcctg	
RM23662	F	gagaggacgatggcactattgg	(GGC)10
	R	cgaggaacttgattcgcatgg	
RM23877	F	tgccacatgttgagagtgatgc	(CA)30
	R	tacgcaagccatgacaattcg	
RM24103	F	actgacgagagagacatggatgg	AC)17
	R	ccggcacacaatgaataggg	
RM328	F	catagtggagtatgcagctgc	(CAT)5

2.5 Screening for drought tolerance

The experiment to evaluate drought-tolerance was performed in a greenhouse under two different conditions (normal and drought stress), completely randomized design and repeated 3 times in the research field of Cuu Long Delta Rice Research Institute, Viet Nam, during 2015-2016. For control purposes, more than more than 30 lines/varieties from areas under drought conditions was collected, as was some purebred rice with high yield potential. These varieties were evaluated and checked for resistance towards drought. At the same time the yield traits and yield components of germplasm were evaluated under difficult conditions caused by drought stress in order to figure out which lines/varieties were expressing the best yield, before continuing to conduct the next stages. Drought reactions were scored after stress using a 0-9 scale of a standard evaluation system for rice (Table 2) [11]. the

Table 2. Drought score at vegetative stage.

Scale	Description	Rate
0	No symptoms	Highly resistant
1	Slight tip drying	Resistant
3	Tip drying extended to ¼ length in most leaves	Moderately resistant
5	¼ to ½ of the leaves fully dried	Moderately susceptible
7	More than 2/3 of all leaves fully dried	Susceptible
9	All plants apparently dead	Highly susceptible

2.6 Quantitative traits

The following quantitative traits were measured:

1. Panicle length (cm): length of panicle at maturity measured from the base to the tip of the panicle (from 10 randomly selected primary panicles per accession per replication).
2. Panicles per plant (number): total number of panicles per plant (from 10 randomly selected primary panicles per accession per replication).
3. 1000-grain weight: weight in grams of 1000 well-developed grains at 14% MC (from 5 randomly selected primary panicles per accession per replication).
4. Days to maturity: days from seeding when 80% of the grains are fully ripened on a per replication basis.
5. Filled grains (number): obtained from counts of total number of filled grains per panicle (from 5 randomly selected primary panicles per accession per replication).
6. Unfilled grains (number): obtained from counts of total number of unfilled grains per panicle (from 5 randomly selected primary panicles per accession per replication).
7. Yield was obtained from harvested plants in each replication. Harvested grains were threshed, cleaned, dried, and weighed for each accession per replication. Moisture content (MC) per plot was determined immediately after weighing using a moisture meter, and yield was adjusted for moisture content.

2.7 Data analysis

Analysis of variance

The agro-morphological data collected were initially analyzed through analysis of variance to verify genetic variation in the traits measured. The few traits with insignificant genetic variation, based on the F-test, were not considered for further analyses.

Correlation analysis

Correlation coefficient (r) is a measure of the association between two or more variables. It is a measure of symmetrical association between variables and does not measure the dependence of one variable on another. Correlation among agro-morphological traits was calculated by using SAS software.

3. Results

3.1 Molecular screening

The polymorphic level of the SSR markers was different for each population. Markers (RM219, RM201 RM105, RM23602, RM23877, RM24103 and RM328) were used for identification to the drought tolerance gene on chromosome 9 based on information from the genetic map of Lang et al. [6]. Some examples were found of high degree of polymorphism between the parents (Fig. 1, 2, 3, 4, 5, 6, 7).

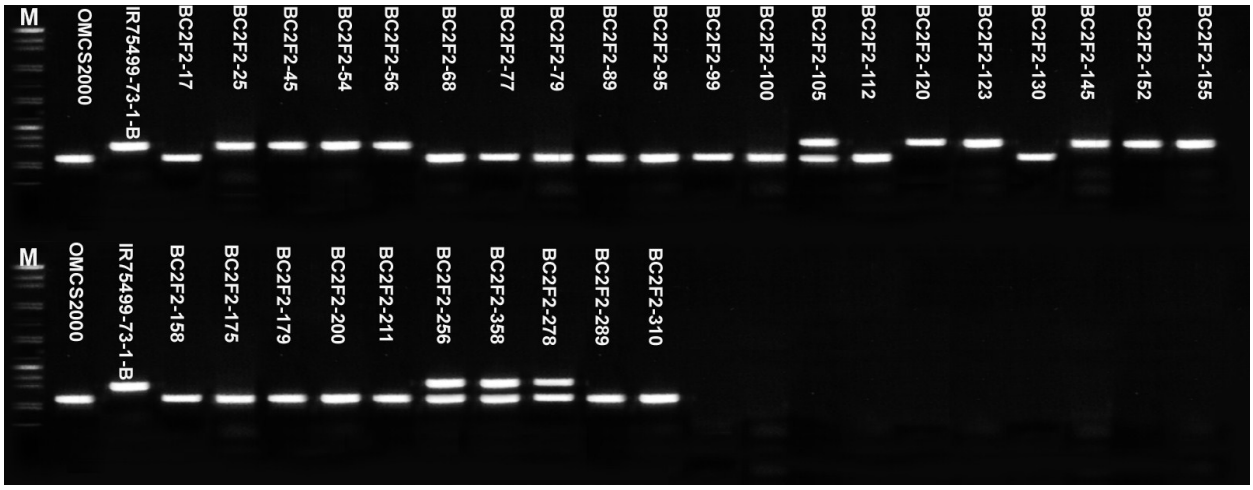

Fig. 1. Representative gel picture of foreground selection for drought tolerance individuals in BC_2F_2 generation of OMCS2000/IR75499-73-1-B// OMCS2000 with RM105.

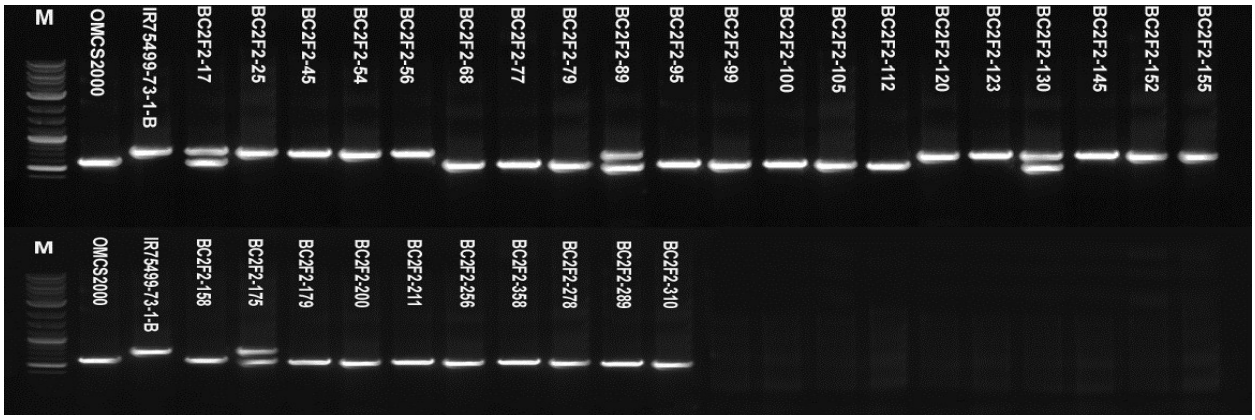

Fig. 2. Representative gel picture of foreground selection for drought tolerance individuals in BC_2F_2 generation of OMCS2000/IR75499-73-1-B// OMCS2000 with RM201.

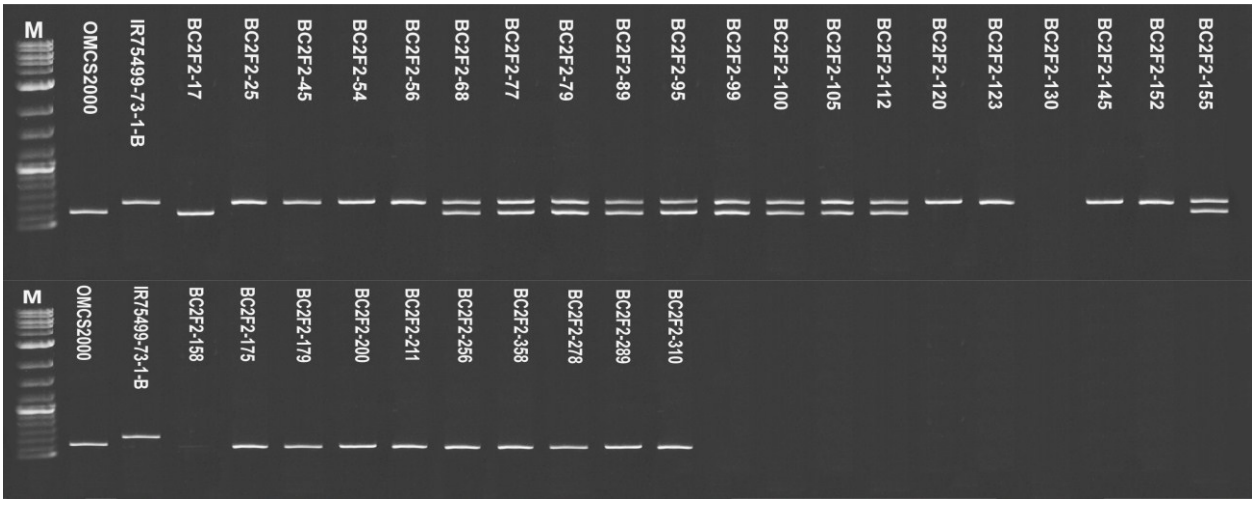

Fig. 3. Representative gel picture of foreground selection for drought tolerance individuals in BC_2F_2 generation of OMCS2000/IR75499-73-1-B// OMCS2000 with RM219.

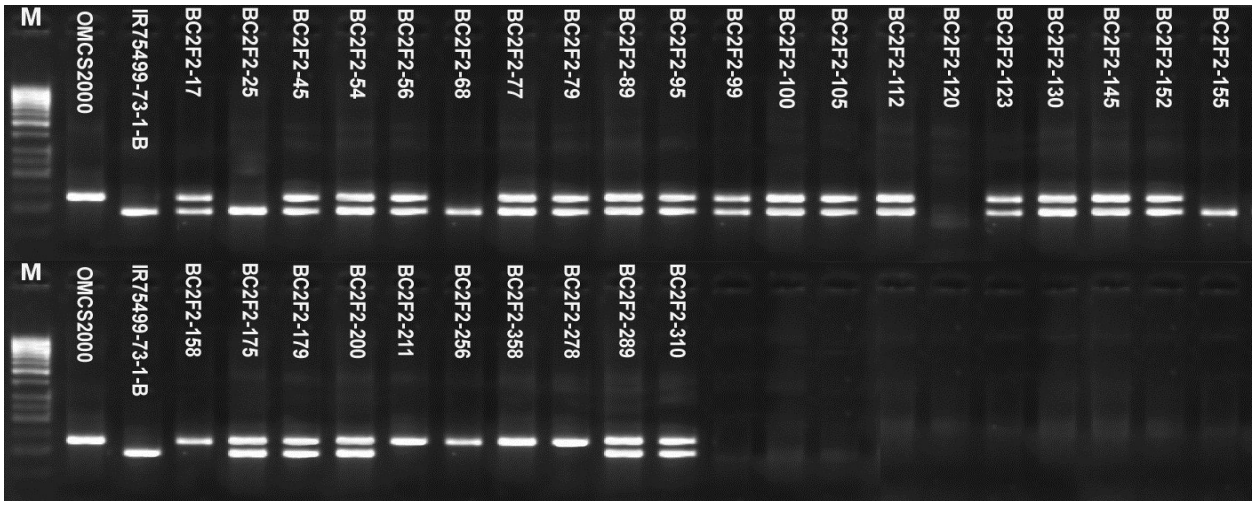

Fig. 4. Representative gel picture of foreground selection for drought tolerance individuals in BC_2F_2 generation of OMCS2000/IR75499-73-1-B// OMCS2000 with RM328.

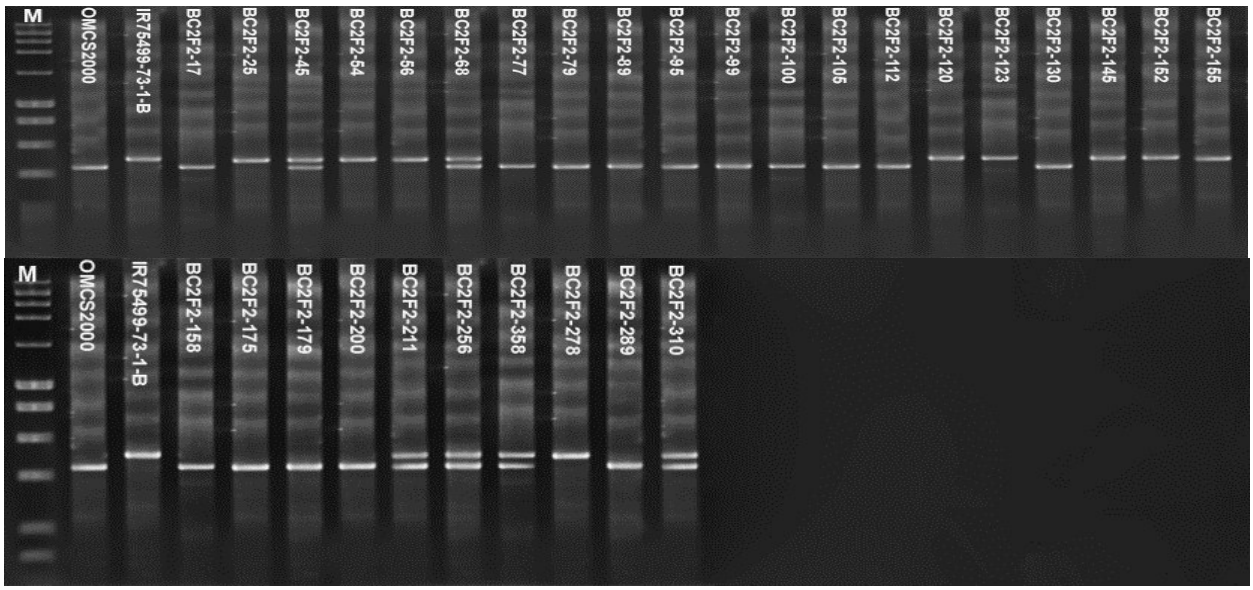

Fig. 5. Representative gel picture of foreground selection for drought tolerance individuals in BC_2F_2 generation of OMCS2000/IR75499-73-1-B// OMCS2000 with RM32662.

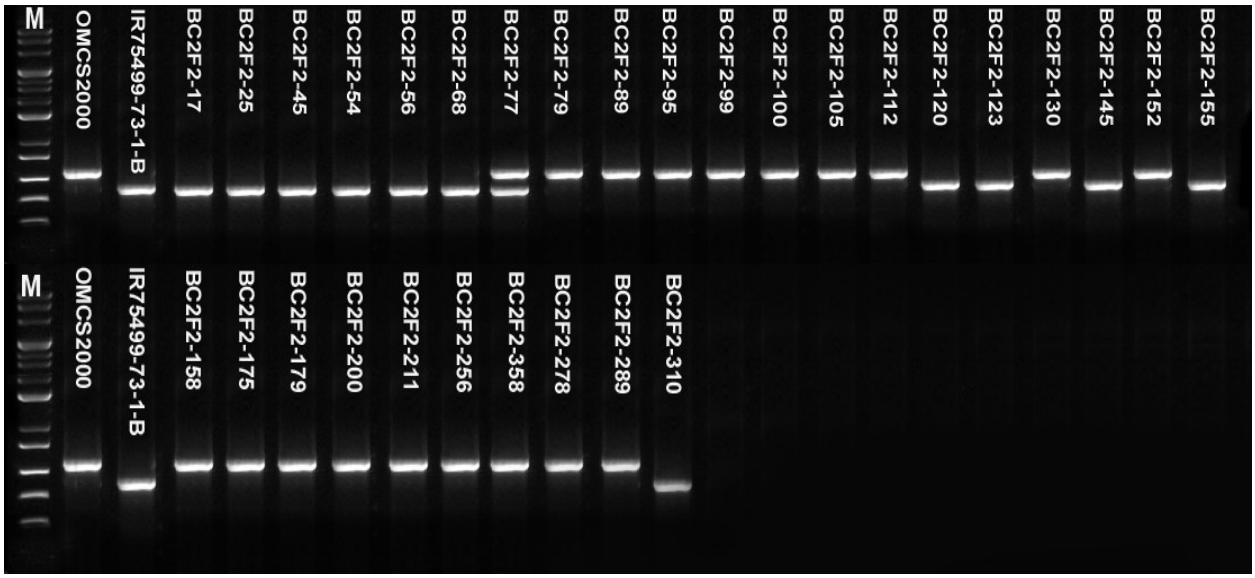

Fig. 6. Representative gel picture of foreground selection for drought tolerance individuals in BC_2F_2 generation of OMCS2000/IR75499-73-1-B// OMCS2000 with RM23877.

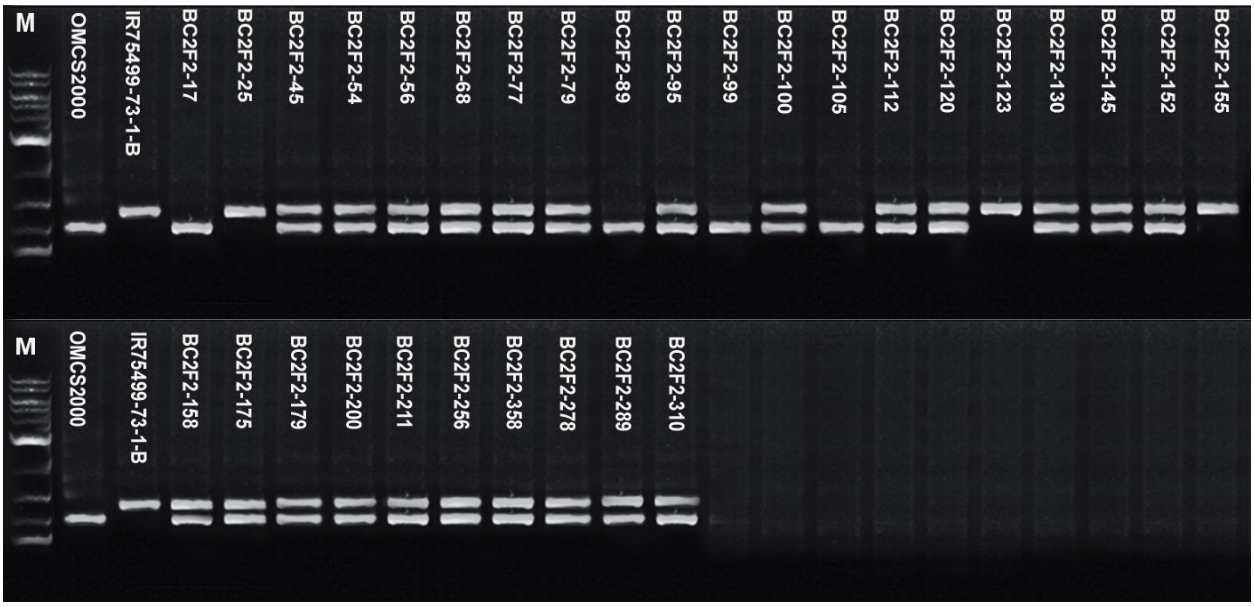

Fig. 7. Representative gel picture of foreground selection for drought tolerance individuals in BC_2F_2 generation of OMCS2000/IR75499-73-1-B// OMCS2000 with RM 24103.

Primer RM23877 confirmed that 11 lines possessed homozygous donor alleles (B), 18 line as homozygous recipient alleles (A) and 1 line had heterozygous alleles (H) (Fig. 6). For the two primers, RM105 and RM201, 9 lines had homozygous donor alleles, 17 lines were similar to homozygous recipient alleles, and 4 lines had heterozygous alleles (H) (Fig. 1 and 2). In case of RM328 and RM24103, the rest of 3 lines had homozygous donor alleles (B) (Fig. 4 and 7). Eight lines showed similarity to homozygous donor alleles for RM219, nine lines were found to be homozygous donor alleles for RM32662 (Figs. 3 and 5). The highest number of heterozygous recipient alleles was obtained by 23 lines by RM24103 (76.67%).

Out of 30 lines, only one line, BC_2F_2-25, possessed homozygous donor alleles for seven markers and five lines BC_2F_2-99, BC_2F_2-179, BC_2F_2-200, BC_2F_2-211, BC_2F_2-289 possessed homozygous recipient alleles for five markers and heterozygous alleles for two markers. Likewise, line BC_2F_2-158 possessed heterozygous alleles for one marker (RM24103) (Table 3).

3.2 Drought phenotyping

Phenotype evaluation of drought tolerance was done in BC_2F_2 of OMCS2000/ IR75499-73-1-B. The result is shown in table 3; one line had score 1 (BC_2F2-25) similar to IR75499-73-1-B, one line had score 0 (BC_2F_2-155), 10 lines had score 3, one line had score 5, 3 lines had score 7, and 14 lines had score 9 like OMCS2000.

Evaluation of survival rate and some traits of the BC_2F_2 lines was performed after lines were fixed before and after the 20 days of drought (Table 4). Results showed that the ability of lines to survive ranged from 4.6 -90.1% after the drought stress 20 days; lower than under normal condition (100%). The lines with the highest survival rate (%) were: BC_2F_2-25, BC_2F_2-155 (75.6%, 90.1 %) with tolerance level 1 and 0 and line BC_2F_2-152, with a survival rate of 66.5% (with a tolerance level of 3). The remaining lines had lower survival rates than the control variety IR75499-73-1-B (59%) including OMCS2000 which had a survival rate of 12.5%.

The plant height of lines ranged from 19 cm (BC_2F_2-211) to 56.3 cm (BC_2F_2-310). Two lines had an average height which was higher than both the parents OMCS2000 (25.6 cm) and IR75499-73-1-B (55.3cm), consisting of BC_2F_2-56 (55.6 cm) and BC_2F_2-310 (56.3 cm). In comparison, lines corresponding to normal condition ranged from 42.3 cm (BC_2F_2-152) to 77 cm (BC_2F_2-68) in height, and there was considerable reduction in number of plants in most of the lines. Fluctuations between the lines in the two environments were quite small with CV% <10% recorded as 3.8 and 5

Table 3. Phenotypic and genotypic analysis of 30 lines BC_2F_2 generation along parents.

No	Lines	Phenotypic analysis	Genotypic analysis						
		Score	RM105	RM201	RM219	RM328	RM32662	RM23877	RM24103
P1	P1	9	A	A	A	A	A	A	A
P2	P2	1	B	B	B	B	B	B	B
1	BC_2F_2-17	7	A	H	A	H	A	B	A
2	BC_2F_2-25	1	B	B	B	B	B	B	B
3	BC_2F_2-45	3	B	B	B	H	H	B	H
4	BC_2F_2-54	3	B	B	B	H	B	B	H
5	BC_2F_2-56	3	B	B	B	H	B	B	H
6	BC_2F-68	9	A	A	H	B	H	B	H
7	BC_2F_2-77	9	A	A	H	H	A	H	H
8	BC_2F_2-79	9	A	A	H	H	A	A	H
9	BC_2F_2-89	7	A	H	H	H	A	A	A
10	BC_2F_2-95	9	A	A	H	H	A	A	H
11	BC_2F_2-99	9	A	A	H	H	A	A	A
12	BC_2F_2-100	9	A	A	H	H	A	A	H
13	BC_2F_2-105	9	H	A	H	H	A	A	A
14	BC_2F_2-112	9	A	A	H	H	A	A	H
15	BC_2F_2-120	3	B	B	B	-	B	B	H
16	BC_2F_2-123	3	B	B	B	H	B	B	B
17	BC_2F_2-130	5	A	H	-	H	A	A	H
18	BC_2F_2-145	3	B	B	B	H	B	B	H
19	BC_2F_2-152	3	B	B	B	H	B	A	H
20	BC_2F_2-155	0	B	B	H	B	B	B	B
21	BC_2F_2-158	9	A	A	-	A	A	A	H
22	BC_2F_2-175	9	A	H	A	H	A	A	H
23	BC_2F_2-179	9	A	A	A	H	A	A	H
24	BC_2F_2-200	9	A	A	A	H	A	A	H
25	BC_2F_2-211	9	A	A	A	A	H	A	H
26	BC_2F_2-256	3	H	A	A	A	H	A	H
27	BC_2F_2-358	3	H	A	A	A	H	A	H
28	BC_2F_2-278	3	H	A	A	A	B	A	H
29	BC_2F_2-289	9	A	A	A	H	A	A	H
30	BC_2F_2-310	7	A	A	A	H	H	B	H

A: homozygous recipient allele; B: homozygous donor allele; H: heterozygous allele
P1: OMCS2000; P2: IR75499-73-1-B

Tillering ability of the experimental lines before and after complete drought stress was assessed through evaluation of the number of tillers /10 hills of each line (Table 4). The number of tillers/10 hills varied from 3 (BC_2F_2-200) to 47 (BC_2F_2-155) while at normal condition, the number of tillers of 10 hills ranged form 26 to 69. Three lines had a higher number of tiller/10 hills than both of the parents IR75499-73-1-B (41) and OMCS2000 (14).

Table 4. Survival rate and some traits of the BC_2F_2 lines derived from OMCS2000/ IR75499-73-1-B before and after the drought stress 20 days.

No	Code	Rate of survival (%)		Plant height (cm)		Number of tiller/10 hills		Root length (cm)	
		Normal condition	DS	Normal conditions	DS	Normal conditions	DS	Normal conditions	DS
	P1	100	12.5 klm	57 bc	25.6 m	37 m	14 ef	12.5 fgh	5.6 kl
	P2	100	89.2 a	59 b	55.3 a	42 jkl	41 b	15.6 b-f	18.9 abc
1	BC_2F_2-17	100	26.3 i	44 kl	41.6 fgh	40 lm	9 ghi	17.5 abc	10.2 ij
2	BC_2F_2-25	100	75.6 b	42.6 l	45.5 cde	49 gh	41 b	17.6 ab	15.6 def
3	BC_2F_2-45	100	50.6 g	49.8 hi	47.6 bcd	42 jkl	28 d	16.8 a-d	18.9 abc
4	BC_2F_2-54	100	59.8 de	56.5 bc	48.6 bc	49 gh	29 d	16.4 a-e	17.9 a-d
5	BC_2F_2-56	100	56.7 ef	55.8 bcd	55.6 a	44 ijk	33 c	16.5 a-e	19.5 ab
6	BC_2F-68	100	4.6 o	77 a	36 jk	45 ij	11 fgh	14.5 b-g	12.2 ghi
7	BC_2F_2-77	100	7.6 no	45.6 jkl	34 k	41 kl	9 ghi	16.5 a-e	11.2 hi
8	BC_2F_2-79	100	12 klm	48.5 hij	35 k	49 gh	8 hij	17.5 abc	7.5 jk
9	BC_2F_2-89	100	38 h	45.6 jkl	45.6 cde	52 efg	11 fgh	15.6 b-f	9.6 ij
10	BC_2F_2-95	100	12.6 klm	47.6 ijk	22.5 mn	51 fg	7 ijk	17.5 abc	7.6 jk
11	BC_2F_2-99	100	14.5 jkl	45.6 jkl	24.5 m	49 gh	6 i-l	15.6 b-f	7.4 jk
12	BC_2F_2-100	100	11.2 lmn	50.3 hi	23.2 m	53 ef	4 kl	14.6 b-g	9.8 ij
13	BC_2F_2-105	100	12.6 klm	51.6 e-h	19.2 n	66 ab	4 kl	19.5 a	14.2 e-h
14	BC_2F_2-112	100	13.5 j-m	54.6 c-f	22.2 mn	52 efg	9 ghi	16.5 a-e	16.5 b-e
15	BC_2F_2-120	100	56.8 ef	51.2 f-i	29.5 l	58 c	29 d	14.5 b-g	17.8 a-d
16	BC_2F_2-123	100	50.6 g	50.3 hi	45.6 cde	54 def	26 d	15.6 b-f	16.3 cde
17	BC_2F_2-130	100	47.5 g	50.4 ghi	50.2 b	55 cde	17 e	14.6 b-g	14.8 d-g
18	BC_2F_2-145	100	60.8 d	51.2 f-i	42.6 e-h	54 def	45 a	14.2 c-g	16.5 b-e
19	BC_2F_2-152	100	66.5 c	42.3 l	40.2 ghi	52 efg	41 b	9.7 h	19.6 ab
20	BC_2F_2-155	100	90.1 a	50.6 ghi	40.5 f-i	51 fg	47 a	16.8 a-d	20.6 a
21	BC_2F_2-158	100	10 mn	50.2 hi	37.6 ijk	57 cd	8 hij	17.7 ab	9.5 ij
22	BC_2F_2-175	100	14.3 jkl	55.3 b-e	43.5 efg	52 efg	36 c	15.3 b-f	17.7 a-d
23	BC_2F_2-179	100	14.5 jkl	54.2 c-g	41.2 f-i	26 n	5 jkl	15.7 b-f	17.7 a-d
24	BC_2F_2-200	100	14.3 jkl	52.3 d-h	36 jk	44 ijk	3 l	13.3 egf	4 l
25	BC_2F_2-211	100	16.5 j	51.5 e-h	19 n	42 jkl	14 ef	15.6 b-f	11.2 hi
26	BC_2F_2-256	100	50.2 g	50.2 hi	44.2 def	45 ij	44 ab	14.7 b-g	14.2 e-h
27	BC_2F_2-358	100	54.6 f	51.4 f-i	41.6 fgh	46 hi	33 c	15.6 b-f	12.6 f-i
28	BC_2F_2-278	100	54.7 f	51.6 e-h	39.5 hij	47 hi	36 c	11.5 gh	5.7 kl
29	BC_2F_2-289	100	15.6 jk	51.6 e-h	44.2 def	65 b	12 fg	14.2 c-g	5.6 kl
30	BC_2F_2-310	100	35.6 h	51.2 f-i	56.3 a	69 a	11 fgh	13.6 g-g	11.6 hi
CV (%)		**-**	**5.3**	**3.8**	**5**	**19.5**	**3.9**	**9.2**	**10.8**
F			**	**	**	**	**	**	**

Superscript letters indicated Duncan text. Same letters in the same column are not significantly differed **: Significant at 0.01 level. P1: OMCS2000; P2: IR75499-73-1-B; DS: Drought stress

Among all of the tested lines, the lines BC_2F_2 -56, BC_2F_2-152 and BC_2F_2-155 had a long root length (19.5 cm, 19.6 cm, and 20.6 cm respectively), which was higher than both of the parents IR75499-73-1-B (18.9 cm) and OMCS2000 (5.6 cm).

The correlation between traits was estimated by regressing phenotypic values of one of these traits with the other traits, as show in Table 5.

Table 5. Correlation coefficient matrix of some targets of phenotypic after completed drought stress 20 days of BC_2F_2 generation.

Traits	Rate of survival	Plant height	Number of tiller	Root length
Rate of survival	1			
Plant height	0.5911*	1		
Number of tiller	0.8588**	0.4993ns	1	
Root length	0.6162*	0.3901ns	0.6136*	1

Note: *, ** significant at P < 0.05, 0.01 respectively; ns: not significant

The significant positive correlated traits was observed in survival rate x number of tillers/10 hills (r = 0,8588**; $P < 0.01$), survival rate x root length and number of tillers/10 hills x root length (r = 0.6162*; 0.6136*; $P < 0.05$), respectively. This increase in root length and their function during complete drought conditions is almost certainly beneficial and different from the increase in plant height.

3.3 Agro-morphological characters

The lines were screened at seedling stage after surviving drought stress and were screened at flowering stage. Breeding lines were tested under two treatments: normal conditions and drought stress for 30 days (Table 6).

Under drought tress, some lines still segregated, and a few lines were fully dead (BC_2F_2-68, BC_2F_2-77, BC_2F_2-79, BC_2F_2-95, BC_2F_2-99, BC_2F_2-100, and BC_2F_2-158). Results indicated that plant height of the lines ranged from 75 cm (BC_2F_2-155) to 114 cm (BC_2F_2-25, BC_2F_2-278 and BC_2F_2-310). Nineteen lines were significantly taller, the highest being IR75499-73-1-B (100 cm), while two lines (BC2F2-155 and BC_2F_2-158) had shorter plant height than OMCS2000 (86 cm). Number of tillers varied significantly among lines. Most of lines had lower tillering ability than both parents; IR75499-73-1-B (12) and OMCS2000 (8). The highest number of tillers was recorded in only one line, BC_2F_2-358 (13). The number of filled grains/panicle was significantly different under drought stress and normal condition (P<0.05). The highest number of filled grains/panicle was recorded by line BC_2F_2-54 (155), which was a higher number of filled grains/panicle than of both parents IR75499-73-1-B (142) and OMCS2000 (23). One line, BC_2F_2-56, had a higher number of filled grains/panicle (>100). The rate of unfilled-grain/panicle was significantly different under drought stress and normal conditions (P<0.05). Under drought stress, the rate of unfilled-grain/panicle varied from 13.6 % (line BC_2F_2-89) to 64.2 % (line BC_2F_2-278). There was one line (BC_2F_2-89) which had a lower rate of unfilled-grain/panicle than both of the parents IR75499-73-1-B (15.6 %) and OMCS2000 (86 %). Yield/hill of BC lines after the drought stress (30 days), recorded lower yield/hill than under normal conditions. Yield/hill of line BC_2F_2-45 was the highest (30 g) followed by line BC_2F_2-54 (29 g). These lines had higher yield/hill than both of the parents, IR75499-73-1-B (27 g) and OMCS2000 (3 g) (Table 6).

The degree of correlation among traits is as important a factor as yield. The results of correlation analysis among agronomic traits after completed drought stress for 30 days are shown by coefficients of correlation (Table 7). The yield/hill was found to be positively correlated with plant height (r = 0.6162*), number of tillers (r = 0.5393*), and filled grain (r = 0.7695**). The number of tillers showed significantly positive correlation with unfilled-grain (0.6893*).

Table 6. Agronomic traits of BC lines after the drought stress at (30 days).

No.	Code	Plant height (cm)		Number of tillers		Filled grains		Unfilled-grain (%)		Yield (g/hill)	
		Normal condition	DS	Normal condition	DS	Normal condition	DS	Normal condition	DS	Normal condition	DS
	P1	110 ij	86 m	14 a-d	12 ab	115 ij	23 l	13.5 f	86.6 a	42.3 ab	3 ij
	P2	119 bcd	100 fgh	14 a-d	12 ab	160 b	142 b	15 ef	15.6 ij	32.5 efg	27 abc
1	BC_2F_2-17	114 fgh	102 efg	13 bcd	7 efg	117 i	85 d	26 bcd	18.6 hi	39 bc	16.5 def
2	BC_2F_2-25	119 bcd	114 a	14 a-d	5 g	156 c	87 d	24 cd	20.6 h	34 def	25 c
3	BC_2F_2-45	117 c-f	113 ab	15 abc	9 cde	140 f	88 d	25 bcd	15.6 ij	35 de	30 a
4	BC_2F_2-54	112 hi	105 de	16 ab	9 cde	168 a	155 a	26 bcd	63.5 b	37 cd	29 ab
5	BC_2F_2-56	116 d-g	111 ab	17 a	7 efg	104 k	102 c	23 d	48.6 c	36 cde	26.6 bc
6	BC_2F-68	100 k	92 kl	14 a-d	0 h	156 c	0 n	24 cd	0 k	37 cd	0 j
7	BC_2F_2-77	107 j	96 ij	15 abc	0 h	100 l	0 n	27 bc	0 k	31 fg	0 j
8	BC_2F_2-79	108 j	98 hij	16 ab	0 h	144 de	0 n	16 ef	0 k	25 ij	0 j
9	BC_2F_2-89	115 e-h	97 hij	14 a-d	6 fg	123 h	68 f	15 ef	13.6 j	26 hij	14.6 ef
10	BC_2F_2-95	114 fgh	90 l	12 cd	0 h	100 l	0 n	18 e	0 k	27 hi	0 j
11	BC_2F_2-99	110 ij	99 ghi	13 bcd	0 h	100 l	0 n	16.5 ef	0 k	26 hij	0 j
12	BC_2F_2-100	113 ghi	95 jk	14 a-d	0 h	112 j	0 n	16.3 ef	0 k	27 hi	0 j
13	BC_2F_2-105	114 fgh	102 efg	15 abc	8 def	89 n	75 e	26.2 bcd	32.5 f	24 ij	16.5 def
14	BC_2F_2-112	116 d-g	103 ef	14 a-d	6 fg	95 m	77 e	32.2 a	33.5 ef	29 gh	14.5 ef
15	BC_2F_2-120	117 c-f	104 de	12 cd	7 efg	105 k	66 f	15.6 ef	36.5 de	27 hi	13.2 fg
16	BC_2F_2-123	116 d-g	112 ab	11 d	9 cde	114 ij	76 e	13.5 f	37.5 d	16 k	25.6 c
17	BC_2F_2-130	102 k	102 efg	16 ab	10 bcd	116 i	69 f	17.5 e	63.2 b	17 k	5.5 hi
18	BC_2F_2-145	115 e-h	103 ef	14 a-d	10 bcd	147 d	46 j	16.9 ef	25.6 g	25 ij	4.8 hi
19	BC_2F_2-152	116 d-g	110 bc	14 a-d	12 ab	123 h	88 d	26.4 bcd	24.5 g	33 ef	6.9 h
20	BC_2F_2-155	114 fgh	75 o	17 a	11 abc	145 de	57 h	26.7 bcd	26.7 g	42 ab	3.5 i
21	BC_2F_2-158	118 b-e	76 no	14 a-d	0 h	162 b	0 n	28.3 b	0 k	41 ab	0 j
22	BC_2F_2-175	120 bc	79 n	15 abc	0 h	122 h	99 c	26.4 bcd	27.5 g	44 a	11 g
23	BC_2F_2-179	117 c-f	100 fgh	14 a-d	10 bcd	142 ef	56 h	26.3 bcd	26.4 g	18 k	19 d
24	BC_2F_2-200	113 ghi	102 efg	17 a	10 bcd	132 g	52 i	35.6 a	25.7 g	16 k	14.5 ef
25	BC_2F_2-211	118 b-e	107 cd	11 d	11 abc	114 ij	55 hi	15.4 ef	39.7 d	22.5 j	16.5 def
26	BC_2F_2-256	120 bc	111 ab	14 a-d	10 bcd	96 m	14 m	14.8 ef	39.8 d	26.7 hi	14.7 ef
27	BC_2F_2-358	121 b	103 ef	15 abc	13 a	98 lm	26 l	16.9 ef	62.5 b	24.6 ij	18.9 d
28	BC_2F_2-278	116 d-g	114 a	16 ab	12 ab	78 o	35 k	17.8 e	64.2 b	25.8 hij	18.5 d
29	BC_2F_2-289	117 c-f	102 efg	14 a-d	10 bcd	96 m	69 f	16.5 ef	61.7 b	24.3 ij	17.4 de
30	BC_2F_2-310	125 a	114 a	15 abc	6 fg	78 o	61 g	17.3 ef	62.3 b	26.7 hi	16.5 def
CV (%)		1.7	1.9	11.5	15	1.6	3.1	9.2	5.6	6.6	12.9

Superscript letters indicated Duncan text. Same letters in the same column are not significantly differed at the 5% level. P1: OMCS2000; P2: IR75499-73-1-B; DS: Drought stress

Table 7. Correlation coefficient among agronomic traits after completed drought stress 30 days of BC_2F_2 generation.

Traits	Plant height (cm)	Number of tillers	Filled grains	Unfilled-grain (%)	Yield/hill (g)
Plant height	1				
Number of tillers	0.4084ns	1			
Filled grains	0.3243ns	0.4677ns	1		
Unfilled-grain	0.3117ns	0.6893*	0.3449ns	1	
Yield	0.6319*	0.5393*	0.7695**	0.4095ns	1

Note: *, ** significant at $P < 0.05$, 0.01 respectively; ns: not significant

4. Discussion

New breeding BC-derived lines were screened for drought tolerance using phenotyping and molecular markers. For self-pollinated crops, an important aim may be to fix alleles in their homozygous state as early as possible. For example, in bulk and single-seed descent breeding methods, screening may be performed at the BC_2F_2 generations where most loci are homozygous. Using co-dominant DNA markers, it is possible to fix specific alleles in their homozygous state as early as the F_2 generation. However, this may require large population sizes. Thus, in practical terms, a small number of loci may be fixed at each generation [12]. An alternative strategy is to 'enrich' rather than fix alleles-by selecting homozygotes and heterozygotes for a target locus-within a population in order to reduce the size of the required breeding populations [13].

Percent genetic maps for crop plants have been generated using a variety of DNA markers, the most common of which are Simple Sequence Repeats (SSR). SSR technology combines the best characteristics of these commonly used markers while avoiding their disadvantages. In this study, the SSR technique generated rapid, numerous, reproducible and codominant markers. Although a BC_2 population developed from *Indica* parents, OMCS2000 and IR75499-73-1-B was used, and SSR markers were generated using a 7 primer combination.

Grain yield in rice is a complex trait determined by its three component traits: number of panicles, number of grains per panicle, and grain weight [14]. The number of grains per panicle is usually highly proportional to the spikelet number. To understand number of grains per panicle, it is essential to understand the basic biological processes of panicle development, as well as the differentiation of meristems into spikelets at drought in rice. From an agronomic perspective, the number of spikelets per panicle can be attributed to two components: the duration of panicle differentiation and the rate of spikelet differentiation [15]. Further evaluations of the selected lines are being conducted to select the next generations.

Conclusions

The results from testing drought tolerance in selected rice lines were confirmed by both genotypic and phentypic analysis. Two advanced breeding lines (BC_2F_2-54 and BC_2F_2-45) were adapted to drought stress. These lines will be released after a shorter period of time in the selection process than the ones in the previous breeding program.

A marker assisted selection was applied to the selection of new lines in developing backcross drought tolerant populations. New drought tolerant populations are continuously being developed for selection for drought tolerant characteristics and yield potential in the next generations.

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