Doctoral Dissertation

Application of Marker – Assisted Selection for Breeding Drought –Tolerant Rice (*Oryza sativa* L.) in Vietnam

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2. **Pham Thi Thu Ha**, Do Tan Khang, Phung Thi Tuyen, Tran Bao Toan, Nguyen Ngoc Huong, Nguyen Thi Lang, Bui Chi Buu, Tran Dang Xuan. Development of new drought tolerant breeding lines for Vietnam using marker-assisted backcrossing. International Letters of Natural Sciences, 59: 1-13, 2016.
3. **Ha Thi Thu Pham**, Khang Tan Do, Minh Ngoc Truong, Xuan Dang Tran, Lang Thi Nguyen and Buu Chi Bui. Path analysis for yield traits in F2 generation and molecular approaches for breeding rice tolerant to drought and submergence. African Journal of Agricultural Research, 11(26): 2329-2336, 2016.
4. **Pham Thi Thu Ha**, Do Tan Khang, Phung Thi Tuyen, Truong Ngoc Minh, Tran Dand Xuan, Nguyen Thi Lang, Bui Chi Buu. Study on physical-chemical characters and heritability for yield components in rice (*Oryza sativa* L.)", International Letters of Natural Sciences, 57: 67-78, 2016.

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List of Abbreviations

AC	Amylose content
AFLP	Amplified fragment length polymorphism
BC	Backcross
BR	Brown rice
Chr	Chromosome
CLRRI	Cuu Long Delta Rice Research Institute
CV	Coefficient of variation
DA	Drought avoidance
DF	Drought at flowering stage
DRF	Drought at flowering
DT	Drought tolerance
DTS	Drought tolerance at seedling
DTV	Drought tolerance at vegetative
FG	Filled grain
GC	Gel consistency
GD	Growth duration
GT	Gelatinization temperature
GW	Grain wide
GY/C	Grain yield/cluster
H	Heterozygous
h^2	Heritability
HI	Harvest Index

HR	Head rice
IRRI	International Rice Research Institute
LR	leaf roll
MAS	Marker-assisted selection
MB	Molecular breeding
MC	Moisture content
MR	Milled rice
NFG/P	Number of filled grain/panicle
NP/C	Number of panicle/cluster
NTSYS-PC	Numerical Taxonomy and Multivariate Analysis System
NUFG/P	Number of unfilled grain/panicle
P	Panicle
PC	Protein content
PCR	Polymerase chain reaction
PH	Plant height
PIC	Polymorphic information content
PL	Panicle length
Pr	Phenotype correlation coefficients
QTL	Quantitative trait locus/loci
RDW	Root dry weigh
RFLP	Restriction fragment length polymorphism
RL	Root length
SSR	Simple sequence repeat

SubS	Submergence at seedling
UG	Unfilled grain
UPGMA	Un weighted pair group method using an arithmetic
W-1000	Weight of 1000 grains

Abstract

Rice, (*Oryza sativa* L.) is one of the most important crops providing staple food for a large segment of the world population. Climate change is likely to adversely impact on rice production in Tropical Asia. There are two broad areas of environmental stresses: abiotic factors (salinity, heat, drought, cold, submergence, radiation, and heavy metals) and biotic factors (pathogens and herbivore). Especially environmental stresses including heat, cold, drought, and salinity factors extremely affected on average agriculture yield in the world. Among this indicator, drought is one of the most common stresses, causing a remarkable loss of crops.

To be more specific, drought stress is one of the main constraints to rice production and yield stability in rainfed upland ecology. It is estimated that 70% of the yield losses could be due to water scarcity affecting approximately 27 million ha of rainfed upland. Vietnam has been predicted to become one of the most vulnerable countries and significantly impacted countries due to the influences of climate change. Farmers in these regions usually utilized rainfall to cultivate rice plants. The drought resistance of plants is one of the most complex biological processes, which involves numerous changes at the physiological, cellular, and molecular levels. The effect of drought on rice plants considerably varies with genotypes, developmental stages, and degree and duration of drought stress. Many genes have been identified to be involved in the response to drought stress in plants. However, as yield is a complex trait, there is a necessity for a genetic and physiological analysis of yield contributing traits under drought stress.

Currently, several methods have been used to develop drought-tolerant crops: conventional breeding, conventional breeding utilizing marker-assisted selection (MAS)

and genetic engineering. So far, genetic engineering has been successfully discovered many genes which are involved in the plant responses to drought stress. However, due to irregular rain, rice plants were damaged by drought during seedling, and from flowering to maturity stage. The injuries on leaves at seedling stage, sterile spikelet at the reproductive stage and stem shoot under drought stress condition are key traits related to drought tolerance. Therefore, selection of drought-tolerant individuals should not only observe morphological traits but also physiological, biochemical and genotype by environment interaction.

The fact is that, the genetic bases of drought tolerance (DT) and drought avoidance (DA) at reproductive stage in rice were analyzed using a combination of inbred line population from a cross between *indica* lowland and a tropical *japonica* upland cultivar. A genetic linkage map consisting of 245 SSR markers was constructed for mapping QTL for these traits. A total of 27 QTLs were resolved for 7 traits of the relative performance of fitness and yield, 36 QTLs for 5 root traits under control, and 38 for 7 root traits under drought stress conditions. Only a small portion of QTL for fitness- and yield-related traits overlapped with QTLs for root traits indicate that DT and DA had distinct genetic bases.

It is obvious that, the physiology and molecular biology information of rice stress tolerance is beneficial the biotechnological improvement of rice productivity. Of which, DNA biotechnology is becoming more prevalent by its great contribution in this field. The progress of developing rice varieties for the unfavorable areas has proved that the modern breeding tools can address many problems of farmers. The development of the high-yielding drought-tolerant rice varieties is considered the most economical mode, which enhances and stabilizes the productivity of drought contaminated areas. Recent

advances in plant breeding with the development of agricultural technologies have provided numerous tools for breeders to improve phenotypic screening, ranging from marker-assisted selection (MAS) of key traits to molecular breeding (MB) and genetic engineering. To be more specific, markers are used for selecting qualitative as well as quantitative traits. The number of empirical researchers applied broadly those tools in improving tolerant rice cultivars to drought stress in China, India, and Thailand. The important prerequisites for successful selection of the early generation with MAS are the population sizes and heritability levels of the selected traits. From conventional breeding techniques, genetic functional markers could shorten rice varietal development. In other words, this study provided the detailed information on the relative importance of marker-assisted selection of drought tolerance.

In brief, the specific objectives of the study include:

- Selection of parental materials for drought tolerance.
- Detection of differences in quantitative trait loci (QTL) in their relative effect on components of quantitative drought tolerance.
- Development of drought-tolerant populations.

In the first study, the rice germplasms were screened using a total of 165 SSR markers used for characterization and evaluation of genetic diversity. Among them, 73 SSR markers were found to be polymorphic among the accessions. Maximum PIC (polymorphic information content) values were detected in three markers including RM11125, RM21, and RM5629, which were between 0.78 and 0.79. Cluster analysis of microsatellite markers revealed that by a genetic distance of 0.63, the rice varieties were separated into three clusters. In the integration of drought tolerance, agro-morphological

traits, and genetic diversity, four cultivars OM4900, IR78913-B-22-B-B-B, OM6162, and IR75499-73-1-B performed as the most promising parental donors for developing agronomic and drought-tolerant rice.

Secondly, rice germplasms were analyzed both physical and chemical properties. Among 44 rice germplasms, IR79008-B-11-B-B-1 showed overall good physical characteristics (head rice, grain length, grain width, chalkiness), and three varieties (IR75499-73-1-B, OM6162 and OM4900) had good chemical characteristics (amylose content, gel consistency, protein content, gelatinization temperature, and aroma). The important highlight of the study was that progenies of the cross between OM6162/ SwarnaSub1 had a low amylose content, high gel consistency, high protein content, and low chalkiness.

Thirdly, path analysis appears as the best method to evaluate the relationship between yield and relevant traits. Path analysis permits estimation of direct effects of various traits on yield as well as their indirect effects via other components traits. Based on path analysis, traits as the number of filled grains/panicles, the number of filled-grain/panicle, and harvest index had strong and direct positive effect correlation with grain yield. Adaptability of rice to the drought and submergence stresses is the most important objective of the rice breeding program. Additionally, rice yield can be improved with a comprehensive combination of both conventional and molecular breeding techniques. Totally 16 markers were used to screen for parental polymorphism. Polymorphism of parents of 6 pairs of crosses served for backcross. Currently, at least two populations determined the usefulness of this powerful approach to identify associations between traits of interest such as yield potential, drought-tolerant characteristics and

genetic markers using diverse genotypes (OM6162/swanasub1// OM6162 and OMCS2000/IR75499-73-1-B//OMCS2000).

The final study, 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 necessary. Therefore, the objective of this study was to investigate the effect of drought stress on seedling and reproductive stages in the development of drought tolerance. Totally seven markers (RM219, RM201 RM105, RM23602, RM23877, RM24103, and RM328) were used for an identifying to drought tolerance on chromosome 9 for the BC_2F_2 generation of OMCS2000/IR75499-73-1-B//OMCS2000. New breeding BC-derived lines were screened for drought tolerance using phenotyping and molecular markers. Two advanced breeding lines (BC_2F_2-45 and BC_2F_2-54) were adapted to drought stress by both genotypic and phenotypic analysis. The plant height, number of tillers, and filled grain had the positive correlation with yield/hill under drought stress.

In the current study, the results have been obtained from various experiments, it can be concluded that these markers were earlier identified to be linked to drought resistance and yield traits in rice through conventional QTL mapping efforts. This research also successfully introduced both (OM6162/swanasub1//OM6162) and (OMCS2000/IR75499-73-1-B//OMCS2000) populations. This study focused initially on finding the right combination of lines to give high yields under drought stress. Two lines (BC_2F_2 and BC_2F_2-54) provide the urgent objective for breeders released as new cultivars in providing higher incomes to the Vietnam farmers.

Chapter 1

General Introduction

Climate change is likely to adversely impact on rice production in Tropical Asia. There are two broad areas of environmental stresses: abiotic factors (salinity, heat, drought, cold, submergence, radiation, and heavy metals) and biotic factors (pathogens and herbivore) (Gomez, 2013). Especially environmental stresses including heat, cold, drought, and salinity factors extremely affected by average agriculture yield in the world (Wang et al., 2003). Among this indicator, drought is one of the most common stresses, causing a remarkable loss of crops (Tester and Bacic, 2005).

To be more specific, drought stress is one of the main constraints to rice production and yield stability in rainfed upland ecology. It is estimated that 70% of the yield losses could be due to water scarcity (Bray et al., 2000, Bimpong et al., 2011), which amounts to approximately 27 million ha of rainfed land (IRRI, 2011). Vietnam has been predicted to become one of the most vulnerable countries and significantly impacted countries due to the influences of climate change. According to Wang et al. (2011), the effect of drought on rice plants considerably varies depending on genotypes, developmental stages, and degree and duration of drought stress. Furthermore, grain yield under drought has been reported to be a function of biomass production and harvest index at the vegetative and reproductive stage respectively (Atlin et al., 2008). It was shown that the vigorous growth of roots with an increase in length and thickness is correlated with drought tolerance and grain yield of rice (Jeong et al., 2010). In addition, grain yield under drought stress is a complex quantitative trait whose repeatability is thought to be low relative to yield in non-stress environments, reducing selection efficiency (Venuprasad et al., 2007). Jeong et al. (2010) found that rice

plants significantly enhanced drought tolerance at the reproductive stage, with a grain yield increase of 25 % to 42 % over the controls under field drought conditions. Recent researchers suggested that the grain yield has been to utilize as a direct selection criterion under drought stress (Kumar et al., 2008; Verulkar et al., 2010) instead of indirect selection, which based on secondary traits (Jongdee et al., 2002). However, as yield is a complex trait, there is a necessity for a genetic and physiological analysis of yield contributing traits under drought stress (Sellamuthu et al., 2015).

Currently, several methods have been used to develop drought-tolerant crops: conventional breeding, conventional breeding utilizing marker-assisted selection (MAS) and genetic engineering. In MAS, specific DNA fragments (markers) are identified, which are closely linked to either single genes or to quantitative trait loci (QTLs). The use of DNA markers for screening and selection of plants in a breeding program has many advantages and thus attract plant breeders. Specifically, this method can be employed to detect polymorphism among different genotypes or alleles of a gene for a particular sequence of DNA in a population or gene pool (Andersen, 2013).

In recent years, conventional breeding of drought resistance has become a basic approach and there have been the number of achievements in various crops such as maize (Hoisington et al., 1996), rice (Zhang et al., 2006), and wheat (Zhao et al., 2000). Many genes have been identified to be involved in response to drought stress in plants (Zhang et al., 2012). However, due to irregular rain, rice plants were damaged by drought from seedling, during flowering to maturity stage. The injuries on leaves at seedling stage, sterile spikelet at the reproductive stage and stem shoot under drought stress condition are key traits related to drought tolerance. Therefore, selection of drought-tolerant individuals should not only observe morphological traits but also physiological, biochemical and genotype by

environment interaction.

The fact is that, the genetic bases of drought tolerance (DT) and drought avoidance (DA) at the reproductive stage in rice were analyzed using a combination of an inbred line population from a cross between *indica* lowland and a tropical *japonica* upland cultivar (Yue et al., 2006). A genetic linkage map consisting of 245 SSR markers was constructed for mapping QTL for these traits. A total of 27 QTLs were resolved for 7 traits of the relative performance of fitness and yield, 36 QTL for 5 root traits under control, and 38 for 7 root traits under drought stress conditions. Only a small portion of QTL for fitness- and yield-related traits overlapped with QTLs for root traits indicating that DT and DA had distinct genetic bases (Yue et al., 2006). Salunkhe et al. (2011) reported that SSR marker used for MAS as RM8085 which mapped on chromosome 1 at 139.9 cM linked to leaf rolling and leaf drying under drought stress. According to Shamsudin et al. (2016), the three drought yields including QTLs, *qDTY2.2*, *qDTY3.1*, *and qDTY12.1* consistently effected on grain yield under reproductive stage drought stress in gene pyramiding.

It is obvious that, the physiology and molecular biology information of rice stress tolerance is beneficial the biotechnological improvement of rice productivity (Gomez, 2013). One of the most useful biotechnological tools that were developed throughout the years is DNA-based markers. The progress of developing rice varieties for the unfavorable areas has proved that the modern breeding tools can address many problems of farmers. The development of drought-tolerant rice varieties is considered as the most effective method and economic mode, which enhances and stabilizes the productivity areas prone to drought. Recent advances in plant breeding with the development of agricultural technologies have provided numerous tools for breeders to improve phenotypic screening, ranging from marker-assisted selection (MAS) of key traits to molecular breeding (MB) and genetic

engineering (Collard and McKill, 2008). To be more specific, markers are used for selecting qualitative as well as quantitative traits. To date, previous researchers used these tools to develop enhanced drought tolerance in rice cultivars in China, India, and Thailand (O'Toole, 2004). The important prerequisites for successful selection of the early generation with MAS are the population sizes and heritability levels of the selected traits. From conventional breeding techniques, genetic functional markers could shorten rice varietal development. In other words, this study provided the detailed information on the relative importance of marker-assisted selection of drought tolerance.

In brief, the specific objectives of the study include:

- ✓ Selection of parental materials for drought tolerance.
- ✓ Detection of differences in quantitative trait loci (QTL) in their relative effect on components of quantitative drought tolerance.
- ✓ Development of drought-tolerant populations.

Chapter 2

Correlation Among Agro-morphological Variation and Genetic Diversity of Rice (*Oryza sativa* L.) under Drought Stress

2.1 Abstract

In this study, the correlation coefficients among agro-morphological variation, genetic diversity, and drought tolerance in 44 rice cultivars were analyzed. The drought tolerance at seeding stage (DTS) was significantly proportional to drought tolerance at vegetative stage (DTV) (r = 0.60). In addition, DTS and DTV had strong significant positive correlation to leaf roll (r = 0.87 and 0.54, respectively). Means of unfilled grains and tillering per panicle were proportionally correlated to DTS (r = 0.22 and 0.25, respectively), and DTV (r = 0.20 and 0.36, respectively). However, weight of 1000 grains and filled grains were recorded no correlation to DTS and DTV. At a homologous coefficient of 16.85 integrated from cluster analysis of agro-morphological, quantitative characteristics and drought tolerant scores, the rice cultivars were divided into five groups. Maximum polymorphic information content (PIC) values were detected in three markers including RM11125, RM21, and RM5629, which ranged from 0.78 to 0.79. Cluster analysis of microsatellite markers revealed that by a genetic distance of 0.63, the rice varieties were separated into three clusters. The results provide valuable information for rice breeders to select donors in breeding rice integrated with drought tolerance and good agronomic characteristics.

2.2 Introduction

Rice (*Oryza sativa* L.) is the staple food of more than three billion people in the world. Recent estimates on climate change predict that because of water deficiency, the intensity and frequency of drought are becoming a serious problem for crop production, especially in rice cultivation (Bates et al., 2008; Wassman et al., 2009). Rice is highly sensitive to water stress (O'Toole, 1982; Venuprasad et al., 2007). Due to irregular rain, rice plants can be damaged by drought during seedling, flowering, and maturity stages. The injuries normally occur on leaves at the seedling stage and on sterile spikelets at the reproductive stage. Particularly, at the reproductive stage, floral fertility in rice is extremely sensitive to water deficiency. Conventional plant breeding approaches for yield improvement under drought conditions are time-consuming and laborious because the field conditions are required to be carefully managed (Yoshida and Hasegawa, 1982). Recent studies at the IRRI (International Rice Research Institute) found that there is moderate to high heritability of grain yield under drought stress (Venuprasad et al., 2007; Berneier et al., 2007; Kumar et al., 2008). Basically, the main objectives of a breeding program for drought-tolerant rice are to determine standard tolerant varieties, to identify important traits, and to evaluate the tolerant levels at seedling and reproductive stages. Therefore, the information of agro-morphological and genetic diversity among the drought-tolerant rice varieties to select potential parental donors is essential for breeding programs. Recent advances in molecular biology, principally the development of the polymerase chain reaction (PCR) for amplifying DNA or DNA sequencing has resulted in powerful techniques which can be exploited for screening, characterizing and evaluating genetic diversity of rice. Several types of molecular markers have been extensively applied for evaluating the genetic

variation in rice (Ni et al., 2002). These include restriction fragment length polymorphism (RFLP) (Botstein et al., 1980) random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and microsatellites or simple sequence repeats (SSRs) (Lang, 2002; Land et al., 2009; McCouch, 1988; Temnykh et al., 2000). Commonly, SSR markers have been extensively used in genetic diversity in rice because of their high level of polymorphism to establish relationships among individuals even with fewer markers (McCouch et al., 1997). This study was carried out to assess drought tolerant levels, agro-morphological and quantitative variations, and genetic diversity of 44 rice varieties collected from the Genebank of Cuu Long Rice Research Institute (CLRRI), Vietnam and the International Rice Research Institute (IRRI). The correlation coefficients among them were also analyzed to provide information for breeding drought-tolerant rice integrated with agro-morphological traits.

2.3. Materials and Methods

2.3.1 Plant materials

Forty-four rice varieties were obtained from the Gene Bank of CLRRI and IRRI. They are either drought tolerant or high yield commercial rice, and selected from preliminary experiments.

2.3.2 Screening of drought tolerance

The experiment of drought tolerance evaluation was laid out in a randomized complete block design with three replications at the reproductive-stage under drought stress (Figure 2.1). These seeds were soaked, germinated in plastic trays in an incubator. Each experimental plot included 30 m^2/ variety. After 15 days, they were transplanted into basins which were built by cement. The row- to- row and plant-to-plant spacing 20 cm x 15 cm was maintained. Ten days after transplanting, the drainage through drain taps was set up, without

provided water until flowering. Fertilizer was applied at rate 100-40-30 kg N-P_2O_5-K_2O ha^{-1}. The drought tolerance of rice was evaluated following the standard evaluation system from IRRI (IRRI, 1996) with scores 0-3: tolerance and score 5-9 for susceptible and agro-morphological characters. Finaly, grain yield was recorded (Table 2.1).

Figure 2.1 Screening for drought tolerance at seedling stage.

Table 2.1 *Leaf rolling level.*

Score	Description
0	Leaves healthy.
1	Leaves starts to fold.
3	Leaves folding (deep V- shaped)
5	Leaves fully cupped (U- shaped)
7	Leaves margins touching (O-shaped)
9	Leaves tightly rolled

2.3.3 Agro-morphological character evaluation

The agronomic characters and quantitative traits, including panicle length (cm), panicles per plant, 1000-grain weight (g), days to maturity, filled grains, unfilled grains, and yields, were recorded from all treatments. The yields were determined by the following formula:

Yield = weight of harvest grain (g)/no. of hills x no. of possible hills x MF

Where: $MF = \frac{100 - MC}{86}$ of the harvest grains

Moisture content (MC) per plot was determined immediately after weighing using a moisture meter.

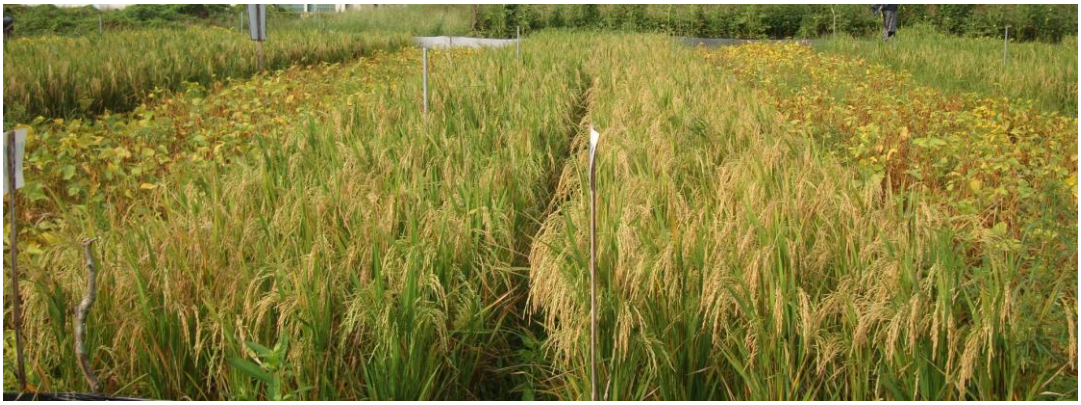


Figure 2.2 Selection for grain yield at reproductive stage under drought stress.

2.3.4 Evaluation of genetic diversity using SSR markers

DNA extraction was prepared according to a method described by McCouch et al. (1997). 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 adding 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 replaced 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 TE buffer.

Microsatellite primers were used to analyze the genetic polymorphism among the samples. A total of 165 primers were randomly selected from the currently available microsatellite markers currently for rice (Temnykh et al., 2000). The PCR reactions were conducted with mineral oils and they were processed in a Programmable Thermal Controller programmed for 35 cycles of 1 min at 94 $^{\circ}$C, 1 min at 55 $^{\circ}$C and 2 min 72 $^{\circ}$C, with a final extension at 75 $^{\circ}$C for 5 min. After amplification, 10 µl of stop solution was added to the PCR products, which was then denatured at 94° C for two min. Eight microliters of each reaction were run on polyacrylamide gel for observing the amplified DNA bands.

DNA band detection and scoring were conducted as follows: plates were separated using a plastic wedge and removed from the tank. The polyacrylamide gel was soaked in ethidium bromide staining solution for 15 to 20 min. Bands in the stained gels were detected and photographed under UV light. Allelic bands were scored as 1 or 0 for presence or absence, respectively. Pair-wise comparisons of the lines based on the presence or absence of unique and shared polymorphic products were used to calculate genetic similarity coefficients. Similarity of coefficients was calculated using (Nei and Li, 1979) distance measure in the NTSYS–PC (Numerical Taxonomy and Multivariate Analysis System (Rohlf, 1990). The lines were clustered using the unweighted pair group method using an arithmetic averages (UPGMA) clustering algorithm.

2.3.5 Data analysis

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 over other. Correlation among agro-morphological traits was calculated by using an SAS program.

In this research, all trials were conducted three times in a completely randomized complete block design. Cluster analysis was carried out for an agro-morphology–based genetic distance matrix using an UPGMA clustering method in the NTSYS program. The distance matrix was calculated by means of Euclidean Distance Coefficient (Sneath and Sokal, 1973):

$$Eij = [\sum_k (X_{ki} - X_{kj})^2]^{1/2}$$

Where: Eij = 0 to ∞, the larger the value, the more distant degrees of relationship; Xi and Xj are the standardized values for the ith and jth characters in kth varieties.

Polymorphic information content (PIC) value that provides an estimate of the discriminatory power of a locus or loci, by considering not only the number of alleles expressed, but also relative frequencies of those alleles, was estimated using the formula suggested by Nei (1973):

$$PIC = 1-\sum x^2k$$

Where, x^2k represents the frequency of the kth allele.

2.4. Results

2.4.1 Evaluation of drought tolerance

The drought tolerant levels are presented in Table 2.2. One of the most important characters of drought tolerance evaluation is leaf roll (LR), which was scored from 0-9.

There were 38 varieties, which obtained LR scores of 0-3, whereas 6 varieties had LR scores of 5-9. Regarding drought-tolerance at the seedling stage, 36 varieties were in the 0-3 score range, 14 varieties scored 5, whereas 4 varieties were in the 7-9 score range. At the vegetative stage, the number of susceptible varieties slightly increased.

Table 2.2 *Results of drought-tolerant evaluations of 44 rice lines/varieties*.

No.	**Name of line/variety**	**LR Score**	**DTS score**	**DTV score**	**Origin**
1	OM4900	3	1	3	CLLRI[1]
2	OM1490	9	7	7	CLLRI
3	AS996	1	3	5	CLLRI
4	M362	0	0	5	IRRI[2]
5	Basmati	1	3	7	IRRI
6	Basmati DB	3	5	5	IRRI
7	OM6162	3	5	3	CLLRI
8	SwarnaSub1	7	9	9	IRRI
9	IR 64Sub1	5	9	7	IRRI
10	IRGA318-11-6-9-2B	3	5	7	IRRI
11	IR78966-B-10-B-B-B-2	1	1	3	IRRI
12	IR78913-B-10-B-B-B	3	5	5	IRRI
13	IR75499-73-1-B	0	0	0	IRRI
14	IR78913-B-19-B-B-B	3	5	5	IRRI
15	Azucena	3	1	5	IRRI

Table 2.2 *Continue*

No.	Name of line/variety	LR Score	DTS score	DTV score	Origin
16	IR78933-B-24-B-B-2	0	1	5	IRRI
17	IR78933-B-24-B-B-3	1	1	5	IRRI
18	IR78933-B-24-B-B-4	0	0	3	IRRI
19	IR79008-B-11-B-B-1	5	5	3	IRRI
20	IR75499-38-1-B	0	0	5	IRRI
21	V3M-92-1	0	0	1	IRRI
22	IR75499-21-1-B	0	0	1	IRRI
23	V3M-109-2	0	0	1	IRRI
24	WAB272-B-B-8-H1	0	0	3	IRRI
25	WAB340-B-B-2-H2	1	1	3	IRRI
26	WAB176-42-HB	1	1	3	IRRI
27	IR78937-B-20-B-B-1	5	5	7	IRRI
28	WAB880-1-38-18-20-P1-HB	1	5	0	IRRI
29	WAB881SG9	1	1	3	IRRI
30	IR78997-B-16-B-B-B-SB2	0	0	1	IRRI
31	IR78966-B-10-B-B-B-SB1	0	1	3	IRRI
32	IR78944-B-8-B-B-B	3	5	5	IRRI
33	IR78941-B-16-B-B-B	3	3	3	IRRI
34	IR78948-B-21-B-B-B	1	1	1	IRRI
35	IR78942-B-2-B-B-2	1	3	5	IRRI

Table 2.2 *Continue*

No.	Name of line/variety	LR Score	DTS score	DTV score	Origin
36	IR78937-B-20-B-B-3	1	3	7	IRRI
37	IR78985-B-13-B-B-B	1	3	7	IRRI
38	IR78933-B-24-B-B-1	3	5	7	IRRI
39	WABC165	3	5	5	IRRI
40	IR80315-49-B-B-4-B-B-B	3	5	7	IRRI
41	IR78966-B-16-B-B-B	0	1	3	IRRI
42	IR78913-B-22-B-B-B	1	5	3	IRRI
43	OMCS2000	9	7	5	CLRRI
44	IR78939-B-9-B-B-B	3	5	5	IRRI

CLRRI: Cuu Long Rice Research Institute; IRRI: International Rice Research Institute

The record plant recovery for each entry following 0-9 score of the standard evaluation system (IRRI,1996) (0: 3 tolerance; 5- 9: susceptible)

2.4.2 Variance of agro-morphological characters

The agro-morphological characteristics were evaluated as shown in Table 2.3 and 2.4. The growth duration (GD) varied from 85 to 140 days. Seven rice varieties had short GD < 90 days. The plant height ranged from 96.7 to 142.0 cm. Three varieties had the low plant heights < 100 cm including OM1490, OMCS2000 and AS996.

Table 2.3 *Descriptive statistics of quantitative traits among 44 lines/varieties.*

Traits	**Max**	**Min**	**Mean**	**CV**	**P**
Growth duration (day)	140	85	103.02		<0.05
Plant height (cm)	142	96.7	116.98	1.4	<0.05
No. of panicles	17.6	4.3	11.57	12.0	<0.05
Filled grains/panicle	212.5	99.4	150.15	9.5	<0.05
Unfilled grains/panicle	62.3	4.6	20.41	8.7	<0.05
Grain weight 1000 (g)	28.6	23.4	26.58	2.2	<0.05
Yield (ton/ha)	8.6	3.5	5.59	9.6	<0.05

Significantly different at P< 0.05

The number of panicles per hill ranged from 4.4 to 17.6. There were 4 varieties with maximum numbers of panicles per hill, ranging from 15 to 17.6. The number of filled grains per panicle varied highly among varieties. Seven cultivars had filled grains per panicle < 115, whereas there were 20 varieties which showed filled grains per panicle > 150. The rate of the unfilled grains per panicle ranged from 4.6 to 62.3%. The low rate of unfilled grain/panicle (from 5-10%) comprised of 2 varieties: V3M-109-2, and WAB272-B-B-8-H. There were 22 varieties with the average rate of unfilled grain/panicle (from 10-20%). The rest of the varieties showed a very high rate of unfilled grain/panicle were very high (more than 30%): Basmati, M362, Basmati DB, IR64Sub1, OM1490, and OMCS2000. The weight of 1000 grains ranged from 23.4 to 28.6 g, and the weight varied among cultivars. There were 17 cultivars with a weight of 1000 grains > 27 g. The productivity ranged from 3.5 to 8.6 tons/ha (Table 2.3). Specifically, there were 16 varieties with the yield from 3 to 5 tons/ha, 21 varieties with the yield from 5 to 7 tons/ha. Seven lines/ varieties had high yield (more than 7

tons/ha) including OM4900, V3M-92-1, IR78985-B-13-B-B-B, WAB881SG9, IR78913-B-22-B-B-B, IR78997-B-16-B-B-B-SB2, and IR75499-73-1-B.

Table 2.4 *Grain yield characters of rice lines/varieties evaluated under drought stress at reproductive*.

No.	Lines/varieties	GD (day)	PH (cm)	No. of P	FG/P	UG (%)	GW (g)	Yield (ton/ha)
1	OM4900	100	109.5 lm	14.1 b-e	209.8 ab	14.2 jk	26.6 e-l	7.2 bcd
2	OM1490	85	96.7 n	14.7 bc	122.3 m-q	51.2 b	26.3 h-l	4.1 mno
3	AS996	85	97.5 n	13.9 b-f	146.9 g-m	28.5 ef	27.5 a-g	5.2 h-l
4	M362	106	113 ijk	5.2 mn	141.8 i-n	32.1 de	26.6 e-l	5.6 g-k
5	Basmati	120	133.9 b	9.4 i-l	155.3 e-k	31.2 de	28.2 abc	4.8 j-n
6	Basmati DB	100	111.5 jkl	11.2 f-i	146.6 g-m	33.4 cd	27.3 b-i	5.2 h-l
7	OM6162	95	108 m	9.2 i-l	127.1 l-q	10.4 lm	28.6 a	6.7 c-f
8	Swarna Sub1	114	116.5 e-h	13.8 b-g	155.5 e-k	12.6 jkl	26.4 f-l	4.2 l-o
9	IR64Sub1	105	109.7 lm	11.3 e-i	147.1 g-m	36.5 c	26.5 f-l	6.2 d-h
10	IRGA318-11-6-9-2B	110	125.9 c	10.1 ijk	186.3 a-d	29.7 ef	28.4 ab	5.9 e-i
11	IR78966-B-10-B-B-B-2	105	124 cd	13.7 b-g	145.6 g-m	24.5 ghi	26.7 d-k	6.3 d-g
12	IR78913-B-10-B-B-B	105	117 ef	11.7 d-i	174.6 c-f	23.1 hi	27.4 a-h	5.9 e-i
13	IR75499-73-1-B	100	114.5 f-i	17.6 a	200.5 abc	24.6 ghi	27.2 b-i	8.6 a
14	IR78913-B-19-B-B-B	120	116 e-i	7.4 klm	144.4 h-m	21.3 i	28.4 ab	4.1 mno
15	Azucena	102	115.7 e-i	10.6 hij	187 a-d	26.5 fgh	26.3 h-l	6.8 cde
16	IR78933-B-24-B-B-2	140	111.7 jkl	11.5 d-i	165.7 d-h	24.5 ghi	25.9 jkl	4 no
17	IR78933-B-24-B-B-3	100	131.2 b	15.9 abc	174.6 c-f	27.3 fg	26.7 d-k	4.2 l-o
18	IR78933-B-24-B-B-4	105	123.7 cd	9.2 i-l	183.1 b-e	22.3 i	26.5 f-l	4.2 l-o
19	WAB326-B-B-7-H1	100	116.7 efg	10.1 ijk	148 g-m	24.1 ghi	26.7 d-k	5.2 h-l

Note: GD: Growth duration day; Plant height: PH; Tilling/P: Tilling/Panicle; FG/P: Filled grain; UG: Unfilled grain

Table 2.4 *Continue*

No.	Lines/varieties	GD (day)	PH (cm)	No. of P	FG/P	UG (%)	GW (g)	Yield (ton/ha)
20	IR79008-B-11-B-B-1	85	115.5 e-i	9.2 i-l	159.9 d-j	21.3 i	25.4 lm	6.7 c-f
21	IR75499-38-1-B	90	116.9 ef	10.7 hij	141.3 i-n	12.1 j-m	26.8 d-k	6.8 cde
22	V3M-92-1	90	125.1 cd	9.1 i-l	135.3 i-o	10.3 lm	27.4 a-h	7.2 bcd
23	IR75499-21-1-B	86	141.5 a	10.1 ijk	158.7 d-j	12.3 jkl	27.9 a-d	6.5 c-g
24	V3M-109-2	104	109.1 lm	10.7 hij	110.3 opq	4.6 n	26.5 f-l	5.2 h-l
25	WAB272-B-B-8-H1	140	122.1 d	16.4 ab	173.4 c-g	8.6 m	28.4 ab	4.1 mno
26	WAB340-B-B-2-H2	140	142 a	11 g-j	122.4 m-q	13.5 jkl	26.9 d-k	4.2 l-o
27	WAB176-42-HB	140	117.9 e	11.3 e-i	119.6 l-q	15.6 j	27.8 a-e	4.6 k-n
28	IR78937-B-20-B-B-1	100	116.9 ef	8.3 jkl	113.2 n-q	14.6 jk	24.3 lm	3.5 o
29	WAB880-1-38-18-20-P1-HB	85	115.9 e-i	7.1 lmn	131.2 k-p	12.3 jkl	27.9 a-d	6.6 c-g
30	WAB881SG9	99	116.7 efg	4.3 n	168.3 d-h	13.5 jkl	24.3 lm	7.4 bc
31	IR78997-B-16-B-B-B-SB2	112	115.8 e-i	9.4 i-l	103.8 pq	14.6 jk	27.6 a-f	8.2 ab
32	IR78966-B-10-B-B-B-SB1	90	123.5 cd	11.1 f-j	111.1 opq	12.6 jkl	25.8 kl	4.9 i-n
33	IR78944-B-8-B-B-B	105	115.8 e-i	14.9 abc	113.9 n-q	11.5 klm	27.1 c-j	4.2 l-o
34	IR78941-B-16-B-B-B	110	113.5 g-k	13.7 b-g	99.4 q	13.2 jkl	23.4 m	5.1 i-m
35	IR78948-B-21-B-B-B	105	115.9 e-i	11.6 d-i	103.6 pq	14.2 jk	25.4 lm	4.1 mno
36	IR78942-B-2-B-B-2	105	116.9 ef	14.3 bcd	122.2 m-q	23.5 hi	26.2 i-l	4 no
37	IR78937-B-20-B-B-3	90	111.1 klm	11.7 d-i	144.9 h-m	22.1 i	26.4 g-l	4.2 l-o
38	IR78985-B-13-B-B-B	85	113.9 f-k	14.9 abc	161.3 d-i	12.3 jkl	27.8 a-e	7.2 bcd
39	IR78933-B-24-B-B-1	95	122.8 cd	13.9 b-f	173.5 c-g	14.4 jk	23.5 m	5.2 h-l

Note: GD: Growth duration day; Plant height: PH; Tilling/P: Tilling/Panicle; FG/P: Filled grain; UG: Unfilled grain

Table 2.4 *Continue*

No.	Lines/varieties	GD (day)	PH (cm)	No. of P	FG/P	UG (%)	GW (g)	Yield (ton/ha)
40	WABC165	100	123.3 cd	15 abc	155 e-k	12.6 jkl	28.4 ab	6.2 d-h
41	IR80315-49-B-B-4-B-B-B	95	109 lm	14.8 abc	173.6 c-g	24.1 ghi	23.4 m	5.7 f-j
42	IR78966-B-16-B-B-B	100	122.2 d	6.7 lmn	150 f-l	12.3 jkl	23.5 m	6.2 d-h
43	IR78913-B-22-B-B-B	95	113.3 h-k	14.7 bc	212.5 a	11.5 klm	26.5 f-l	7.5 bc
44	OMCS 2000	90	97 n	13.4 c-h	186 a-d	62.3 a	26.5 f-l	6.2 d-h
	CV (%)		1.4	12	9.5	8.7	2.2	9.6

Note: GD: Growth duration day; Plant height: PH; Tilling/P: Tilling/Panicle; FG/P: Filled grain; UG: Unfilled grain

2.4.3 Correlation among agro-morphological traits and drought tolerance

The correlation coefficients among agro-morphological traits, drought tolerance at seedling stage (DTS), and drought tolerance at vegetative stage (DTV) are shown in Table 2.5.

Table 2.5 *Correlation coefficients for among agro-morphological traits.*

Traits	GD	PH	Tilling/P	FG/P	UG	1000 w	Yield	LR	DTS	DT V
GD (day)	1									
PH (cm)	0.32ns	1								
Tilling/P	0.02ns	-0.16ns	1							
FG/P	-0.08ns	0.01ns	0.25ns	1						
UG (%)	-0.11ns	-0.41*	0.10ns	0.23ns	1					
1000 w	0.14ns	0.14ns	0.06ns	0.06ns	0.03ns	1				
Yield (t/ha)	-0.42*	-0.08ns	-0.08ns	0.37ns	-0.10ns	0.12ns	1			
LR (score)	-0.04ns	-0.29ns	0.29ns	0.13ns	0.22ns	0.13ns	-0.10ns	1		
DTS (score)	-0.02ns	-0.28ns	0.22ns	0.02ns	0.25ns	0.02ns	-0.17ns	0.87**	1	
DTV (score)	0.13ns	-0.16ns	0.20ns	0.07ns	0.36ns	0.24ns	-0.32ns	0.54*	0.60**	1

Note: DT: drought-tolerance; ns: not significant; DTV: DT at vegetative stage; DTS: DT at seedling stage; GD: Growth duration day; Plant height: PH; Tilling/P: Tilling/Panicle; FG/P: Filled grain; UG: Unfilled grain; 1000 w: 1000 weight; LR: leaf roll; Means with * and ** are significantly different at $P< 0.05$ and 0.01, respectively

It was found that neither DTS nor DTV correlated with filled grains and weight of 1000 grains. Leaf roll values strongly correlated with DTS and DTV ($r = 0.87$ and 0.54, $P< 0.01$, 0.05 respectively), indicating that it should be used as an index for drought tolerance of rice. A negative significant correlation was observed in growth duration x yield ($r = 0.42$). DTS was also found to correlate significantly with DTV ($r = 0.60$). Despite the correlation

coefficients not being markedly different, DTS and DTV were proportional to unfilled grains and tilling per panicle, while they were negatively proportional to rice yield and plant height.

2.4.4 Cluster analysis among 44 varieties based on phenotype

The value of ten agro-morphological characters including seven yield component traits and three parameters of drought tolerance evaluation were used to carry out the analysis. The detailed result is shown in Figure 2.3. The similarity coefficient of the group was ranging from 2.75 to 21.55. At homologous coefficient of 16.85 was the lines/varieties were divided into five main clusters marked A, B, C, D, and E.

At similarity coefficient of 12.15, cluster A was separated into two sub-clusters, A1 and A2. Sub-cluster A1 included two varieties WAB176-42-HB and WAB 340-B-B-2-H2. These varieties had a long GD of 140 days, number of panicles/hill 11 to 12, the number of filled grains/panicle from 122 to 120, rate of unfilled grains/panicle from 13 to 16%, the weight of 1000 grains from 27 to 28 g, yield from 4.2 to 4.6 tons/ha, level of leaf roll from score 1 to score 5, level of drought tolerance at seedling stage from score 1 to score 3, and level of drought tolerance at vegetative stage from score 3 to score 7 (Tables 2.2, 2.4; Figure 2.3). Sub-cluster A2 comprised of eight cultivars. This cluster had GD from 90 to 110 days, plant height from 109 to 123 cm, yield from 4 to 8 tons/ha, level of leaf roll from score 1 to score 3, level of drought tolerance at seedling stage from score 1 to score 3, and level of drought tolerance at vegetative stage (from score 0 to 7) (Tables 2.2, 2.4; Figure 2.3).

Cluster B was divided into two sub-clusters as cluster B1 and B2. Sub- cluster B1 consisted of two varieties, this group had GD 140 days, plant height from 111 to 122 cm, number of panicles/hill from 11 to 16, number of filled grains/panicle from 165 to 173, rate of unfilled grains/panicle from 8 to 25%, weight of 1000 grains from 26 to 28 g, yield of 4 tons/ha, level of leaf roll from score 0 to score 1, level of drought tolerance at seedling score

1, and the level of drought tolerance at vegetative stage from score 3 to score 5 (Tables 2.2, 2.4; Figure 2.3). As well cluster B2 had yield 4 to 7 tons/ha, GD from 85 to 90 days, level of leaf roller from score 0 to score 3, level of drought tolerance at seedling stage from score 0 to 3, and level drought tolerance at vegetative stage from score 1 to 7 (Tables 2.2, 2.4; Figure 2.3).

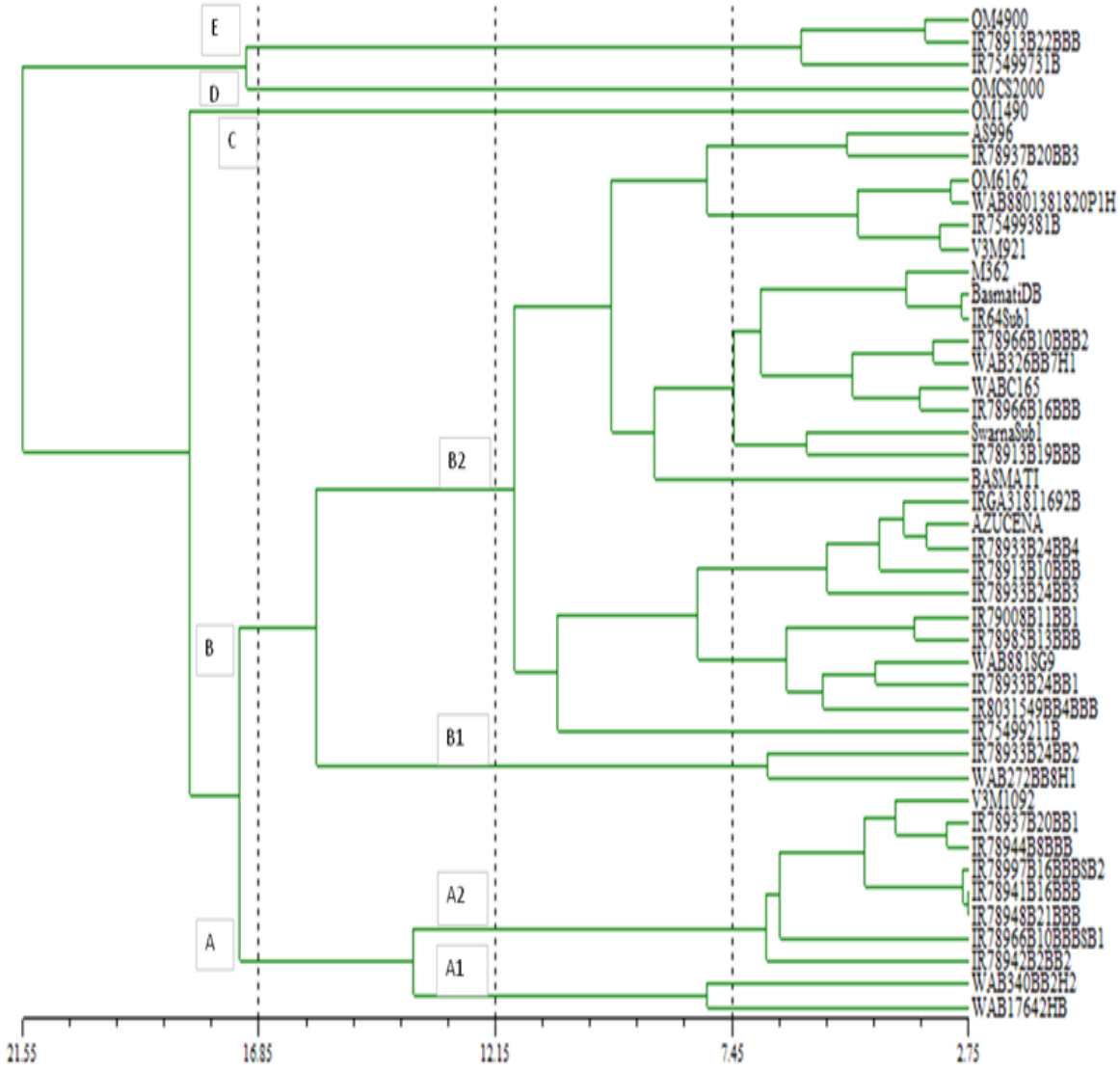


Figure 2.3 Dendrogram of cluster analysis of 44 rice lines/varieties using UPGMA method based on both agro-morphiological characters and drought tolerant scores.

Cluster C contained only one variety (OM1490). This variety had low growth time (85 days) low yield (4.1 tons/ha), level of leaf roller (score 9), drought tolerance at seedling (score 7), level of drought tolerance at vegetative (score 7). Similarly, cluster D had one variety (OMCS2000) with low growth time (90 days), the average yield (6.2 tons/ha), level of drought tolerance at seedling (score 3), level of dried leaf (score 3), level of drought tolerance at vegetative (score 5) (Figure 2.3).

Cluster E included 3 varieties (OM4900, IR78913-B-22-B-B-B, and IR75499-73-1-B) and in this group had GD ranged from 95 to 100 days, the plant height from 110 to 115 cm, number of panicles/hill from 14 to 18, number of filled grains from 200 to 215, rate of unfilled grains/panicle from 12 to 25%, weight of 1000 grains from 26 to 27 g, yield from 7.2 to 8.6 tons/ha), level of leaf roll from score 0 to 9, level of drought tolerance at seedling stage from score 0 to 7, and level of drought tolerance at vegetative stage from score 0 to 5 (Tables 2.2, 2.4; Figure 2.3).

2.4.5 Polymorphism of microsatellite markers

The result of microsatellite analysis showed that there were 73 out of 165 SSR markers found to be polymorphic. The number of amplified fragments ranged from 2 to 9 alleles, therefore the average number of alleles was 5.12 per locus. Three primers RM10890, RM21539 (chromosome 7), and RM222 (chromosome 10) produced the highest number of alleles (9). The lowest PIC values were noted in the primer RM5908 (0.23), followed by RM252 (0.30). Whereas, the highest values were observed in the primer RM11125 (0.79), followed by the RM21 and RM5629 (0.78) (Table 2.6).

Table 2.6 *Results of polymorphic analysis based on SSR markers.*

No.	Primer	Chr	No. of alleles	Size (bp)	PIC value	No.	Primer	Chr	No. of alleles	Size (bp)	PIC value
1	RM105	9	5	210-215	0.46	38	RM154	2	9	160-180	0.71
2	RM10115	1	4	240-250	0.49	39	RM231	3	6	200-210	0.67
3	RM243	1	3	190-210	0.45	40	RM21539	7	9	205-210	0.45
4	RM10649	1	6	180-210	0.45	41	RM122	5	4	205-230	0.64
5	RM24	1	4	200-205	0.63	42	RM510	6	4	220-230	0.42
6	RM7643	1	4	205-220	0.66	43	RM547	8	5	200-210	0.49
7	RM472	1	3	210-242	0.64	44	RM23662	9	8	210-220	0.64
8	RM11125	1	5	160-200	0.79	45	RM219	9	7	200-215	0.65
9	RM10843	1	5	180-200	0.73	46	RM24013	9	8	215-220	0.42
10	RM3412b	1	6	190-200	0.64	47	RM3	6	7	220-225	0.5
11	RM10793	1	6	210-220	0.63	48	RM223	8	8	200-210	0.46
12	Salt 1	1	4	200-220	0.74	49	RM315	1	5	210-230	0.49
13	Salt 2	1	2	210-220	0.45	50	RM13	5	5	190-210	0.63
14	RM 152	8	3	175-200	0.63	51	RM166	2	6	190-200	0.65
15	RM5806	10	6	210-230	0.66	52	RM140	1	4	200-210	0.63
16	RM5806	10	6	230-250	0.64	53	RM220	1	5	210-220	0.64
17	RM211	2	4	200-215	0.65	54	RM227	3	4	200-220	0.65
18	RM17	12	4	160-190	0.79	55	RM148	3	6	190-210	0.43
19	RM310	8	5	200-210	0.72	56	RM471	4	5	213-250	0.6

Chr: Chromosome PIC: Polymorphic Information Content

Table 2.6 *Continue*

No.	Primer	Chr	No. of alleles	Size (bp)	PIC value	No.	Primer	Chr	No. of alleles	Size (bp)	PIC value
20	RM27877	12	6	215-240	0.63	57	RM252	4	5	200-215	0.5
21	RM221	2	5	220-230	0.66	58	RM1155	4	3	200-245	0.4
22	RM28746	12	2	200-210	0.63	59	RM279	2	4	200-263	0.5
23	RM5436	7	3	200-210	0.73	60	RM555	2	2	190-240	0.3
24	RM3867	3	7	210-230	0.74	61	RM71	2	3	210-230	0.56
25	RM6329	3	5	220-230	0.64	62	RM324	2	6	210-235	0.54
26	RM249	5	5	210-230	0.64	63	RM418	7	4	200-215	0.56
27	RM5626	3	5	200-210	0.78	64	RM455	7	5	200-245	0.58
28	RM18	7	4	190-200	0.64	65	RM125	7	6	200-215	0.56
29	RM21	11	4	210-220	0.78	66	RM8300	9	7	200-417	0.54
30	RM163	5	3	255-260	0.45	67	RM24712	9	4	200-332	0.75
31	S11049	11	6	200-210	0.74	68	RM222	10	9	180-245	0.79
32	RM140	1	8	190-200	0.61	69	RM590	10	5	185-236	0.23
33	RM169	5	8	240-250	0.73	70	RM2010	2	2	180-245	0.56
34	RM9	1	6	230-240	0.49	71	RM1024	2	3	190-255	0.45
35	RM10852	1	3	220-230	0.64	72	RM7396	4	6	200-265	0.63
36	RM10890	1	9	205-510	0.66	73	RM463	12	4	210-225	0.52
37	RM10927	1	7	240-245	0.4	Average			5.12		

Chr: Chromosome PIC: Polymorphic Information Content

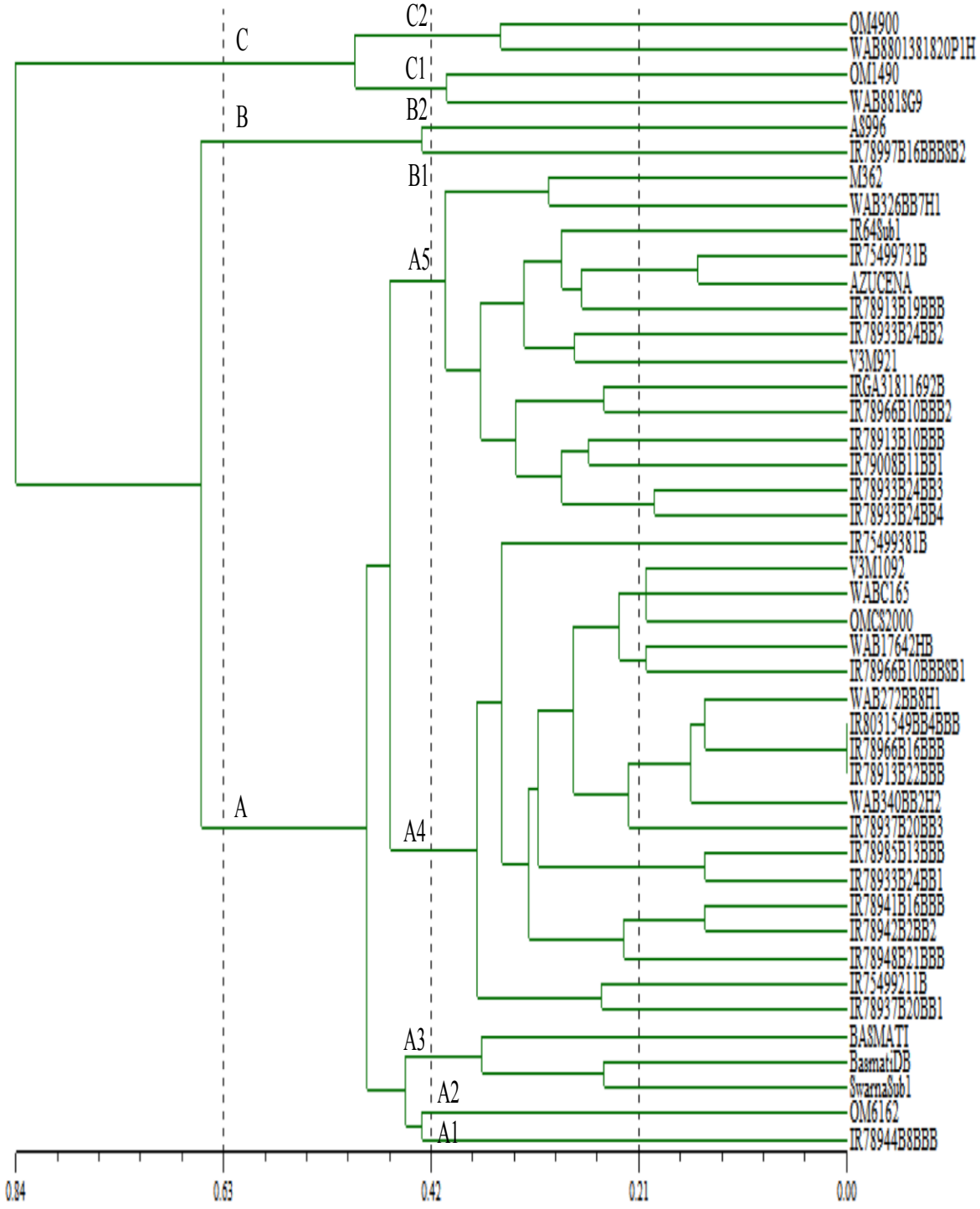


Fig. 4. Cluster dendrogram of 44 rice lines/varieties by the genetic distance of using UPGMA method with the SAHN based on SSR markers.

Figure 2.4 Cluster dendrogram of 44 rice lines/varieties by the genetic distance of using UPGMA method with the SAHN based on SSR markers.

A dendrogram based on cluster analysis using (UPGMA) method with the module of SAHN in the NTSYS-pc package was generated and is showed in Figure 2.4. The genetic variation among rice varieties ranged from 0 to 0.84. At genetic distance of 0.63 there were three major clusters namely, A, B, and C. At genetic distance of 0.42, group A was divided into 5 sub-clusters with group A1 (IR78944-B-8-B-B), group A2 (OM6162), group A3 (Swana Sub1, Basmati DB, and Basmati), group A4 (19 varieties), and group A5 (14 varieties).

At genetic distance of 0.42, group B was separated into two sub-clusters with Group B1 (IR78997-B-16-B-B-B-SB2), and group B2 (AS996). Similarly, group C included two sub-clusters with group C1 (OM1490, and WAB881-SG9), and group C2 (OM4900 and WAB8801381820P1H).

2.5. Discussion

The standard evaluation system of IRRI has been applied to evaluate the drought tolerant level of rice for nearly 20 years with high reliability. The lower of the evaluated scores indicate the higher tolerance levels of rice. Among 44 rice lines/varieties shown in this study, many varieties had the scores between 0 and 3 obtained from IRRI. There were six lines including IR75499-73-1-B, V3M-92-1, IR75499-21-1-B, V3M-109-2, IR78997-B-16-B-B-B-SB2, and IR78948-B-21-B-B-B) achieving high drought-tolerant levels (score from 0 to 1). They are potential parents for breeding of drought-tolerant rice varieties.

Rice is vulnerable to abiotic stress such as drought caused by shortage of water and irregular rain, especially at the reproductive stage. If the duration of the reproductive stage is shorter, the levels of drought stress can be increased (Abarahahr et al., 2011). In several drought stress conditions, the early flowering feature was a very important mechanism to

escape from drought stress (Joongdee et al., 2006). Therefore, selection of drought-tolerant parental varieties for breeding is highly related to agro-morphological characters including pre-mature duration. In this study, maximum values were obtained in IR75499-73-1-B (8.6 t/ha).

Frequency distribution of rice varieties with respect to maturity, plant height, panicles per plant, number of filled grains, number of unfilled grains, 1000 grain weight, yield and level for drought tolerance, showed a great diversity among 44 varieties studied. Phenotypic measurement is very important for identifying QTLs because quantitative traits are much affected by the environment, especially for measuring drought-tolerance (Lang et al., 2009). The variation in agro-morphological characters of rice in this study can provide important information for plant breeders to breed drought-tolerant rice integrated with good agronomic traits.

The genetic basis of drought traits is governed by one recessive allele located on 12 chromosomes. Therefore, application of molecular markers linked tightly to target drought genes is considered as a powerful tool to select drought rice varieties faster and more effective. Studies by Garris et al. (2005) and Ram et al. (2007) showed that the number of alleles per locus, polymorphic information content and gene diversity were 4.8-14.0, 0.6-0.7, and 6.2-6.8, respectively. The average alleles per locus were 3.9 in 416 rice accessions collected from China (Jin et al., 2010).

The PIC values obtained from the markers RM17616 and RM316 were 0.146 and 0.756, respectively, in *Indica* accessions (Nachimuthu et al., 2015). The lowest PIC values were noted in primers RM5908 (0.23), followed RM252 (0.30). The maximum values were at primers RM11125 (0.79), followed by RM21 (0.78) and RM5629 (0.78). The maximum genetic diversity is the most important criteria to select parental varieties for breeding

because it increases the choice of desirable genotypes (Nertan et al., 2007). The findings of this study are similar to previous studies, in which the PIC values were ranging from 0.16 to 0.78 for European Chinese rice collection of 416 accessions (Jin et al., 2010; Courtois et al., 2012). In addition, Chen et al. (2011) screened 300 rice accessions with 372 SNP markers for 0.358 of diversity and found that polymorphicity was 0.285. A gene diversity < 0.68 was reported (Liaket Ali et al., 2011). However, most rice diversity worldwide has the gene diversity of 0.5 to 0.7 (Ni et al., 2002; Garris et al., 2005; Liaket Ali et al., 2011).

2.6 Conclusion

Many among 44 studied rice varieties showed high drought-tolerant levels. They can serve as important parental donors in breeding drought-tolerant rice. In addition, there was a high variation of agro-morphological character and genetic diversity in these 44 cultivars. The detected three clusters of rice in this study are evidence that can form a basis for effort to improve productivity of drought-tolerant rice. In the integration of drought tolerance, agro-morphological traits, and genetic diversity, the three cultivars OM4900, IR78913-B-22-B-B-B, OM6162 and IR75499-73-1-B appear as the most promising parental donors for developing agronomic and drought-tolerant rice.

Chapter 3

Physical - Chemical Characters and Heritability for Yield Components in Rice (*Oryza sativa* L.)

3.1. Abstract

The present study was performed to analyze both physical and chemical properties of rice germplasm and heritability for yield components in combinations. A total of 44 lines/varieties obtained from Cuu Long Rice Research Institute genebank, and 30 F_1 combinations were evaluated. The results showed that the rice line of IR79008-B-11-B-B-1 showed overall good physical characters (head rice, grain length, grain width, chalkiness). In terms of chemical characteristics, the three varieties IR75499-73-1-B, OM6162, and OM4900 had good amylose content, gel consistency, protein content, gelatinization temperature, and aroma. The cross between OM6162/SwarnaSub1 had low amylose content (20.2%), high gel consistency (78.2 mm), high protein content (8.1%), appropriate gelatinization temperature (scale 5), low chalkiness (level 0), high heritability (0.9) for grain yield trait/cluster, and (0.84) for the number of panicles/cluster. The other characters, consisting of plant height, panicle length, number of panicles/cluster, number of filled grains/panicle, number of unfilled/panicle, and grain yield/cluster showed moderate to high heritability of mean for the combination OM6162/SwarnaSub1. The results suggest that the grain yield trait/cluster and the number of panicles/cluster are important factors contributing to yield trait and these information many aid rice breeders in selecting ideal combinations for higher yield and quality of next generations.

3.2. Introduction

Rice is an important staple food for more than half of the world's population, especially in developing countries. For thousands of years, the culture, economy, and history of many countries and regions in the world have been profoundly shaped by this plant. In Vietnam, currently, one of the most important objectives of rice production is to develop new varieties having high quality and highly adapt to climate change. The research strategy in rice breeding is to create of rice varieties with desired traits including long grain, low amylose content (< 20%), less chalkiness, aroma, short growth duration (90-100 days), and stable tolerance to both biotic and abiotic stresses.

Rice grain quality plays an important role in acceptance by consumers and is the second important factor as well as behind high yield in rice breeding objectives (Jewel et al., 2011; Pandey et al., 2014; Ramesh et al., 2000). Furthermore, it determines the market price of rice (Sano et al., 1985). Therefore, evaluation of rice quality involving chemical compositions, cooking quality, gelatinization temperature and physical properties of rice is essential (Zhou et al., 2002). The quality of rice can be categorized into three groups consisting of (1) physical characteristics including moisture content, shape, size, whiteness, translucency, chalkiness, head rice, broken rice, brewers, green kernels and yellow kernels; (2) chemical characteristics including amylose content, protein content, gel consistency, expansion level of cooked rice, water absorption, and cooking time; (3) the sensory aspects of cooked rice including color, aroma, hardness, stickiness, and consistency (Irshad, 2001). The three key components important for cooking and eating are amylose content, gelatinization temperature, and gel consistency. The chemical characteristics which are directly related to cooking and the eating quality include the amylose content (AC),

gelatinization temperature (GT), gel consistency (GC), protein content (PC) and aroma which are directly related to cooking and eating quality (Lise et al., 2000).

Besides the quality of rice, the quantitative trait rice yield is a complex character of any crop. The success of a breeding program depends on the availability on genetic variability in the varieties subjected to selection (Dixit et al., 2014). Genetic variability for agronomic traits is key component of breeding programmes for broadening the gene pool of rice (Akinwale et al., 2011). Rice yield can be improved with different strategies (Wijerathana, 2015). Rice breeding strategies depend on the degree of associated characters as well as its magnitude and nature of variation (Zahid et al., 2006). Commonly, plant breeders select yield components which indirectly increase yield (Akinwale et al., 2011). Moreover, heritability of traits is essential for selection based improvement as it indicates the extent of transmissibility of a character into generations (Sabsan et al., 2009). Therefore, the main objectives of this study were (1) to evaluate the quality levels of physical and chemical characteristics of rice germplasm in order to improve quality of rice cultivars, in which several beneficial traits should be combined; and (2) to estimate the heritability of yield and yield components of F_4 generation.

3.3. Materials and Methods

3.3.1. Plant materials:

Rice germplasms including 44 lines/varieties in the genebank of Cuu Long Delta Rice Research Institute (CLRRI) were used in this study.

The experiment was conducted from 2015 to 2016, in the dry season at Cuu Long Delta Rice Research Institute, Vietnam. When seedlings were 15 days old seedling, each variety was transplanted with one plant per hill in randomized block design with three

replications. The row- to- row and plant-to-plant spaces of 20 cm x 15 cm were maintained. Ten-day after transplanting, the drainage through drain taps was set up; without the water until flowering. The fertilizer was applied at a rate 100-40-30 kg N-P_2O_5-K_2O ha^{-1} for the dry season and 80-40-30 kg N-P_2O_5-K_2O ha^{-1} for the wet season. After harvesting the seeds of each line/variety were dried using solar heat to obtain 14% moisture content, and then dehulled for evaluation of the grain quality in three replications.

The hybrid materials were screened in the field and used for making crosses. Totally, F_1 seeds of 30 combinations were made by a single cross. Base on the data of amylose content (AC), gel consistency (GC), protein content (PC), gelatinization temperature (GT), and aroma were the main major traits for the first step to select combinations.

The F_1-population was developed and F_2 produced. Then F_2 seeds were produced and F_2, F_2 generations selected from 30 combinations during 2015 dry season to 2016 wet season at Cuu Long Delta Rice Research Institute, Vietnam. The F_2 generation was grown and separations selected for generating the F_3 generation, and F_3 plants were selected. F_3 lines were planted to continue to evaluate for the F_4 generation. For the performance test, agronomic characteristics such as plant height, panicle length, number of panicle/cluster, number of filled grain/panicle, number of unfilled grain/panicle, and grain yield/cluster (g) were investigated.

3.3.2. Evaluation of physical characteristics:

Brown rice (BR) and milled rice (MR) ratios were measured by a standard dehusker (Dela Cruz and Khush, 2000). Head rice (HR) was tested by using one hundred grams of dehusked rice grains that had no visible breakage. The percentages of HR and broken rice were then calculated (Dela Cruz and Khush, 2000). The chalk index was determined by

placing ten dehusked rice grains on a light box and visually identifying the chalkiness of each grain. The chalkiness percentage of each rice line/variety was calculated by an average of ten values from ten grains. The following levels were used for classifying endosperm chalkiness of milled rice including level 0 (no chalkiness), level 1 (less than 10% chalkiness), level 5 (10 – 20% chalkiness), and level 9 (more than 20% chalkiness) (Dela Cruz and Khush, 2000). The grain size was measured in length (mm) and width (mm) of the grain.

3.3.3. Evaluation of chemical characteristics:

Amylose content (AC): The amylose contents of different varieties were determined based on a standard graph (Williams 1958; Perez and Juliano 1978). Rice varieties are grouped based on their AC as follows, waxy (0 - 2%), very low (3 - 9%), low (10 - 19%), intermediate (20 - 25%), and high (>25%) by Kumar and Khush, (1996) (Figure3.1).

Figure 3.1 Evaluation of amylose content

Gel consistency (GC): Milled rice samples (10 grains) were ground into a fine powder in the Wig-L Bug grinder. Consistency was measured by the length in a test tube of the cold gel and was held horizontally for 1h after heating in 0.2 N potassium hydroxide (KOH) (2 mL) and categorized as soft (61 - 100 mm), medium (41 - 60 mm), and hard (26 -

40mm) [15] (Figure 3.2).

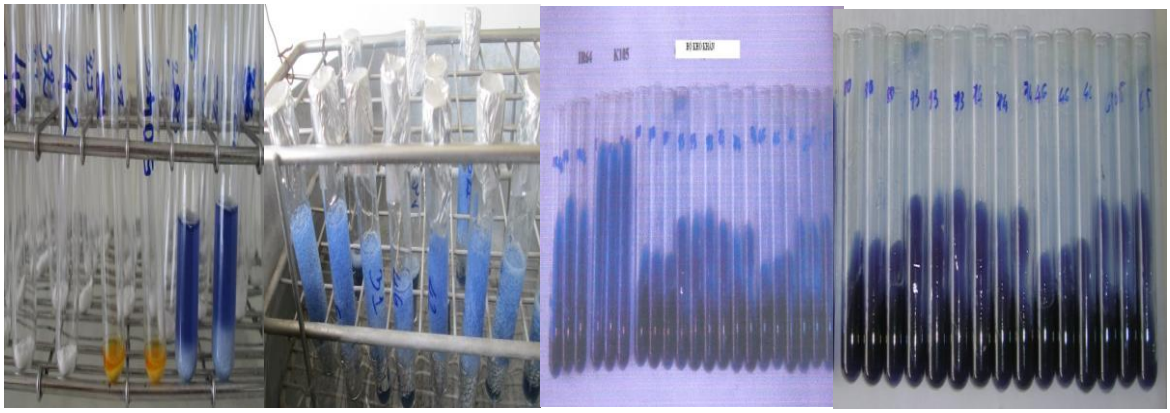


Figure 3.2 Evaluation of gel consistency (GC)

Gelatinization temperature (GT): GT is estimated by the extent of alkali spreading, according to IRRI, (2007). Ten milled rice grains were placed in a Petri dish, and then 10 mL of 1.7% KOH was added. The sample was placed in an incubator at 25 ^{o}C for 24h. The degree of spreading was measured using a 7- point scale as follows: 1 (grain not affected), 2 (grain swollen), 3 (grain swollen, collar incomplete and narrow), 4 (grain swollen, collar complete and wide), 5 (grain split or segmented, collar complete and wide), 6 (grain dispersed, merging with collar), and 7 (grain completely dispersed and intermingled). The scale includes 1-2: high (74.5 - 80 ^{o}C), 3: high intermediate, 4 - 5: intermediate (70 - 74 ^{o}C), and 6 - 7: low (<70 ^{o}C) (Figure 3.3).

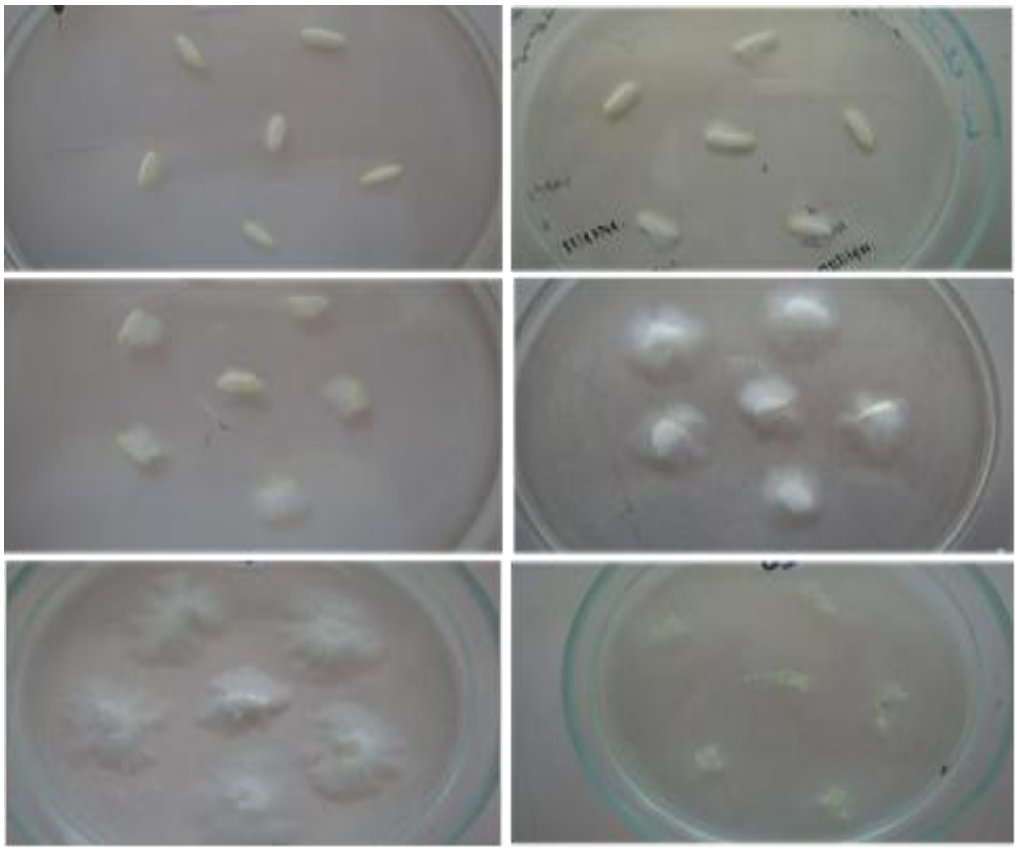


Figure 3.3 Evaluation of gelatinization temperature (GT)

Protein content (PC): Protein content was determined on a dry weight basis based on nitrogen content. The nitrogen content in each grain sample was estimated by the Technicon Autoanalyser (Technicon Instrument Inc. USA). Protein content (%) = Nitrogen content (%) x 5.7, where 5.7 is the conversion factor.

Aroma: Ten leaves of rice were cut into 5 mm long pieces, and then put into a capped test tube. A volume of 5 ml of 1.7 % KOH solution was added and incubated at 50 ^{o}C for 10 min. Five panelists were asked to classify the samples as either aromatic or non-aromatic by their own smell. Aroma classification is defined as score 0 (no aroma), score 1 (slight aroma), score 2 (moderate aroma), and score 3 (strong aroma) (Lang, 2002).

Figure 3.4 Evaluation of aroma

3.3.4. Data analysis

All measurements were conducted in triplicates. An analysis of variance (ANOVA) for all data was performed using the SAS 9.1 software. Broad-sense heritability (h^2) was calculated as the ratio of the genotypic variance to the phenotypic variance using the formula according to Allard, (1960):

$h^2 = \sigma^2 g/\sigma^2 p$

Where h^2 = broad -sense heritability, $\sigma^2 g$ = genotypic variance and $/\sigma^2 p$ = phenotypic variance.

3.4. Results

3.4.1. Physico-chemical characteristics of parental materials

Physical characters: Evaluation of targets grain quality parent materials under drought stress were significantly for the physical-chemical characters. In this study, physical traits were evaluated with described characters in Table 3.1.

The percentage of BR ranged 75.6 to 88%, the lowest was recorded in the line IR78966-B-16-B-B-B and the highest was WAB340-B-B-2-H2. Most of the remaining varieties had the BR percentage higher than 80%.

The percentage of BR ranged from 75.6 to 88%, the lowest was recorded in the line IR78966-B-16-B-B-B and the highest was in the line WAB340-B-B-2-H2. Most of the remaining varieties had the BR percentage higher than 80%. The lowest milling recoveries were observed in four varieties (70.0%) including IR75499-73-1-B, IR75499-21-1-B, WABC165, and IR78966-B-16-B-B-B. The highest was found in WAB340-B-B-2-H2 (77.0%). Forty varieties showed high milling (>70%). HR values ranged from 42.7 to 57.2%. The varieties WAB340-B-B-2-H2 and WAB176-42-HB had the highest head rice and the lowest was found in IR78966-B-16-B-B-B (42%). In this study, the grain length had the highest value in V3M-92-1 (8.4 mm), followed by 22 lines/varieties (7 - 7.9 mm) and the lowest was BASMATI and IR78966-B-10-B-B-B-SB1 (5.2 mm). Among 44 lines/varieties, the chalkiness ranged from level 0 to 5. Most of the lines/varieties had low chalkiness from level 0 to 1 under drought condition, except IR78913-B-10-B-B-B (level 5).

Chemical characters: Amylose content (AC), gel consistency (GC), and gelatinization temperature (GT) are shown in Table 3.2. The results showed that the AC ranged from 12.0 to 27.3 %. The line IR78933-B-24-B-B-2 had the lowest amylose content. There were varieties containing 20 to 25% of AC which is classified as soft and flaky cooked rice. All materials in this study were intermediate when it comes to AC, except IR78937-B-20-B-B-1 (26.3%) and WAB176-42-HB (27.3%).

Table 3.1 *Physical characteristics of rice germplasms.*

No.	Names	Physical characters				
		BR %	MR %	HR %	GL (mm)	Chalkiness (level)
1	OM4900	80.3g	72.0def	52.3cd	6.8a-g	0
2	OM1490	81.5d-g	71.0ef	51.1de	5.3g	1
3	AS 996	80.5fg	70.2ef	51.0de	6.3b-g	0
4	M362	81.2d-g	71.3ef	51.3de	5.6fg	1
5	BASMATI	84.3c	74.2bcd	54.2b	5.2g	1
6	Basmati DB	80.4fg	70.2ef	50.2e	6.4b-g	1
7	OM6162	80.1g	70.1ef	51.2de	6.3b-g	1
8	SwarnaSub1	80.6efg	70.5ef	51.2de	7.9ab	1
9	IR64Sub1	86.2b	75.5ab	55.6ab	7.8abc	1
10	IRGA318-11-6-9-2B	82.3de	72.5cde	52.1d	7.1a-f	1
11	IR78966-B-10-B-B-B-2	82.6cd	72.3c-f	52.1d	7.2a-f	1
12	IR78913-B-10-B-B-B	81.6d-g	71.0ef	51.2de	7.8abc	5
13	IR75499-73-1-B	80.5fg	70.0f	50.2e	7.8abc	0
14	IR78913-B-19-B-B-B	82.3de	72.0def	52.4cd	7.9ab	1
15	AZUCENA	81.3d-g	71.2ef	51.2de	6.8a-g	0

Data within a column by same letter are not significantly different ($P<0.05$); CV: coefficient of variation; BR: brown rice, MR: milled rice, HR: head rice, GL: grain length: GW: grain wide.

Table 3.1 *Continue*

No.	Names	Physical characters				
		BR %	MR %	HR %	GL (mm)	Chalkiness (level)
16	IR78933-B-24-B-B-2	84.2c	74.0bcd	54.2b	6d-g	1
17	IR78933-B-24-B-B-3	86.3ab	72.3c-f	52.3cd	5.9efg	0
18	IR78933-B-24-B-B-4	86.2b	75.6ab	55.6ab	6.6b-g	1
19	IR79008-B-11-B-B-1	86.2b	75.0ab	55.5ab	7.1a-f	0
20	IR75499-38-1-B	82.6cd	72.4c-f	52.3cd	7.5a-e	1
21	V3M-92-1	81.2d-g	71.0ef	51.2de	8.4a	1
22	IR75499-21-1-B	80.6efg	70.0f	50.1e	7.6a-d	1
23	V3M-109-2	84.2c	74.0bcd	54.2b	6.8a-g	0
24	WAB272-B-B-8-H1	84.2c	74.0bcd	51.3de	7.6a-d	1
25	WAB340-B-B-2-H2	88.0a	77.0a	57.2a	7.4a-e	1
26	WAB176-42-HB	87.4ab	76.0ab	56.0a	5.3g	1
27	IR78937-B-20-B-B-1	81.3d-g	71.0ef	51.0de	5.6fg	1
28	WAB880-1-38-18-20-P1-HB	84.2c	74.0bcd	54.0bc	7.1a-f	1
29	WAB881SG9	81.3d-g	71.2ef	51.3de	6.2c-g	1
30	IR78997-B-16-B-B-B-SB2	81.2d-g	71.0ef	54.0bc	7.5a-e	1

Data within a column by same letter are not significantly different ($P<0.05$); CV: coefficient of variation; BR: brown rice, MR: milled rice, HR: head rice, GL: grain length: GW: grain wide.

Table 3.1 *Continue*

No.	Names	Physical characters				
		BR %	**MR %**	**HR %**	**GL (mm)**	**Chalkiness (level)**
31	IR78966-B-10-B-B-B-SB1	84.3c	74.2bcd	54.2b	5.2g	1
32	IR78944-B-8-B-B-B	80.4fg	70.2ef	50.2e	6.4b-g	1
33	IR78941-B-16-B-B-B	80.1g	70.1ef	51.2de	6.3b-g	1
34	IR78948-B-21-B-B-B	80.6efg	70.5ef	51.2de	7.9ab	0
35	IR78942-B-2-B-B-2	86.2b	75.5ab	55.6ab	7.8abc	1
36	IR78937-B-20-B-B-3	82.3de	72.5cde	52.1d	7.1a-f	1
37	IR78985-B-13-B-B-B	82.6cd	72.3c-f	52.1d	7.2a-f	1
38	IR78933-B-24-B-B-1	81.6d-g	71.0ef	51.2de	7.8abc	1
39	WABC165	80.5fg	70.0f	50.2e	7.8abc	1
40	IR80315-49-B-B-4-B-B-B	82.3de	72.0def	52.4cd	7.9ab	1
41	IR78966-B-16-B-B-B	75.6h	70.0f	42.0f	6.2c-g	1
42	IR78913-B-22-B-B-B	81.0d-g	74.0bcd	50.0e	6.6b-g	0
43	OMCS 2000 (check)	80.6efg	76.2ab	51.2de	7.1a-f	0
44	IR78939-B-9-B-B-B	80.5fg	72.5c-f	51.0de	6.3b-g	1
CV%		1.02	1.65	1.62	11.73	-
LSD 0.05		0.36	0.51	0.36	0.35	-

Data within a column by same letter are not significantly different ($P<0.05$); CV: coefficient of variation; BR: brown rice, MR: milled rice, HR: head rice, GL: grain length: GW: grain wide

Table 3.2 *Chemical characteristics of rice germplasms.*

No.	Names	Chemical characters					
		GW (mm)	**AC (%)**	**GC (mm)**	**PC (%)**	**GT (scale)**	**Aroma (score)**
1	OM4900	3.2abc	19.8j-m	82.3d	8.6ab	3	2
2	OM1490	2.8bcd	24.3cd	51.2q	7.6b	3	0
3	AS 996	3.4ab	24.5c	48.7rs	8.9ab	3	0
4	M362	3.0abc	24.1cd	55.2p	8.6ab	3	0
5	BASMATI	3.2abc	22.6d-g	76.3fg	7.93b	3	2
6	Basmati DB	3.2abc	24.3cd	49.3r	8.7ab	3	0
7	OM6162	3.3ab	18.3m	84.2c	8.2ab	3	2
8	SwarnaSub1	3.1abc	24.3cd	49.3r	8.7ab	3	0
9	IR64Sub1	3.2abc	23.5c-f	68.7kl	8.5ab	7	0
10	IRGA318-11-6-9-2B	2.9a-d	24.2cd	48.2rst	7.5b	3	0
11	IR78966-B-10-B-B-B-2	3.0abc	21.0hij	77.5ef	8.3ab	3	0
12	IR78913-B-10-B-B-B	2.3d	20.1jkl	71.0hi	8.5ab	3	0
13	IR75499-73-1-B	3.1abc	18.9klm	78.9e	8.2ab	3	1
14	IR78913-B-19-B-B-B	3.1abc	18.7lm	88.1b	8.4ab	5	0
15	AZUCENA	3.0abc	24.5c	48.9rs	8.4ab	5	1

Data within a column by same letter are not significantly different ($P<0.05$); CV: coefficient of variation; AC: Amylose content; GC: gel consistency; GT: gelatinization temperature; PC: protein content

Table 3.2 *Continue*

No.	Names	Chemical characters					
		GW (mm)	**AC (%)**	**GC (mm)**	**PC (%)**	**GT (scale)**	**Aroma (score)**
16	IR78933-B-24-B-B-2	3.4ab	12.0o	100.0a	8.5ab	3	0
17	IR78933-B-24-B-B-3	3.5a	24.0cde	45.6uv	7.9b	5	2
18	IR78933-B-24-B-B-4	3.2abc	24.5c	52.1q	7.5b	5	1
19	IR79008-B-11-B-B-1	3.3ab	24.2cd	48.3rst	7.9b	7	2
20	IR75499-38-1-B	3abc	20.5ijk	72.5h	8.4ab	5	1
21	V3M-92-1	3.1abc	18.3m	62.8n	7.8b	2	0
22	IR75499-21-1-B	3.2abc	21.2hij	70.3ij	10.3a	2	0
23	V3M-109-2	3.4ab	23.6c-f	65.3m	7.8b	5	2
24	WAB272-B-B-8-H1	3.2abc	16.4n	75.3g	8.5ab	3	0
25	WAB340-B-B-2-H2	3.2abc	24.5c	62.1n	8.1ab	3	0
26	WAB176-42-HB	3.1abc	27.3a	42.8w	7.8b	3	0
27	IR78937-B-20-B-B-1	3.4ab	26.3ab	44.6v	8.2ab	3	0
28	WAB880-1-38-18-20-P1-HB	2.6cd	24.2cd	46.8tu	8.3ab	3	0
29	WAB881SG9	3.2abc	24.1cd	47.6st	8.4ab	3	0
30	IR78997-B-16-B-B-B-SB2	3.2abc	23.5c-f	49.2r	7.6b	3	0

Data within a column by same letter are not significantly different ($P<0.05$); CV: coefficient of variation; AC: Amylose content; GC: gel consistency; GT: gelatinization temperature; PC: protein content

Table 3.2 *Continue*

No.	Names	Chemical characters					
		GW (mm)	**AC (%)**	**GC (mm)**	**PC (%)**	**GT (scale)**	**Aroma (score)**
31	IR78966-B-10-B-B-B-SB1	3.3ab	24.1cd	66.5m	7.6b	3	0
32	IR78944-B-8-B-B-B	3.4ab	25.0bc	60.1o	8.5ab	3	0
33	IR78941-B-16-B-B-B	3.5a	24.5c	66.3m	8.4ab	3	0
34	IR78948-B-21-B-B-B	3.1abc	16.2n	85.4c	8.9ab	5	2
35	IR78942-B-2-B-B-2	3.2abc	24.0cde	48.6rs	7.6b	3	1
36	IR78937-B-20-B-B-3	2.9a-d	23.4c-f	44.5v	7.7b	3	0
37	IR78985-B-13-B-B-B	3.0abc	23.5c-f	69.7i-l	7.8b	3	0
38	IR78933-B-24-B-B-1	2.3d	24.5c	66.4m	8.5ab	1	0
39	WABC165	3.1abc	21.1hij	70.2ijk	8.7ab	3	0
40	IR80315-49-B-B-4-B-B-B	3.1abc	24.7bc	69.4jkl	8.1ab	3	0
41	IR78966-B-16-B-B-B	3.1abc	23.5c-f	66.5m	7.6b	3	0
42	IR78913-B-22-B-B-B	3.4ab	22.1ghi	68.7kl	7.9b	3	0
43	OMCS 2000 (check)	3.2abc	24.1cd	68.5l	8.1ab	3	0
44	IR78939-B-9-B-B-B	3.4ab	24.3cd	48.7rs	7.93b	5	0
CV%		10.72	3.72	1.23	0.26	-	-
LSD 0.05		0.14	0.36	0.33	0.33	-	-

Data within a column by same letter are not significantly different (P<0.05); CV: coefficient of variation; AC: Amylose content; GC: gel consistency; GT: gelatinization temperature; PC: protein content

The highest GC was found in IR78933-B-24-B-B-2 (100 mm) and the lowest was WAB176-42-HB (42.8 mm). There were 28 lines/varieties belonging to the categories soft rice, 19 lines/varieties belonging to medium. The gelatinization temperature (GT) is the temperature at which the starch granule begins to swell irreversibly in hot water with a simultaneous loss of crystalline. In this study, the GT scales had a large variation among lines/varieties which ranged from scale 1 to 7. The highest GT scale was observed in the line IR78933-B-24-B-B-1, and the lowest was IR64sub1. There were 31 varieties which obtained a GT score of 3 and two varieties IR78933-B-24-B-B-1 and V3M-92-10 had score 2. The following lines/varieties had score 5: IR78913-B-19-B-B-B, AZUCENA, IR78933-B-24-B-B-3, IR78933-B-24-B-B-4, WAB326-B-B-7-H1, IR75499-38-1-B, V3M-109-2, and IR78948-B-21-B-B-B. The protein content of 44 lines/varieties ranged from 7.5 to 10.3%. Twenty-six lines/varieties had protein content higher than 8%. The highest protein content was found in IR75499-21-1-B and the lowest was found in the two varieties IRGA318-11-6-9-2B and IR78933-B-24-B-B-4 ($P<0.05$). The results showed that only six varieties obtained moderate aroma, similar to aroma of Basmati including OM4900, OM6162, IR78933-B-24-B-B-3, IR79008-B-11-B-B-1, V3M-109-2, and IR78948-B-21-B-B-B. Five varieties had slight aroma, consisting of IR75499-73-1-B, AZUCENA, IR78933-B-24-B-B-4, IR75499-38-1-B, and IR78942-B-2-B-B-2 and the remaining lines/varieties had no aroma.

The results demonstrated that the line IR79008-B-11-B-B-1 was the best in term of good physical characteristics, such as head rice, grain length, grain width, and chalkiness. The three lines/varieties that came out on top of the chemical characteristics were

IR75499-73-1-B, OM6162, and OM4900. Identifying those lines that exhibit the best characteristics will be useful for improving the quality of future rice breeding programs. This will be helpful in assessing the varietal characters for selecting parents.

3.4.2. Grain quality characteristics of combinations

The grain quality traits of 30 F_1 combinations were evaluated, shown in Table 3.3. The AC of all combinations ranged from 20 to 26.3%. There were six combinations consisting of IR65191-3B-2-2-2-2/IR64Sub1, IR78933-B-24-B-B-3/IR64Sub1, OM6162/SwarnaSub1, WAB326-B-B-7-H1/IR64Sub1, V3M-170-1/IR64Sub1, and IR75499-29-2-B//IR64Sub1 which had low AC (20 - 21.3%).

The majority of combinations had an average GC of more than 40 mm and they were categorized as soft and medium. Total eleven combinations had good GC (71.2 to 85.3 mm) including:

- ✓ IR78933-B-24-B-B-31/IR64Sub1
- ✓ WAB99-47/IR64Sub1
- ✓ OM6162/SwarnaSub1
- ✓ WAB326-B-B-7-H1/IR64Sub1
- ✓ IR78913-B-10-B-B-B/IR64Sub1
- ✓ IR65191-3B-2-2-2-2/IR64Sub1,
- ✓ IR78913-B-19-B-B-B/IR64Sub1,
- ✓ V3M-170-1/IR64Sub1,
- ✓ IR78913-B-19-B-B-B/IR64Sub1,
- ✓ V3M-167-2-B/IR64Sub1,
- ✓ IR75499-21-1-B/IR64sub1

The protein contents of 30 combinations ranged from 6.8 to 8.5%. A high protein content was observed in nine combinations:

- ✓ IR75499-73-1-B/IR64Sub1
- ✓ V3M-103-2/Sawanasub1
- ✓ IR78933-B-24-B-B-4/IR64Sub1
- ✓ WAB326-B-B-7-H1/IR64Sub1
- ✓ IR75499-84-1-B/IR64Sub1
- ✓ V3M-167-2-B/IR64Sub1
- ✓ IR78913-B-19-B-B-B/IR64Sub1
- ✓ IR65191-3B-2-2-2-2/IR64Sub1
- ✓ OM6162/SwarnaSub1

Therefore, selection of non-chalky grains should be conducted in an earlier generation in rice breeding programs. The chalkiness of combinations ranged from level 0 to 5. The combinations having a minimum level of chalkiness were good quality grains. The best quality grains were observed in the combinations as:

- ✓ WAB326-B-B-7-H1/IR64Sub1
- ✓ IR65191-3B-2-2-2-2/IR64Sub1
- ✓ IR78913-B-10-B-B-B/IR64Sub1
- ✓ OM6162/SwarnaSub1.

Table 3.3 *Cooking quality traits of hybrid combinations.*

No.	Name of combinations	AC (%)	GC (mm)	GT (scale)	Protein (%)	Chalkiness (level)
1	IR75499-21-1-B/IR 64 sub1	24.1bcd	75.8d	3	7.5bcd	3
2	IR78937-B-3-B-B-3/IR64Sub1	25.6ab	68.2ij	3	7.6bc	3
3	WAB326-B-B-7-H1/IR64Sub1	24.6bc	45.3m	3	8.2ab	3
4	IR78913-B-10-B-B-B/IR64Sub1	23.5cde	69.2ghi	3	7.8bc	1
5	IR75499-73-1-B/IR64Sub1	24.5bcd	48.6l	3	7.2bcd	3
6	IR78933-B-24-B-B-3/IR64Sub1	20.6g	85.3a	3	7.5bcd	1
7	IR78933-B-24-B-B-4/IR64Sub1	25.3ab	64.2k	3	7.6bc	5
8	V3M-103-2/Swarnasub1	26.3a	42.3n	3	8.4ab	1
9	V3M-103-2/IR64sub1	23.5cde	69.3ghi	3	7.5bcd	1
10	WAB99-47/IR64Sub1	25.6ab	78.5b	3	7.6bc	5
11	IRAT302//IR64Sub1	23.5cde	68.6hi	5	7.9bc	5
12	IR75499-29-2-B//IR64Sub1	21.3fg	66.8j	3	7.5bcd	5
13	IR75499-84-1-B//IR64Sub1	23.1cde	70.2fgh	5	8.2ab	1
14	V3M-170-1/IR64Sub1	21.2fg	72.1e	5	7.4bcd	3
15	V3M-167-2-B/IR64Sub1	23.2cde	71.2ef	5	8.2ab	1

Note: Data within a column by the same letter are not significantly different ($P<0.05$);CV: coefficient of variation; AC: Amylose content; GC: Gel consistency; GT: Gelatinization temperature.

Table 3.3 *Continue*

No.	Name of combinations	AC (%)	GC (mm)	GT (scale)	Protein (%)	Chalkiness (level)
16	RR166-645/IR64Sub1	24.2bcd	68.2ij	5	8.1ab	5
17	WAB99-5/IR64SUB1	23.5cde	68.9ghi	3	7.5bcd	5
18	IR78982-B-24-B-B-B/IR64Sub1	24.3bcd	68.5i	3	7.7bc	5
19	IR78937-B-3-B-B-3/IR64Sub1	23.5cde	66.8j	1	6.8d	3
20	WAB326-B-B-7-H1/IR64Sub1	21.2fg	77.3b	5	7.8bc	0
21	IR78913-B-10-B-B-B/IR64Sub1	22.3ef	74.5d	5	7.9bc	1
22	IR75499-73-1-B/IR64Sub1	24.3bcd	65.2k	3	8.5a	5
23	IR78913-B-19-B-B-B/IR64Sub1	23de	72.3e	3	8.2ab	3
24	AZUCENA//IR64Sub1	24.3bcd	49.5l	3	7.2bcd	1
25	BP225D-TB-6-8/IR64Sub1	24.1bcd	64.2k	3	8.2ab	1
26	IR78933-B-24-B-B-31/IR64Sub1	23.5cde	71ef	3	7.9bc	0
27	IR78933-B-24-B-B-4/IR64Sub1	24.2bcd	68.2ij	3	8.4ab	1
28	IR65191-3B-2-2-2-2/IR64Sub1	20g	74.5d	3	8.1ab	0
29	BP227D-MR-2-12/IR64Sub1	23.2cde	70.4fg	5	7.8bc	1
30	OM6162/SwarnaSub1	20.2g	78.2b	5	8.1ab	0
CV%		3.42	1.34	-	10.63	-
LSD 0.05		1.31	1.48	-	1.36	-

Note: Data within a column by the same letter are not significantly different (P<0.05);CV: coefficient of variation; AC: Amylose content; GC: Gel consistency; GT: Gelatinization temperature.

Selecting line of the F_3 generation and F_4 seeds: In lines selected generation with combinations were recorded in Table 3.4. Therefore, selection was 10 combinations recorded on phenotype and relatively good yield and growth duration less than 100 days. The F_3 generation recorded through selected individual from many crosses reached that segregation with many different shape, include grain shape and color of grain hulls. Result through the F_3 generation were recorded with 282 of the selected lines were in planting to evaluate for planting the F_4 generation from 10 combinations.

Table 3.4 *Number of individual in the F_1, F_2, F_3 generations.*

No.	Combinations	F_1	F_2	F3
1	IR75499-29-2-B//IR64Sub1	50	200	00
2	IR78937-B-3-B-B-3/IR64Sub1	100	200	00
3	IR75499-84-1-B//IR64Sub1	100	200	20
4	V3M-167-2-B/IR64Sub1	100	200	19
5	WAB326-B-B-7-H1/IR64Sub1	175	200	19
6	IR75499-73-1-B/IR64Sub1	148	152	28
7	IR78913-B-19-B-B-B/IR64Sub1	126	400	35
8	IR65191-3B-2-2-2-2/IR64Sub1	125	400	56
9	BP227D-MR-2-12/IR64Sub1	200	400	50
10	OM6162/ SwarnaSub1	250	1200	55

Selecting line of the F_4 generation: F_4 generation was planted to evaluate phenotype. Totally, 8 combinations that had high yield were selected. Two combinations IR75499-29-2-B//IR64Sub1 and IR78937-B-3-BB-3/IR64Sub1 generating seeds with the beard should continue to be removed. The F_4 generation was grown with 8 combinations (Table 3.5).

Table 3.5 *Selected individual of the F_3, F_4 generations.*

No.	Combinations	F_3	F_4
1	IR75499-29-2-B//IR64Sub1	20	150
2	V3M-167-2-B/IR64Sub1	19	200
3	WAB326-B-B-7-H1/IR64Sub1	19	200
4	IR75499-73-1-B/IR64Sub1	28	200
5	IR78913-B-19-B-B-B/IR64Sub1	35	200
6	IR65191-3B-2-2-2-2/IR64Sub1	56	289
7	BP227D-MR-2-12/IR64Sub1	50	250
8	OM6162/ SwarnaSub1	55	250

3.4.3 Heritability

Phenotypic analysis of genetic traits was estimated in the F_4 population of cross from IR75499-29-2-B//IR64Sub1 with n=150. The analysis results showed that their traits and yield varied from minimum of 80 cm by plant height, 19 cm by panicle length, 3 by panicles/ cluster, 65 by number of filled grains/panicle, 12 by number of unfilled grains/panicle and 10.58 g by grain yield/cluster to a maximum of 119 cm, 29.4 cm, 17 panicles/ cluster, 165 number of filled grains/panicle, 83 number of unfilled grains/panicle and 42.12 g/cluster,

respectively.

Similarly, as the analysis of the F4 combination of OM6162/SwarnaSub1 with n = 250 was analyzed. Data genetic parameters of yield traits and yield components of combination OM6162/SwarnaSub1 gained highest plant height (129.0 cm), lowest plant height (90.0 cm) and medium (105.4 cm). The panicle length was highest (30.4 cm), lowest (20 cm), and medium (25.54 cm). The highest number of panicles/cluster (17), lowest (3) and medium (8.2) were attained by the combination OM6162/SwarnaSub1. The difference from IR75499-29-2-B//IR64 Sub1 was not statistically significant. Data regarding number of filled grains/panicle revealed that highest (183), lowest (65) and medium (153, 19). Trait number of unfilled/panicle in combination OM6162/SwarnaSub showed results highest (63), lowest (5) and medium (18.5).

Analysis of genetic parameters for grain yield trait and yield components is presented in Table 3.6. Significant heritability (h^2) of high yield, panicle length, number of unfilled grain/panicles and plant height at $P<0.01$ was 0.98; 0.74; 0.56; and 0.5, respectively by IR75499-29-2-B//IR64Sub1. The highest heritability was noticed in grain yield trait/cluster. Medium to significant heritability at $P<0.05$ was observed in the number of filled grains/panicle trait.

The high heritability was observed in all most of the traits of combination OM6162/SwarnaSub1 at significance $P<0.01$. The heritability observed for traits ranged from 0.4 to 0.9. High heritability observed for panicle length, number of panicles/cluster, number of filled grains/panicle, number of unfilled/ panicle, and grain yield/cluster. The final result of yield components was grain yield/ cluster. Combination OM6162/SwarnaSub1 attained statistically the highest grain yield/cluster (120 g) whereas combination IR75499-29-2-B//IR64 Sub1 gave (42.12 g), lowest (10.58 g), and medium (26.5 g).

Table 3.6 *Analysis of genetic parameters of yield traits and yield components in combinations.*

Combinations	Traits	Highest	Lowest	Medium	CV	P	h^2
IR75499-29-2-B/IR64 Sub1	PH(cm)	119.0	80.0	105.7	10.09	**	0.51
	PL(cm)	29.4	19.0	25.4	1.19	**	0.74
	NP/C	17.0	3.0	8.2	3.99	ns	-
	NFG/P	163.0	65.0	153.2	39.36	*	0.30
	NUFG/P	83.0	12.0	17.6	13.86	**	0.56
	GY/C (g)	42.1	10.6	26.0	1.31	**	0.98
OM6162/SwarnaSub1	PH(cm)	129.0	90.0	105.4	11.09	*	0.40
	PL(cm)	30.4	20.0	25.5	1.15	**	0.74
	NP/C	20.0	10.0	13.2	4.90	**	0.84
	NFG/P	183.0	65.0	163.9	9.35	**	0.70
	NUFG/P	63.0	5.0	18.5	5.74	**	0.76
	GY/C (g)	120.0	10.6	26.5	1.15	**	0.90

**: significant at $p < 0.01$, *: significant at $P < 0.05$, ns: no significantly; h^2: heritability

PH: Plant height; PL: Panicle length; NP/C: Number of panicles/cluster; NFG/P: Number of filled grains/panicle; NUFG/P: Number of unfilled/ panicle; GY/C (g): Grain yield/ cluster (g).

3.5. Discussion

Grain quality in rice is determined by many factors such as grain appearance, nutritional value, cooking and eating quality (Juliano et al., 1990). The head rice recovery is the main factor effecting on milling quality. In this study, most lines/varieties had more than 50% of HR recovery. However, previous studies showed that the HR should have a value of at least 70% (Ashish et al., 2006). In fact, rice grains in this study were evaluated under drought condition, so the HR average was lower. HR levels depend on different factors, such as grain type, chalkiness, environment during dry conditions, variety, and cultural practices (Ashish et al., 2006; Dipit et al., 2003). The grain size is an important quality in rice trade with different preferences among consumers (Fan et al., 2006). The grain size was controlled by genetic traits (Gupta et al., 2006). Also, the grain size is bred at the level of early generation, and the long grain type is generally preferred in the Mekong Delta. The size and shape were stable varietal properties that could be used to identify a variety (Rickman et al., 2006). The most acceptable grain length is around 6 mm (Kaul, 1970). The shape of milled rice, in terms of length-width ratio is slender > 3.0, medium (2.1 - 3.0), bold (1.1 - 2.0), and round (1.1) (Jennings et al., 1979). Based on these classifications, rice materials could be defined as medium to long grain. Since the grain length and width of rice are important factors involved in the rice industry, these characteristics are seriously considered in the breeding of new varieties (Slaton et al., 2005). Thus, the grain size is the first criteria of rice quality that researchers need to concentrate on the development of new varieties.

The chalkiness is influenced by both genetic and environmental factors, as temperature immediately after flowering and other factors, such as soil fertility, water management (Mackil et al., 1996), drought stress during ripening and blast disease (Lang et

al., 2013). Most of the lines/varieties had low chalkiness from level 0 to 1. The line/varieties having minimum level of the chalkiness can be used as donors for breeding varieties of quality rice from the commercial point of view. Amylose content (AC) is one of the most important characteristics of cooking and processing practices (Juliano and Villareal, 1993). Commonly, consumers like rice with intermediate AC ranged between 20 to 25% (Rachmat et al., 2006). Gelatinization temperature (GT) is another important quality to determine the cooking quality of rice. GT is not associated with other grain traits except amylose content (Jennings et al., 1979). In this study, there were large variations of GT among lines/varieties. The nutritional values of rice depend on the total content of protein. High protein content equates with higher nutritive value. A wide range of protein content (4.5 - 15.9%) was found among 2,674 rice varieties (Kennedy and Burlingame, 2003). The aromatic level in rice is one of the important traits in breeding and may influence the demand in the market (Shilp and Krishnan, 2010). Totally 114 different volatile compounds are responsible for rice fragrance (Yajima et al., 1979). The biochemical basis of aroma was identified as 2-acetyl-1-pyrroline (Kandan and Pattamker, 1938) which stands out as the main fragrance compound in both jasmine and basmati varieties.

Moreover, good quality characteristics should be evaluated for grain quality and more advanced lines at early generations for nutritional factors in the breeding processes. Among 30 hybrid combinations, there were six combinations consisting of IR65191-3B-2-2-2-2/IR64Sub1, IR78933-B-24-B-B-3/IR64Sub1, OM6162/SwarnaSub1, WAB326-B-B-7-H1/IR64Sub1, V3M-170-1/IR64Sub1, and IR75499-29-2-B//IR64Sub1 obtaining low AC (20 - 21.3%). According to Jennings et al. (1979) (homozygous dominant (AA) defines the high AC, and the homozygous recessive (aa) produces low AC, so the heterozygous unsteadily generates the average AC.

The chalkiness is influenced by both genetic and environmental factors (Mackil et al., 1996). If selection non-chalky is made only in the late generations ($F_6 – F_7$), it is difficult to eliminate. Therefore, selection of non-chalky grains should be made in an earlier generation in rice breeding programs.

It is important to evaluate the association between characteristics to determine the direction of selection and number of traits to be considered for rice yield. Heritability provides the better genetic advance for selecting plant material regarding these traits. The highest heritability was noticed for grain yield trait/cluster both of two combinations. Results showed that high heritability for the number of spikelets per panicle, 1000 grain weigh and number of the panicles per plant (Bhatti et al., 1998). In this study, high heritability was observed in the combination OM6162/SwarnaSub1 0.74 for panicle length, 0.84 for the number of panicles/cluster, 0.7 for the number of filled grains/panicle, 0.76 for number of unfilled/panicle, and 0.9 for grain yield/cluster. A previous study also reported that high heritability 41.74% for number of panicles per plant (Ei-Malky et al., 2008). Results of high heritability and genetic advance of grain yield/plant are also in accordance with those reported by Li et al. (1991); Jha and Ghos, (1998); Singha and Dash, (2000). Heritability serves as a good index for transmission of traits from one generation to the next and it should be considered in terms of the selected concept (Hanson et al., 1956). Since high heritability does not always indicate high genetic gain, heritability with genetic advance considered together should be used in breeding (Ali et al., 2002).

3.6. Conclusion

Among 44 rice germplasms, IR79008-B-11-B-B-1 showed good physical characteristics (head rice, grain length, grain width, chalkiness), three varieties (IR75499-73-1-B, OM6162 and OM4900) showed good chemical characteristics (amylose content, gel consistency, protein content, gelatinization temperature, and aroma). The important highlight of the study was that progenies of the cross between OM6162/SwarnaSub1 had a low amylose content, high gel consistency, high protein content, and low chalkiness.

These characters along with plant height, panicle length, number of panicles/cluster, number of filled grains/panicle, number of unfilled/panicle, and grain yield/cluster showed moderate to high heritability of mean for combination OM6162/SwarnaSub1. Most of above traits have resulted that highest heritability was noticed for grain yield trait/cluster, and the number of panicles/cluster. Therefore, the results suggest that the grain yield trait/cluster and the number of panicles/cluster are important yield contributing traits and selection based on these characters would be most effective for rice breeding.

Chapter 4

Path Analysis for Yield Traits in F_2 Generation and Molecular Approach for Breeding Rice Tolerant to Drought and Submergence

4.1. Abstract

Drought and submergence are the two major limiting factors to reduce rice production. In this study, correlation of yield traits through path analysis under drought and submergence conditions to improve grain yield of rice, from dry season 2014-2015 and genotypic analysis using SSR markers was evaluated, during 2015-2016. Path analysis indicated that the number of panicles/clusters had the highest and a direct positive effect on the grain yield, followed by the number of filled-grain/panicle, and the harvest index compared to other component traits. These traits could be used as selection criteria for high yield and drought tolerance in rice. There were two markers including RM201 (210-225 bp) and RM219 (210-215 bp) chosen to select parents in backcrossing because of production of polymorphic bands relevant to submergence and drought tolerance genes. By the BC_1F_1 and BC_2 F_1 generations of the cross OM6162/Swarnasub1//OM6162, primers RM201 and RM219 were identified drought and submergence-tolerant individuals. These lines will be used in the breeding programs for the release of both drought and submergence-tolerant rice with considerable yield in the next step. Findings of this study are promising to develop rice drought and submergence-tolerant rice varieties, and can help to reduce some detrimental impacts from climate changes to rice production.

4.2. Introduction

Rice is currently grown in varied environmental conditions where it shows different levels of response to abiotic stress, depending on the environmental conditions of origin and cultivation (Rananwake and Hewage, 2014). The effects of climate changes, such as drought, flooding, salinity and high temperature have detrimental impacts on rice production, especially in developing countries. Abiotic stresses as drought and submergence have been identified as the two constraints to cause most rice loss (Bernier et al., 2008; Devereux, 2007; Dey and Upadhyaya, 1996; Gauchan and Pandey, 2012; Pandey and Bhadari, 2009; Venupeasad et al., 2007). Flooding is a major cause for low yields in rainfed lowland rice areas of the Mekong Delta, Vietnam, where occupies more than 1 million ha. High water level is a problem for about haft of rainfed areas. Rice production is damaged by both short-term submergence (up to 2 weeks) and by longer-term stagnant flooding at water depth above 40 cm. Thus, the drought and submergence stresses are the most important objective of the rice breeding program. Additionally, rice yield can be improved with a comprehensive combination of both conventional and molecular breeding techniques (Khush, 2005).

Marker-assisted selection (MAS) method is proposed by Tanksley (1983) to investigate the introgression of tolerant genes (Mechinger, 1990). It includes the marker-assisted evaluation of breeding materials, marker-assisted introgression, and marker-assisted pyramiding. Although markers can be used any stage of plant breeding program, the number of plants retained due to their early generation performance can be decreased and ensured a high probability of retaining superior lines by MAS to improve the selection for early generations (Eathington et al., 1997). So, it is recommended that the

population size and heritable level of the selected traits are important prerequisites for improvement of rice breeding program (Lande and Thompson, 1990). According to Kuchel (2007) and Bonnett et al. (2005), that a larger number of plants can be achieved at a much lower cost with the aid of MAS, compared with the conventional breeding. The previous researchers, MAS have successfully introduced the bacterial blight resistance gene Xa21 (Chen et al., 2000) and waxy gene (Zou et al., 2003) to target for commercial rice breeding. The combination of QTLs of for abiotic stress tolerance, especially studies in genetics of rice showed that the submerged-tolerant trait of the FR13A variety is controlled by a polygene and affected by environment, designated *sub1* (Xu and Mackill, 1996). This gene was identified recently as an ethylene-responsive like factor (ERF) and designated *Sub1A* (Xu et al., 2006). The most widely submerged-tolerant donors are IR64sub1 and Swanasub1. Swanasub1 was pyramided with the *sub1* gene for drought and submergence tolerance. *Sub1* versions of popular rice varieties were developed through the marker-assisted backcrossing (MABC) approach (Neeraja et al., 2007; Septinnighsih et al., 2009; Iftekharuddaula et al., 2015; Lang et al., 2015). Nguyen et al. (2004) developed 85 markers for mapping of QTL regions for drought tolerance in rice and identifying putative candidate genes. One QTL region controlling osmotic adjustment on chromosome 3 and 14 affects root traits which are located on chromosomes 1, 2, 4, 5, 6, 7, 8, 9, 10 and 12. In a previous study, Kumar et al. (2014) reported that two markers, RM201 and RM328, were linked with drought-tolerant genes ($qDTY_{1.1}$, $qDTY_{2.1}$, and $qDTY_{3.1}$).

Path analysis was developed as the best method to evaluate the relationship between yield and relevant traits (Board et al., 1997). Estimate path analysis gave correlation coefficients of direct effects of various traits on yield as well as their indirect effects via other yield components traits (Mohsin et al., 2009). In plants breeding, the diallel theory which

was first developed by Hayman (1954), is widely used for path analysis (Krihna Veni and Shobha Rani, 2005; Eradasappa et al., 2007). The diallel analysis is used to get information about the genetic structure of populations and helps to explore the genetic mechanism of various traits in plants (Griffing, 1956; Rahimi et al., 2010; Muthuramu et al., 2010). Therefore, the path coefficients analysis is utilized helpful as a basic idea of direct and indirect contribution traits for selection in rice breeding program.

Development of high yield cultivars that combine drought and submergence tolerance could be the ideal to reduce detrimental effects of climate change on rice production. IRRI has started drought and submergence breeding programs to develop germplasm for this target population (Kumar et al., 2008; Septiningsih et al., 2009). Therefore, the introduction of both drought and submerged-tolerant characteristics to target rice cultivars is an important task for rice breeders. Thus, the objectives of this study were (1) to clarify direct and indirect effects of yield traits under drought and submergence stresses and (2) molecular breeding to develop rice that combines tolerance to both drought and submergence.

4.3. Materials and Methods

4.3.1. Plant materials

The materials consisted of 36 F_2 combinations by the crossing of six parents IR64sub1, OMCS2000, OM6162, OM1490, Swarnasub1, and IR78933-B-24-BB-4 in a diallel mating design. The variety OM6162 was crossed with Swannasub1 which was used as the donor for both *qDTY* and *Sub1* genes to obtain a backcross population for MAS.

4.3.2. Path analysis

Evaluation of agronomic characters and grain yield to rice under drought stress

Seeds of the F_2 diallel lines were soaked, germinated in an incubator, and sown into plastic trays. After 15 days, they were transplanted into cement basins. The row-to-row and plant-to-plant space of 20 cm x 15 cm was maintained. Ten days after transplanting, water was not provided until flowering. Fertilizer was applied at the rate 100-40-30 kg of $N\text{-}P_2O_5\text{-}K_2O$ ha^{-1}. The plant recovery for each entry followed the 0-9 score of the standard evaluation system (IRRI, 1996) with scores 0-3: tolerance and score 5-9: susceptible, and agro-morphological characters and grain yield were recorded.

Screening for submergence tolerance

Seeds of the F_2 diallel lines were soaked, germinated in an incubator, and sown into plastic trays. Ten-day-old seedlings were transplanted using 1 plant/hill and with space of 20 x 15 cm in submergence tanks. At the seventh-day after transplanting, plants were completely submerged for 14 days at 10 cm water depth which was then increased by 10 cm at every 10 days interval. Finally, 50 cm water level was maintained up to the soft dough stage. Four plants were tagged for tiller counting. Surviving plants were counted just after the recession of water and their tillers were counted before and after submergence at 7 days intervals. The standard evaluation system (SES) scores for submergence tolerance followed by IRRI (1988), 1 to 9 (1: all plants survive; 9: all plants completely dead).

Agro-morphological character evaluation

All agro-morphological traits including panicle/cluster, filled grain/panicle, the weight of 1000 grains (g), root length (cm), yield/cluster (g) were recorded. Biomass-weight of 10 plants harvested from each accession per replication was also recorded. Harvested plants were dried before weighing for calculating the Harvest Index as follows:

Harvest Index = Economic yield/Biological yield x 100,

where economic yield is the total weight of grain harvest from 10 plants per accession per replication, and biological yield is the total grain weight and biomass from 10 plants per accession per replication.

The correlation coefficient (*r*) among traits was calculated by using SAS 9.1 program. The correlation coefficient 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.

4.3.3. Marker-assisted selection

Microsatellite primers were used to survey polymorphism on the samples based on information of the gene mapping data overview of markers as determined by of Lang and Buu (2008). For submerged-tolerant genes, the molecular markers were selected based on the genetic mapping information of previous study by Lang et al. (2015). Sixteen microsatellite primers were selected from microsatellite primers mentioned above (Table 4.1).

In BC_1F_1 and BC_2F_2 generations, the selection was initially carried out by markers through screening parental polymorphism at both *qDTY* and *Sub-1* loci.

4.3.4. DNA extraction

Leaves were collected 2-3 weeks after planting for extraction of DNA. Standard molecular grade chemicals and general techniques for preparing stock solutions, buffers, reagents, and equipment were followed according to Sambrook et al. (1989).

DNA extraction was prepared according to a method described by McCouch et al. (1997) and conducted at the Department of Genetics and Plant Breeding at Cuu Long Rice Research Institute, Can Tho, Vietnam.

DNA quality was checked using 1% agarose (melting 3 g agarose in 300 ml TAE buffer). The mixture was heated in the microwave for 5-6 minutes and then cooled to around 55-60 ^{o}C. This was then poured into a prepared electrophoresis box with combs. Gels were ready and the combs were removed after about 45 min. Seven microliters of DNA sample and 3 µl loading buffer (Tris 1M pH = 8.0, glycerol, EDTA 0.5M pH = 8.0, xylene cyanol 0.2%, bromophenol blue 0.2% and distilled water) were 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. The gel was then taken out and stained with ethidium bromide. The gel image was visualized under UV light.

4.3.5. Amplification of microsatellites and detection of their polymorphisms

PCR amplification was performed in a mixture of 10 mM Tris-HCl (pH=8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 1 unit of TAKARA *Taq*, 4 nmole of dNTPs, 10 *p*mole of primers, with 30 ng of genomic DNA per 25 µl using a thermal cycler 9600 (Perkin-Elmer,

USA). The PCR reactions were denatured at 94 ^{o}C for 4 min, followed by 35 cycles at 94 ^{o}C for 1 min, 55 ^{o}C for 1 min and 72 ^{o}C for 2 min. The final extension was at 72 ^{o}C for 5 min. After PCR, 13 µl of loading buffer (98% formamide, 10 mM EDTA, and 0.025% bromophenol blue, 0.025% xylene cyanol) were added. Polymorphisms in the PCR products were detected by ethidium bromide staining after electrophoresis on 3% agarose gel.

4.4. Results

4.4.1. Path analysis

The correlation coefficient was conducted out 6 to 36 crosses of diallel from the varieties IR64sub1, OMCS2000, OM6162, OM1490, Swarnasub1, and IR78933-B-24-BB-4 (Table 4.2). The trait of panicle/cluster showed a strong and positive association with root length, drought tolerance at seedling, and grain yield. Positive and high phenotypic correlations coefficient of yield with the number of panicles/cluster were obtained in this study. While the significant negative association with 1000 weight was observed. Moreover, the association among yield traits revealed that filled grains/panicle showed significant positive association with HI and root length. Whereas, the significant negative correlation was observed with the weight of 1000 grains and drought tolerance at the flowering stage. The number of filled grains/panicle also showed a strong positive and high phenotypic correlation on grain yield. The weight of 1000 grains was recorded to have a positive significant association with drought tolerance at the flowering stage while significant negative correlated with drought tolerance at the seedling stage. The weight of 1000 grains and submergence at seedling was not correlated with grain yield. Correlation between the HI

and drought responses at the flowering stage was significantly negative, and phenotypic correlation of HI with yield trait was 0.69. This finding suggests that, the HI could be used as a reliable criterion for improving yield. The trait root length exhibited a significant and positive association with drought tolerance and was associated with the yield trait. The trait test for drought tolerance at the flowering stage was found positively correlated with drought tolerance and high phenotypic correlation coefficient (0.65) at the seedling stage and while the significant negative association with grain yield. However, drought tolerance at the seedling stage did not show significant phenotypic association with yield.

Path coefficient analysis devises the genetic correlation between yield and yield component traits direct and indirect effect and has been used to estimate better traits as selection criteria for developing grain yield in rice. The path cofficient analysis shows in Table 4.3 revealed that the number of panicles/cluster has the highest positive direct effect on grain yield followed by the number of filled grains/panicle, HI, and weight of 1000 grains, which indicated that these traits were more contributors towards yield in these combinations, but there has not been stability in the results in various experiments or in different populations

4.4.2. Approach marker assisted selection

Total 16 markers were used to screen for drought and submergence in paring (Table 4.1). The parental polymorphic survey was performed among the parental genotypes OM6162 and Swarnasub1**.** Two SSR markers RM201 and RM219 produced polymorphic bands (Figures 4.1 and 4.2).

In the BC_1F_1 generation of OM6162/Swarnasub1//OM6162, plants were screened to drought tolerance by the robust tightly-linked marker RM201, a marker linked QTL regions for drought tolerance. There were two amplified bands, type P1 of the 225 bp band and type P2 of the 210 bp band. Out of 38 plants, eight lines showed "B" score similar to the homozygous donor allele by the 210 bp band, 15 lines showed heterozygous "H" score and 15 lines showed "A" score (Figure 4.1a). Thus, eight plants from cross OM6162/Swarnasub1//OM6162 were self-fertile to develop BC_2. These plants with the "H" score for tightly linked marker were subjected to phenotypic selection.

In the BC_2F_1 generation, segregation of plants into drought tolerant and susceptible can be seen clearly in the gel picture with linked RM201 by type P1 of the 215 bp band and type P2 of the 210 bp band. The 21 plants with " A" score similar to the homozygous recipient allele. Eighteen plants with "B" score as homozygous donor allele of Swarnasub1 were produced due to the accidental failure of backcrossing (Figure 4.2a).

In the case of RM219, a marker was linked to the submergence tolerance QTL *sub1*. there were two amplified bands, the 210 bp, and the 215 bp band. Twenty plants showed "A" score, 8 plants showed heterozygous "H" score and only six plants of OM6162/Swarnasub1//OM6162 had the band similar to the Swarnasub1 variety. (Figure 4.1b). In the BC_2F_1 generation of OM6162/Swarnasub1//OM6162, the marker RM219 linked to type P1 of the 210 bp band and type P2 of the 215 bp band. A total of eighteen plants had homozygous donor allele as Swarnasub1 (Figure 4.2b).

4.5. Discussion

The positive direct effect was found in the number of panicles/cluster. The results of the importance of the direct effect of panicles per plant were reported by (Bagheri et al., 2011; Madhavilatha et al., 2005; Yogameenaskshi and Vivekanandan, 2010). This result indicates that getting the higher number of panicles must be considered for developing high yielding rice. In this section, the analysis aimed to determine important traits directly correlated to the yield or indirectly through other traits because they are able to help improve rice yield. Vaishali (2003) showed that grain yield had a strong significant positive correlation coefficient with the number of productive tillers per plant. On the other hand, the panicle/cluster had a strong and positive correlation coefficient with root length. This result was in consonance with previous study of Jeena and Mani (1990) for root traits and grain yield on some upland rice varieties and indicated that high root length density and root weight were important for breeding drought tolerance genotypes. The results get from the findings of O'Toole and De Datta (1986); Thangaraji et al. (1990), Sharma et al. (1994), and Sinclair and Muchow (2001) who showed significant genetic variability in some root traits has been demonstrated and implicated for improving drought tolerance in crop plants

In summary, according to the principle of correlation, On the basis of results were evaluated by path analysis, and the results are displayed in Table 4.3. If the correlation coefficient between the cause and the result are equivalent to its direct value, the correlation can be explained as a really close relationship and direct selection through phenotypic correlation coefficent among traits. If the correlation coefficient is positive, but directly affected values are negative or negligible, the indirect values be the causes of the correlation. In this case, the indirect causes must be simultaneously considered in the selection. For example, for the length of roots, we must consider its indirect factors simultaneously if the

traits for selection are the number of panicles/cluster, filled grains/panicle and drought tolerance at the flowering and seedling stages. Commonly, phenotype correlation of grain yield, yield components, and drought-related traits provides the information to determine the direction of association this relasioship between various traits (Sunderraj et al., 1972).

Molecular markers can be used in many steps of rice breeding program. Markers are also used to examine parental polymorphism with desirable genes and gene combinations. This approach has the potential to make parental selection more efficient, to expand the gene pool of modern cultivar and to speed up the development of new varieties. Lang and Buu (2008) found that the markers RM201 and RM328 were linked to drought-tolerant traits. Under drought stress treatment, it was confirmed that this root length QTL with target segment on chromosome 9 was segregated in the BC population of OM1490/WAB 880-1-38-18-20P1-HB; OM1490/WAB881 SG9, and OM4495/IR65195-3B-2-2-2-2 (Lang et al., 2013). If BC_1F_1 generation more than on individual satisfying the strong condition is found, selection between them can be performed on the basis of analysis of other marker loci to determine the most desirable individual for producing BC_2 (Tanksley et al., 1989). SSR marker, RM219 has been mapped for 3.4 cmRM219 to *sub 1* locus (Xu et al., 2004). Rathanayake et al. (2012) studied that the 220 bp of allele of RM219 was used as diagnostic alleles or gel bands to monitor Sub-1 in IRRI119/Bw363 cross. For the Swarna variety, a combination of three QTLs ($qDTY_{1.1}$, $qDTY_{2.1}$, and $qDTY_{3.1}$) was pyramided *Sub1*, the large effected QTL for tolerance of submergence (Kumar et al., 2014).

Exploitation of the initial materials is very important in breeding. Because of the drought and submergence-tolerant genes are multi-gene, so evaluation of initial materials to select the parents serving studies of hybridization is urgent to select good hybrid material for achieving targets in breeding. Currently, at least one population was determined the

usefulness of the two markers (RM201 and RM219) for selection both of submergence and drought tolerance genes. These lines were selected by genotype, which will be the reference to pick for the next generation. At the same time, phenotypic testing of final products for drought and submergence tolerance needs careful planning the start in to confirm the transfer of QTL identification process based on MAS.

4.6. Conclusion

Most of the above traits showed that traits as root length, the number of panicles/cluster, and a number of filled grains/panicles at harvest had a strong and positive correlation with grain yield. Based on path analysis, the traits include a number of filled grains/panicles, the number of filled-grain/panicle, and harvest index had strong and direct positive effect correlation with grain yield.

The present study established the utilization of marker-assisted selection for developing new varieties by combinations of drought and submergence tolerance. Fortunately, both *qDTY* and *Sub1* can be combined in the same variety. These best lines will be used for development of further breeding. This type of variety was approached as a first step to develop new varieties for gathering genes of drought and submergence tolerance.

Table 4.1 *The information of molecular markers used in diagnosis of submergence and drought.*

No.	Markers	Sequence code (5' - 3')	Chromosome	Repeating sequence
1	RM201	F: ctcgtttattacctacagtacc R: ctacctcctttctagaccgata	9	(CT)17
2	RM511	F: cttcgatccggtgacgacac R: aacgaaagcgaagctgtctc	12	(GAC)7
3	RM11125	F: ccaagaaccctagctccctctcc R: tcgacgagatcctcctcgtaaacc	1	(CT)22
4	RM10713	F: atgaacccggcgaactgaaagg R: ctggctccctcaaggtgattgc	1	(AGA)12
5	RM3252	F: ggtaactttgttcccatgcc R: ggtcaatcatgcatgcaagc	1	(CT)13
6	RM10115	F: acaagacgaggtaacacgcaagc R: gcgaaggatcaacgatgatatgg	1	(CTT)24
7	RM105	F: gtcgtcgacccatcggagccac R: tggtcgaggtggggatcgggtc	9	(CCT)6
8	RM219	F: cgtcggatgatgtaaagcct R: catatcggcattcgcctg	9	(CT)17

Table 4.1 *Continue*

No.	Markers	Sequence code (5' - 3')	Chromosome	Repeating sequence
9	RM23662	F: gagaggacgatggcactattgg R: cgaggaacttgattcgcatgg	9	(GGC)10
10	RM23877	F: tgccacatgttgagagtgatgc R: tacgcaagccatgacaattcg	9	(CA)30
11	RM547	F: taggttggcagacctttcg R: gtcaagatcatcctcgtagcg	8	(ATT)19
12	RM249	F: ggcgtaaaggttttgcatgt R: atgatgccatgaaggtcagc	5	(AG)5A2(AG)14
13	RM24103	F: actgacgagagagacatggatgg R: ccggcacacaatgaataggg	9	(AC)17
14	RM25181	F: aaagagcttccctaatggcttcg R: gagagaatgacctctcccaagacc	10	(TTC)22
15	RM1125	F: ggggccagagttttcttcag R: gtacgcgcagaaaatgagag	10	(AG)12
16	RM328	F: catagtggagtatgcagctgc R: ccttctcccagtcgtatctg	9	(CAT)5

Table 4.2 *Correlations coefficients among the traits with yield of F_2 diallel generation.*

Traits	**Panicle /cluster**	**FG**	**W-1000**	**HI**	**RL**	**SubS**	**DF**	**DS**	**Yield**	
									R	**Pr**
Panicle /cluster	1	0.20	-0.28*	0.19	0.59**	-0.13	0.15	0.43**	0.78**	0.88
FG		1	-0.38*	0.68**	0.79**	-0.04	-0.68**	0.25	0.76**	0.67
W-1000			1	-0.19	-0.07	-0.04	0.82**	-0.39*	-0.24	0.37
HI				1	0.08	0.25	-0.17	-0.69**	-0.89**	0.69
RL					1	-0.04	0.28	0.37**	0.84**	-0.39
SubS						1	0.06	0.11	-0.14	-0.08
DF							1	0.99**	-0.68**	0.65
DS								1	-0.20	-0.19

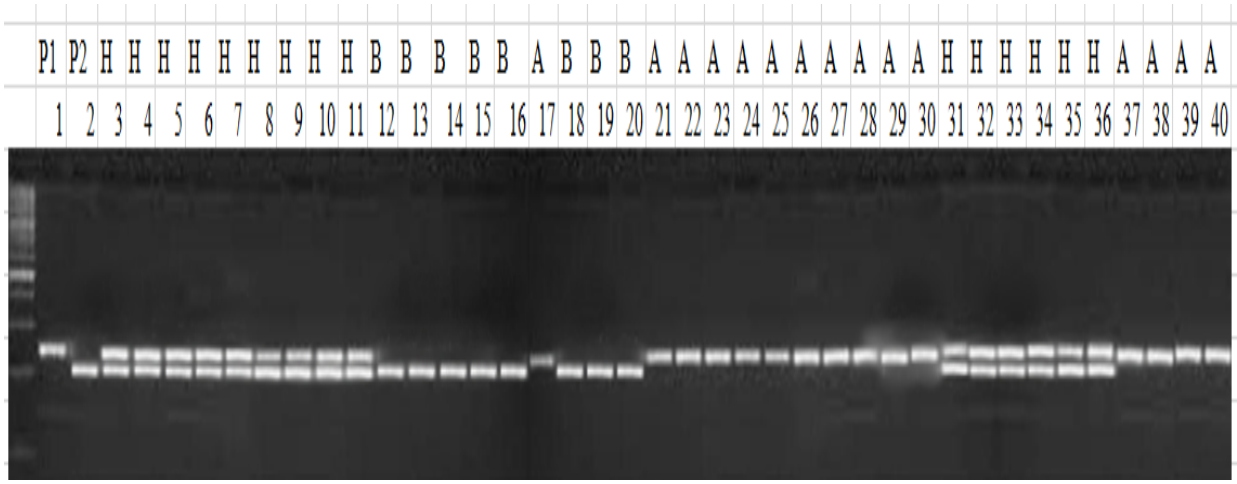
: significant at P<0.01. *: significant at P<0.05; r: correlations coefficients; Pr:** phenotype correlation coefficients

Note: DF**:** Drought at flowering stage; DS: Drought at seedling stage; RL: Root length; SubS: Submergence at seedling; HI: harvest index; W-1000: Weight of 1000 grains: FG: Filled grain/panicle

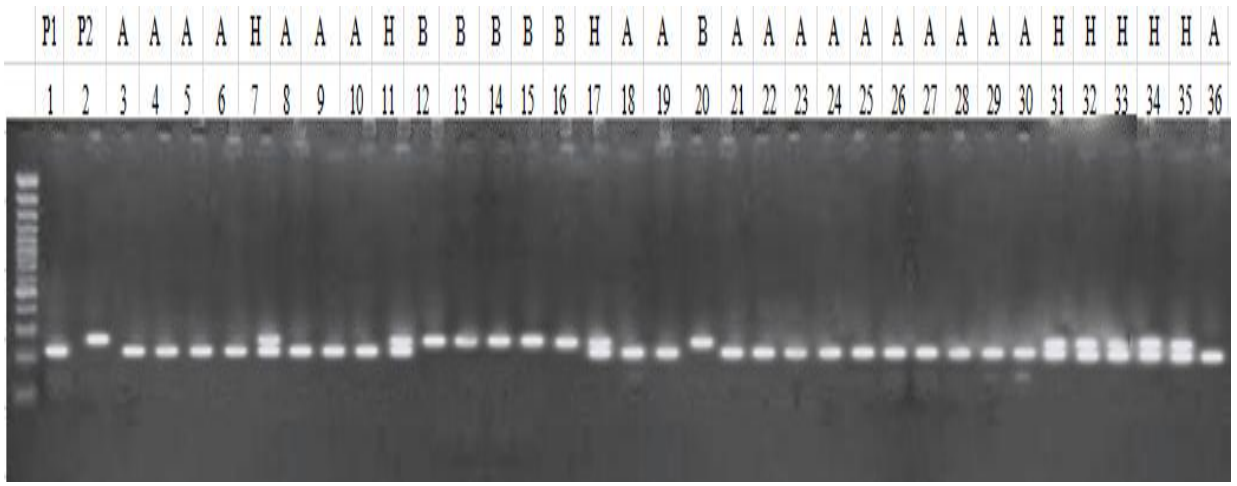
Table 4.3 *Analysis of correlation by path (path analysis) between the grain yield traits of rice in F2 diallel generation.*

Traits	**Panicle /cluster**	**FG**	**W-1000**	**HI**	**RL**	**SubS**	**DF**	**DS**	**r total**
Panicle /cluster	0.85	0.15	-0.10	0.06	0.00	0.00	0.00	0.00	0.68
FG	0.19	0.67	-0.11	0.34	-0.14	0.07	0.11	-0.01	0.73
W-1000	-0.27	-0.20	0.46	-0.10	0.02	0.04	0.00	0.01	-0.21
HI	0.15	0.37	-0.06	0.59	-0.01	-0.01	0.00	0.02	0.41
RL	0.44	0.27	-0.02	0.01	-0.44	0.00	0.00	-0.01	0.52
SubS	-0.12	-0.03	-0.01	0.13	0.02	-0.06	-0.00	0.00	-0.16
DF	0.09	-0.31	0.29	-0.05	-0.07	0.00	0.00	-0.03	0.15
DS	0.16	0.11	-0.10	-0.41	-0.40	-0.10	0.01	-0.06	0.17

Note: DF**:** Drought at flowering stage; DS: Drought at seedling stage; RL: Root length; SbS: Submergence at seedling; HI: harvest index; W-1000: Weight of 1000 grains: FG: Filled grain/panicle.

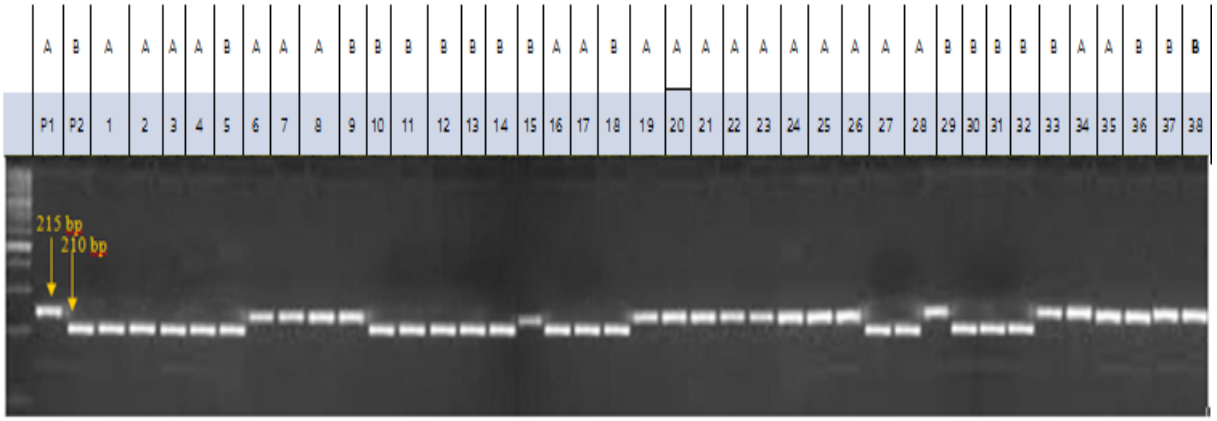


(a)

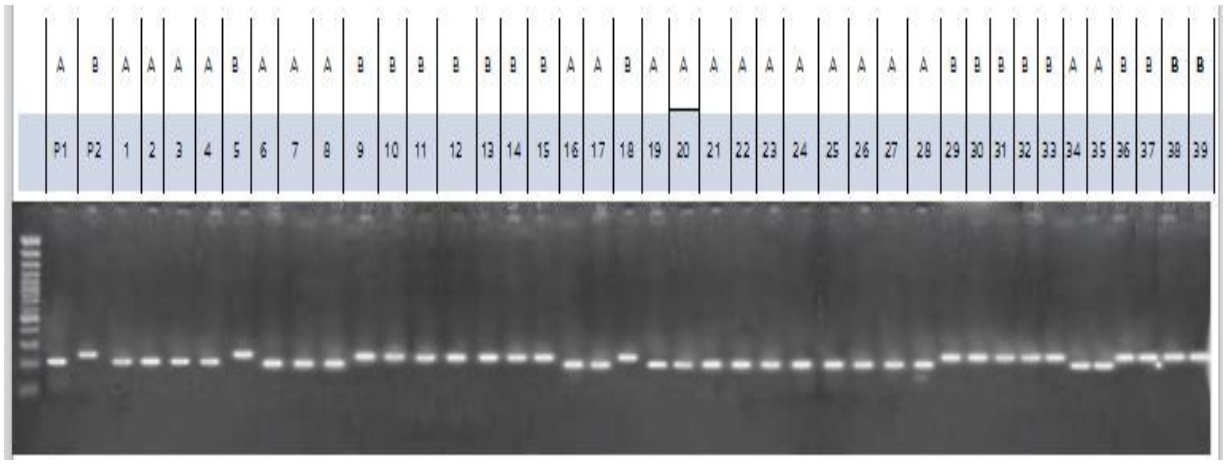


(b)

Figure 4.1 PCR profiles of some lines genotype in BC_1F_1 of OM6162/Swarnasub1//OM6162; (a) RM211 linked drought-tolerant gene: single band linked lane 2 as P1: the recipient parent (225 bp), P2: the donor parent (210 bp), A: similar homozygous recipient allele B: homozygous donor allele and H: double band indicated heterozygous allele; (b) RM219 linked submergence-tolerant gene: same as "a"; P1: the recipient parent (210 bp), P2: the donor parent (215 bp).



(a)



(b)

Figure 4.2 PCR profiles of some lines genotype in BC_2F_1 of OM6162/Swarnasub1//OM6162; (a) RM211 linked drought-tolerant gene: single band linked lane 2 as P1: the recipient parent (210 bp), P2: the donor parent (215 bp), A: similar homozygous recipient allele B: homozygous donor allele and H: double band indicated heterozygous allele; (b) RM219 linked submergence-tolerant gene: same as "a"; P1: the recipient parent (215 bp), P2: the donor parent (210 bp).

Chapter 5

Development of Drought Tolerance Rice Lines Using Marker-Assisted Backcrossing in Vietnam

5.1 Abstract

Development of high yield and abiotic-stress tolerance rice varieties is one of the urgent and essential demands for people living in the areas of Mekong Delta, Vietnam, where being seriously affected by climate changes, especially long-day drought and hot-temperature situations. This experiment used IR75499-73-1-B as a drought-tolerant donor and OMCS2000 as a recipient parent for breeding new rice lines with good drought-tolerant capacity and high yield. Seven SSR markers (RM219, RM201 RM105, RM23602, RM23877, RM24103, and RM328) were used for identifying drought-tolerant target genes in breeding populations, including backcrossing populations (BC_2F_2). As a result, primer RM23877 showed the highest number of homozygous allele bands (11 lines), corresponding to donor line. Followed by RM105 and RM201 appeared 9 lines. Drought screening in BC_2F_2 generation indicated that BC_2F_2-45 and BC_2F_2-54 lines resulted in strong drought-stress resistance and high productivity. Outputs of this study suggested that application of marker-assisted backcrossing (MABC) method might be a new approach for Vietnamese scientist to develop new agricultural crops possessing good agronomic traits as well as adapting to climate changed regions in Vietnam.

5.2 Introduction

Drought stress is one of the major constraints to rice yield stability in rainfed upland areas, and estimates 70% of the yield losses could be due to water scarcity situation (Bray et al., 2000). Development of rice cultivars tolerance to abiotic stresses is being a new direction to help people to improve rice production adapting to climate changes, including drought stress. Application of SSR (Simple Sequence Repeat) markers not only helps to identify quantitative trait loci (QTLs) associated with drought resistance genes, but also supports for molecular breeding, creating new drought resistance varieties with other desired targets, such as good quality and high yield.

Recent advances in plant breeding have provided breeders useful molecular tools to support for genotypic screening, in which SSR marker-assisted selection (MAS) has been one of the potential tools (Collard and McKill, 2008). Currently, China, India, and Thai land are among pioneer countries applying MAS method combined with conventional breeding techniques to produce good quality and drought-tolerant rice cultivars for the commercial market (O'Toole, 2004). 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. (2007) detected a QTL resistance to drought 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. Similarly, a QTL correlated with drought tolerance was found on chromosome 3 of a hybrid rice cultivar between tolerant Apo variety and susceptible Swanna variety (Venuprasad et al., 2009). Besides, Lang et al. (2013) reported fine mapping of QTL regions for drought tolerance in rice on chromosome 9.

Conventional breeding methods combined with genetic techniques using molecular markers have been applied for many rice breeding programs in Vietnam. Rice breeding for drought tolerance could be accelerated by marker-assisted selection (MAS). Commonly, rice plants usually dryly die within a week under water scaricity conditions; however, some drought tolerance cultivars can survive for from 3 to 4 weeks, depending on different varieties. Currently, several strong tolerant varieties (WAB, IR i) are developed from IRRI with drought-tolerant linked genes are identified on chromosome 9 (Lang et al., 2013). Typically, the variety IR74371-70-1-1 was developed through conventional breeding and it was disseminated to farmers in drought affected areas. The yield of these varieties can provide about 1 ton/ha under severe drought stress (Verulkar et al., 2010). In this context, conventional breeding techniques were combined with MAS, and several potential drought-tolerant rice lines were selected as a release of commercial varieties to food crop market.

Drought situation is unavoidable under the perspective of climate change in the Mekong Delta, Vietnam. Development of new rice lines with combined traits, such as good quality, high yield, and tolerance to drought stress is a big challenge for plant breeding scientists. Thus, deep understanding of plant physiological and molecular mechanisms against abiotic stresses is very necessary. The objective of this study is to investigate the effects of drought stress on seedling and reproductive stages. Simultaneously, this approach of marker-assisted backcrossing (MABC) will be applied to improve some drought tolerance rice lines adapting to climate changes in Mekong delta areas of Vietnam.

5.3 Materials and Methods

5.3.1 Rice materials

Backcrossing population is developed from OMCS2000 and IR75499-73-1-B. IR75499-73-1-B is chosen as the drought-tolerant donor, while OMCS2000 is an improved variety in Cuu Long Rice Research Institute (CLRRI), Vietnam, which is susceptible to drought stress.

5.3.2 Development of the backcross population

About 100 BC_1F_1 seeds were produced from crossing between OMCS2000 and IR75499-73-1-B, and then were planted in an experimental field to produce F_2 generation seeds. In the BC_2F_1 generation, selection of genetic background was carried out based on phenotypic and genotypic evaluation. Plant height, flowering date, and drought tolerance of each cultivar were recorded in the BC_2F_2 generation. 200 segregating lines were harvested as recombinant inbred lines. A total of 30 lines were advanced and evaluated for yield and yield components using OMCS2000 and IR75499-73-1-B as the check. After assessment of drought tolerance, selected potential plants would be continuously screened and isolated by DNA analysis using molecular markers SSR (simple sequence repeat).

5.3.3 DNA extraction

The 30 lines/varieties were grown in pots in a greenhouse under optimum conditions. Rice leaves were collected from 2 to 3 weeks after planting to extract DNA. The extraction method included standard chemicals and molecular techniques were prepared and conducted following the protocol of Sambrook et al. (1989) and McCouch et al. (1997), respectively. The laboratory experiments were performed from 2015 in

Genetics and Plant Breeding Department, Cuu Long Delta 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. The gels were ready and the combs were removed after about 45 min. Seven microliters 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, 60 mA for 45 min or until loading buffer dye moved far from the wells. Gel was then taken out and stained with ethidium bromide. The image of gel was visualized under UV light.

5.3.4 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 5.1) (Temnykh et al., 2000).

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 5.1 *The information of molecular markers used in diagnosis of drought on chromosome 9.*

Name of markers	**Forward primers (F)/ Reversed primer (R)**	**Sequences (5' - 3')**	**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

5.3.5 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, Vietnam, 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 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 5.2) (IRRI, 1996).

Table 5.2 *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

5.3.6 Quantitative traits

The following quantitative traits were measured:

(1) Panicle length (cm): length of panicle at maturity is 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 the 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 the 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 the number of unfilled grains per panicle (from 5 randomly selected primary panicles per accession per replication).

(7) The 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.

5.3.7 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.

5.4 Results

5.4.1 Molecular screening

The polymorphic level of SSR markers was different from each population. The markers included RM219, RM201 RM105, RM23602, RM23877, RM24103 and RM328 were used to identify drought tolerance genes on chromosome 9, based on information from the genetic map of Lang et al. (2013). Some examples were found a high polymorphism, as compared with their parents (Figures. 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, and 5.7).

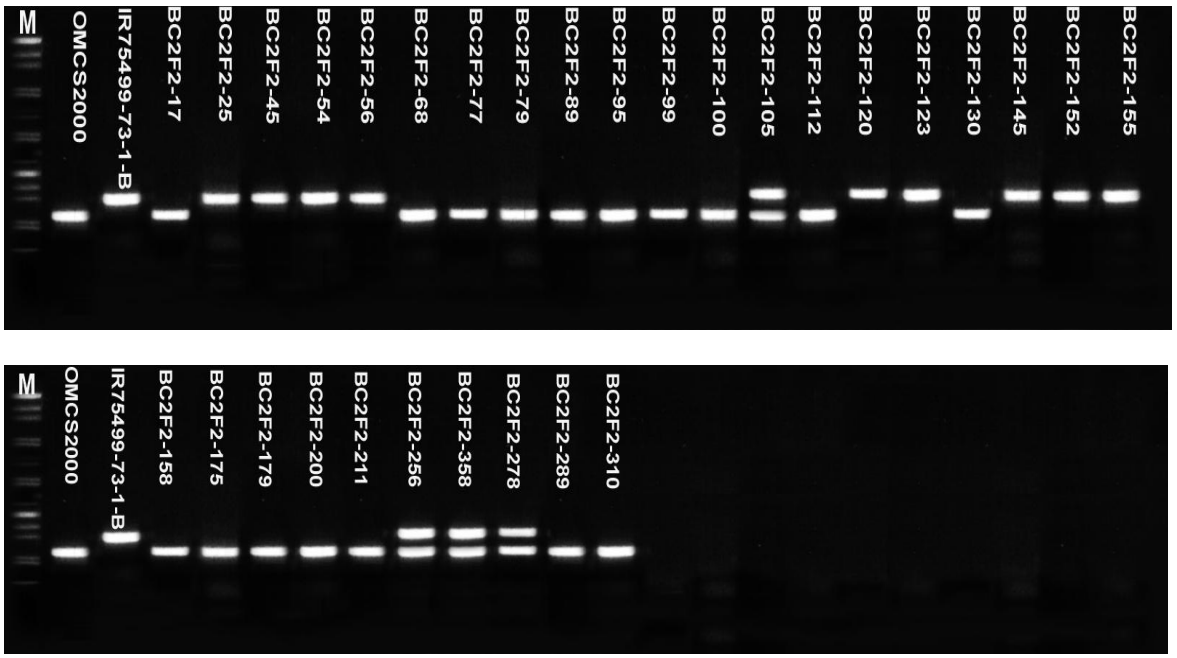


Figure 5.1 Representative gel picture of foreground selection for drought tolerance individuals in the BC2F2 generation of OMCS2000/IR75499-73-1-B// OMCS2000 with RM105.

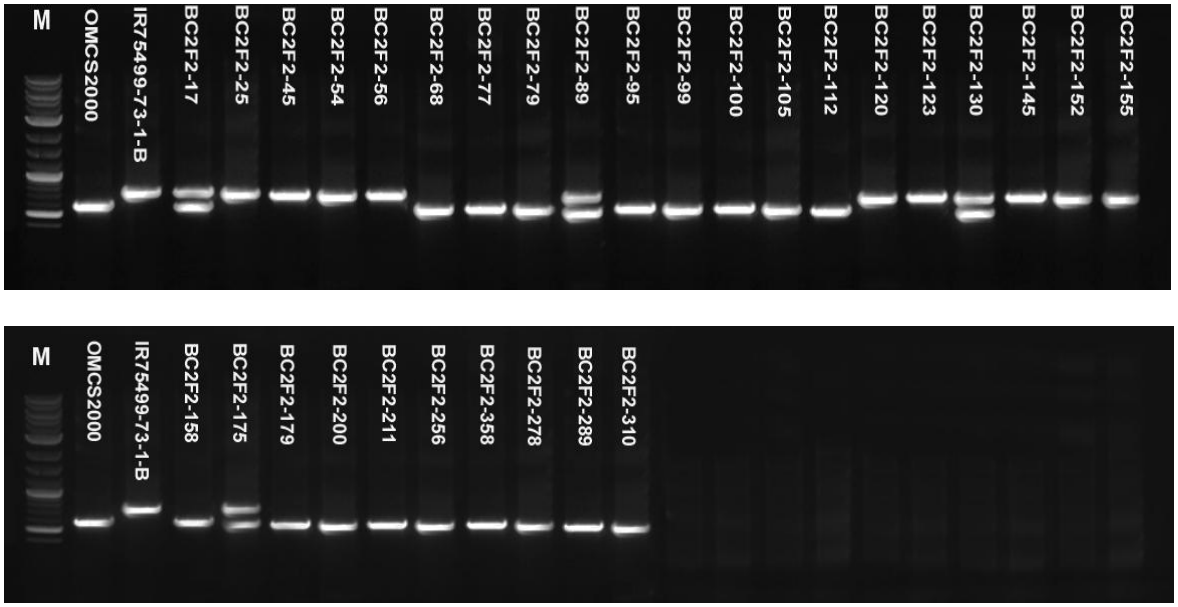


Figure 5.2 Representative gel picture of foreground selection for drought tolerance individuals in the BC_2F_2 generation of OMCS2000/IR75499-73-1-B// OMCS2000 with RM201.

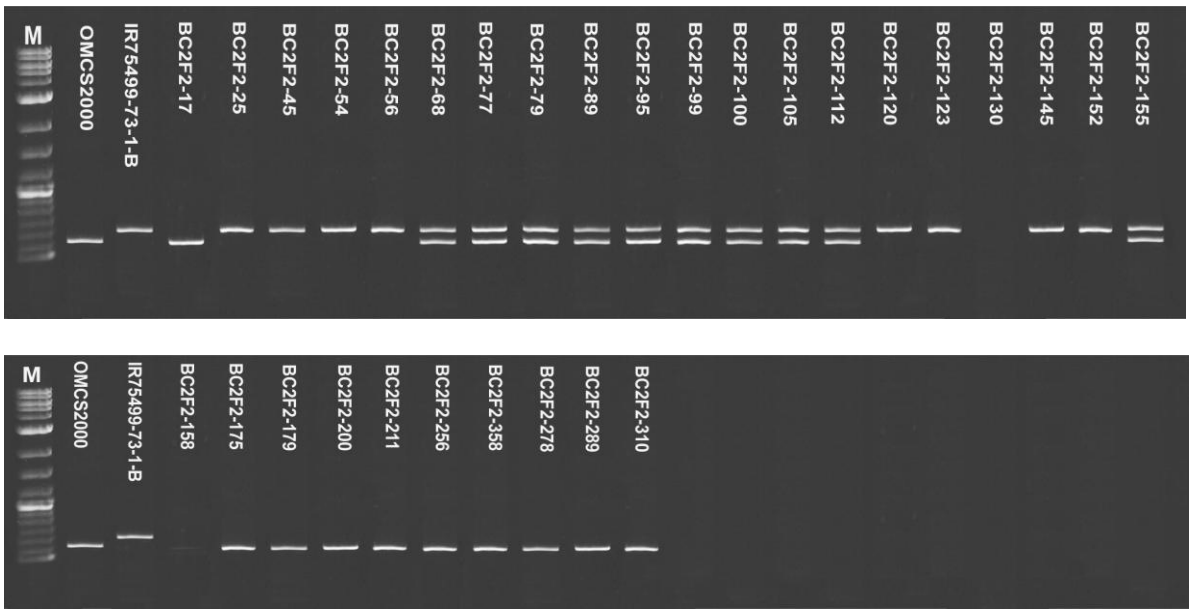


Figure 5.3 Representative gel picture of foreground selection for drought tolerance individuals in the BC_2F_2 generation of OMCS2000/IR75499-73-1-B// OMCS2000 with RM219.

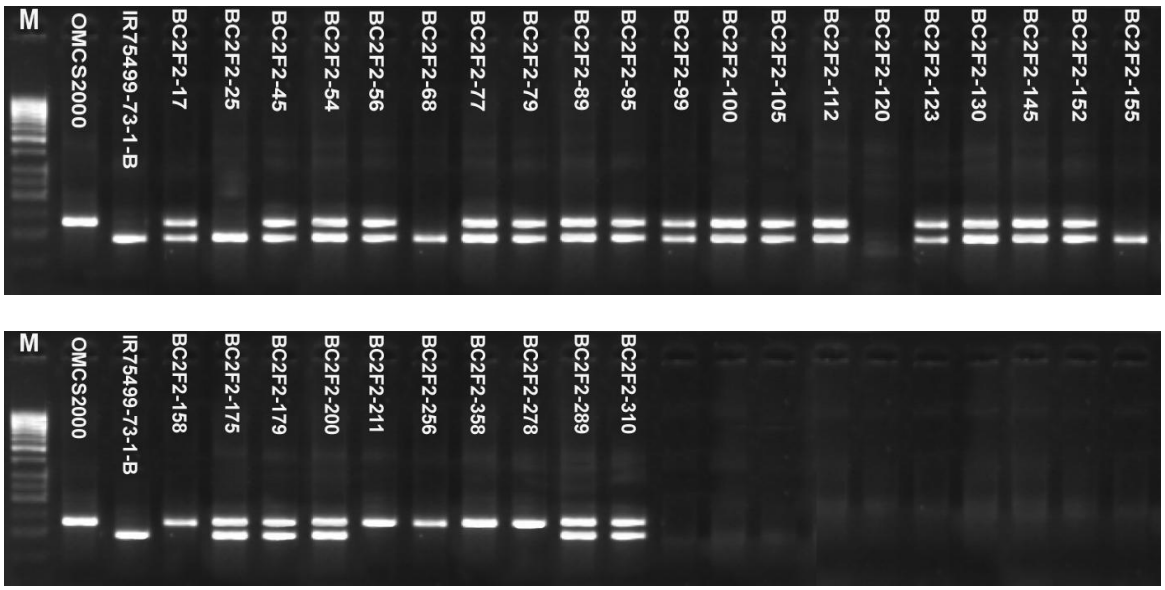


Figure 5.4 Representative gel picture of foreground selection for drought tolerance individuals in the BC_2F_2 generation of OMCS2000/IR75499-73-1-B// OMCS2000 with RM328.

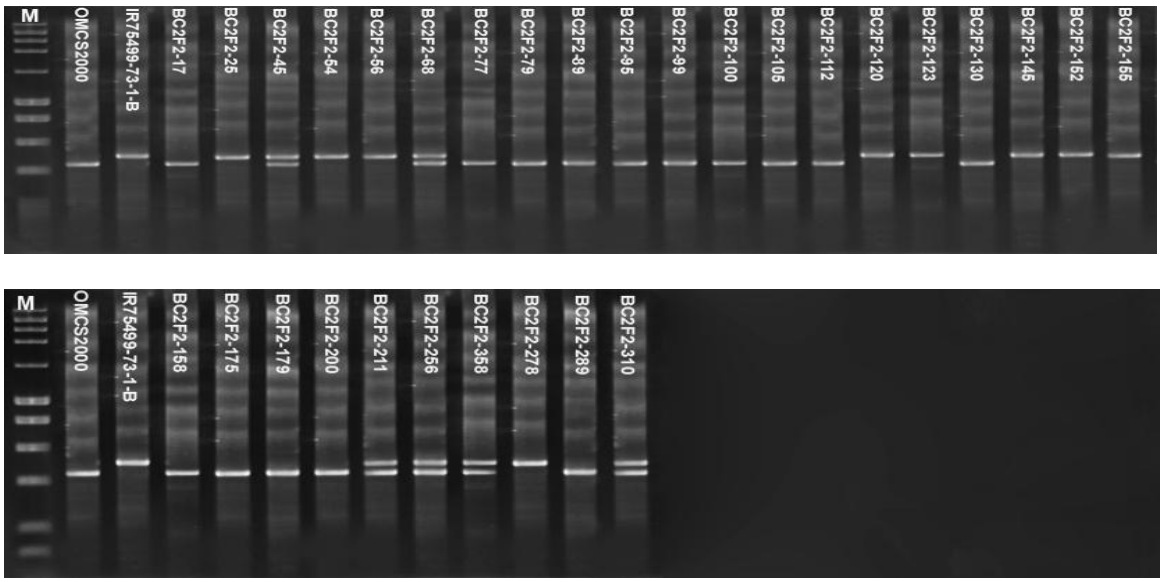


Figure 5.5 Representative gel picture of foreground selection for drought tolerance individuals in the BC_2F_2 generation of OMCS2000/IR75499-73-1-B// OMCS2000 with RM32662.

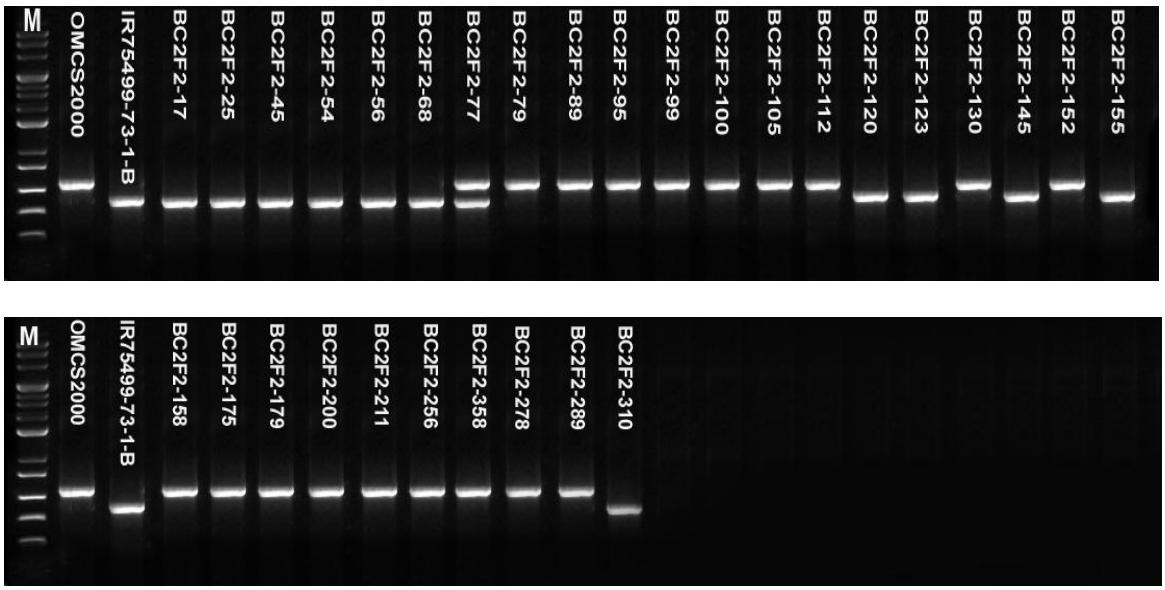


Figure 5.6 Representative gel picture of foreground selection for drought tolerance individuals in BC_2F_2 generation of OMCS2000/IR75499-73-1-B// OMCS2000 with RM23877.

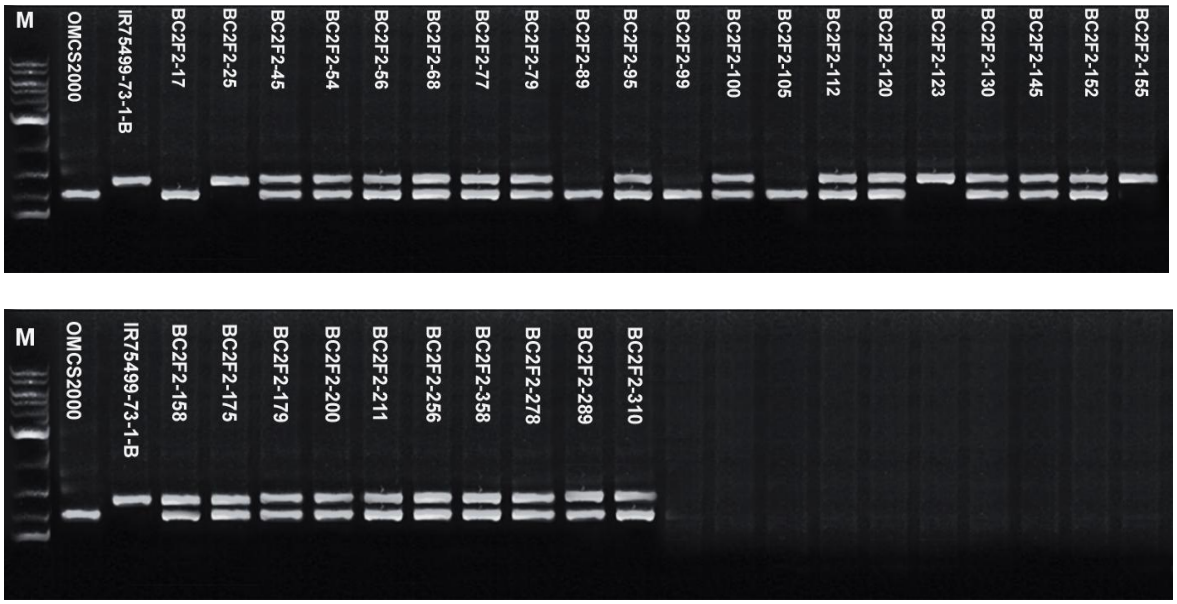


Figure 5.7 Representative gel picture of foreground selection for drought tolerance individuals in BC_2F_2 generation of OMCS2000/IR75499-73-1-B// OMCS2000 with RM 24103.

Primer RM23877 confirmed that 11 lines possessed homozygous donor alleles (B), 18 lines as homozygous recipient alleles (A) and 1 line had heterozygous alleles (H) (Figure 5.6). For the two primers, RM105 and RM201, 9 lines had homozygous donor alleles, 17 lines were similar with homozygous recipient alleles, and 4 lines had heterozygous alleles (H) (Figures 5.1 and 5.2). In case of RM328 and RM24103, the rest of 3 lines had homozygous donor alleles (B) (Figures 5.4 and 5.7). Eight lines showed similarity to homozygous donor alleles for RM219, nine lines were found to be homozygous donor alleles for RM32662 (Figures 5.3 and 5.5). 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 5.3).

Table 5.3 *Phenotypic and genotypic analysis of 30 lines BC_2F_2 generation along parents.*

No.	Lines	Phenotypic analysis Score	Genotypic analysis						
			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

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

Table 5.3 *Continue*

No.	Lines	Phenotypic analysis Score	Genotypic analysis						
			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
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

5.4.2 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 with 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 5.4). 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 the 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.

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 5.4). 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 from 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 5.4 *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

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

Table 5.4 *Continue*

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
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

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

DS: Drought stress

Table 5.4 *Continue*

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
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. 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 5.5.

Table 5.5 *Correlation coefficient matrix of some targets of phenotypic after completed drought stress 20 d 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 significantly 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 completely drought conditions is almost certainly beneficial and different from the increase in plant height

5.4.3 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 5.6).

Table 5.6 *Agronomic traits of BC lines after the drought stress 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	11 7 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

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 5.6 *Continue*

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
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

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 (BC_2F_2-155 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 5.6).

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 5.7). 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 5.7 *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

5.5 Discussion

BC breeding lines were screened for drought tolerance based on phenotypic indicators and genotypic analysis using molecular markers SSR. In self-pollinated crops, it is necessary to fix alleles in 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 could fix specific alleles in their homozygous state as early as in F_2 generation. However, this may require a large size of the breeding population. Hence, in term of practical, a small number of loci may be fixed at each generation (Koebner and Summers, 2003). An alternative strategy is to 'enrich' rather than fix alleles by selecting homozygotes and heterozygotes for a target locus within a population to reduce the size of the required breeding

populations (Bonnett et al., 2005).

Besides the use of SSR to identify target genes in food crops, application of these molecular markers could also aim for QTL/gene fine mapping to detect the location of gene on chromosomes. SSR markers usually correlate with good traits that help to improve the quality, productivity or tolerant capacity of food crops. In this study, BC_2 population developed from *indica* parents, OMCS2000 and IR75499-73-1-B with using a combination of 7 primers.

Rice grain yield is a complex trait determined by three 3 factors: number of panicles, number of grains per panicle, and grain weight (Yongzhong and Qifa, 2010). The number of grains per panicle is usually correlated tightly to the spikelet number. Therefore, to calculate the number of grains per panicle, it is necessary to understand the basic biological processes of panicle development, as well as the differentiation of meristems into spikelets under drought condition. In term of agronomy, the number of spikelets per panicle can be attributed to two components including the differentiation of panicle and spikelet (Huang et al., 2006). This is main background knowledge to aim for selection of noble lines possessed valuable traits and create strong stress-tolerant varieties as well in further investigations.

5.6 Conclusion

Through phenotypic screening and genotypic analysis, BC_2F_2-54 and BC_2F_2-45 were selected as two potential drought tolerance lines.

The use of marker-assisted selection (MAS) method to collect new rice lines tolerance to drought stress could be also applied for the development of new varieties or other crops with good characteristics, such as high yield, good quality, and various abiotic stresses tolerance.

Chapter 6

General Discussion

Recent developments in molecular marker technology and genomics have provided new approaches for discovering and tagging novel genes and alleles. These tools can enhance the efficiency of breeding programs through their use in marker-assisted selection (MAS). Marker-assisted selection is the process of using DNA markers to assist in the selection of plant breeding material (Francia et al., 2005; Collard et al., 2008). MAS offers potential to assemble target traits in same genotype more precisely with fewer unintentional losses in fewer selection cycles (Xu and Crouch, 2008). MAS accelerate recovery of recurrent parent genome and reduce the number of backcrosses required for gene introgression. Besides, this tool also minimizes the dependence on environmental conditions during the selection procedure (Tuberosa and Salvi, 2006). The development of drought-resistant varieties could be made more efficient by MAS to introgress alleles of QTL conferring improved drought resistance into the genome of widely used cultivars through backcrossing (Bernier et al., 2007). When the traits that need to be improved are low in heritability, MAS may be more efficient than phenotypic selection (Asins, 2002).

In 2007, the University of Agricultural Sciences (UAS), Bangalore, crossed a deeprooted upland *japonica* rice variety from the Philippines with a high yielding *indica* variety. Bred with MAS, the new variety consumes up to 60% less water than traditional varieties. In addition, MAS 946-1 gives yield comparable with conventional varieties (Gandhi, 2007).

In 2009, IRRI recommended two new drought-tolerant rice lines for release, which are as high yielding as normal varieties: IR4371-70-1-1 (Sahbhagi dhan) in India and a sister line, IR4371-54-1-1 for the Philippines (Reyes, 2009). Field trials in India are being

reported as very successful, with the rice tolerating a dry spell of 12 days (BBC, 2009)

In Vietnam, drought contaminated areas were estimated approximately 1,200000 ha in the Mekong Delta. Farmers in this region usually utilize rainfall to cultivate rice. However, due to irregular rain, rice plants were damaged by drought during seedling, from flowering to maturity stage. Lang and Buu (2008) identified SSR technique combined with selective genotyping associated with drought tolerance in rice. Two hundred and twenty-nine lines (BC_2F_2) derived from the cross of OM1490/WAB880-1-38-18-20-P1-HB were evaluated for drought at flowering (DRF), root dry weigh (RDW), and root length (RL). A microsatellite map of this population was used with 232 markers to detect the linkage to the target traits. QTL mapping was used to determine effects of loci associated with drought-tolerant traits. SSR markers located the drought recovery score genes between RM201 and RM328 on chromosome 9. They suggested that RM201 is the only marker that related to increased root length and drought tolerance under drought conditions.

The selective efficiency of drought-tolerant rice varieties was very limited because of the complexities of investigated regions, environmental factors and difficult to control. Recently OM6162, OM6161, and OM7347 were found to recover rapidly and give high yield at target areas. In coastal areas of the Mekong Delta, OM4900 and OM6677 were developed with large scale from 2009-2012 and ongoing. Howerver, two varieties as OM6162 and OM6677 were only developed in several years because of poor grain quality.

Thus, the objectives of breeding program are to determine the standard of drought -tolerant rice variety breeding, to identify important traits, tolerance mechanism at seedling and at the reproductive stage. The injuries on leaves at seedling stage, sterile spikelets at reproductive stage and stem shoot under drought stress conditions are key traits related to

drought tolerance. Selection of drought-tolerant individuals should not only observe morphological traits but also physiological, biochemical traits and genotype by environment interaction.

Conclusively, evaluation of germplasm for resistance genes plays a major role in selection of parental lines and the development of new breeding material. Information on target loci obtained from markers will greaty facilitates the efficiently using of germplasm. In addition, there was a high variation of agro-morphological character and genetic diversity in these 44 cultivars by the genetic distance of using UPGMA method with the SAHN based on SSR markers. Seven microsatellite primers currently available used for MAS such as RM201; RM105; RM219, RM23602, RM23877, RM24103 and RM328 linked to drought-tolerant gene based on information from the genetic map of Lang et al. (2013). The results further indicate that since the SSR markers are neutral and co-dominant, they are powerful tools to access the genetic variability of the cultivars under study. Use of marker and phenotype information together offers an efficient tool to the breeders in selecting parents for various breeding programs and marker-assisted selection (MAS).

In the current study, the results have obtained from various experiments conducted with the objectives: (i) to find out the extent of genetic variation in drought response among rice germplasm, select donors in breeding rice integrated with drought tolerance and good quality characteristics; (ii) the utilization of marker assisted selection for developing new lines by combinations between drought and submergence tolerance**.**

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This research also successfully (iii) introduced both (OM6162/swanasub1//OM6162) and (OMCS2000/IR75499-73-1-B//OMCS2000) populations. This effectively powerful approach identifies the associations between traits of interest yield potential, drought-tolerant characteristics and genetic markers using diverse genetic background. Two lines (BC_2F_2-45 and BC_2F_2-54) provide urgent objective for breeders released as new cultivars in providing higher incomes to the Vietnam farmers.

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