SELECTION OF PROMISING LINES WITH COMBINATION OF DROUGHT AND SUBMERGENECE TOLERANT TRAITS IN F7 GENERATION THROUGH MOLECULAR MARKERS IN RICE

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ABSTRACT

From 7 selected combinations evaluated on 2 coordinated targets of drought and submergence resistance gens, resulted in selecting 12 lines hybrid combinations in F7 generation. To continue to select pure lines, 23 molecular markers on chromosome 9 and one marker RM42 on chromsome 7 were used in order to identify pure lines. The results indicated that there were lines having great polymorphisms with molecular markers such as RM105, RM201, RM38 and RM42. Analysis of yield integrating short-maturity lines with high-yielding potential including lines number (F7-23), (F7-28-1) and (F7-28-2). These lines continue to be evaluated on quality, the following lines with low amylose content were that line F7 -13 (18,2%) of IR75499-84-1-B//IR64Sub1 crosses, line of F7-28-1 (16,2%) of IR65191-3B-2-2-2-2/IR64Sub1 crosses, line of F7-30-1 (18,2%) and line of number F7-30-2 (18,6%) of OM6162/ Swarna Sub1 hybrid combination. In addition, there were two scented lines namely F7-30-1 and F7-30-2 in OM6162/ Swarna Sub1 hybrid combination. These lines need to be continued for further utilized in rice production.

Keywords: *amylose, aroma, drought, submergence, marker*

INTRODUCTION

Sea level rise will translate into higher water levels in vast parts of these deltas resulting in more flooding. There is also need for vulnerability and risk analysis and risk management specific to agricultural ecosystems and for improving weather/climate and flood forecasting and warning. Higher risks of rice production in Mekong delta and flash flood occurred over a short time, and resulted in reduced profitability of rice production. Flash floods occur mostly in the dry season during November and December. Flash floods last for 7-10 days at the early growth stage of the rice plants. Several factors contribute to flash floods: relatively low level of the rice field, high tides, heavy rains, typhoon effects. (Farmers were not able to predict flash flood situation.) Thus it caused death of rice plants, usually at the early stage, and farmers had to replant or filling the gaps with seedlings or broadcast seed again (Lang *et al.*, 2013b). Tolerance of abiotic stresses are a primary target for MAS, because of the difficulty in screening for them, and also because a number of QTLs with fairly large effect have been found (Mackill, 2006). Among the abiotic stresses, drought is by far the most widely prevalent in rice, and it is also one of the most complicated genetically and physiologically. There has been considerable effort to map QTLs for drought tolerance in rice (Lang *et al.*, 2013a). Prior to crossing (hybridization) and line development, there are

several applications in which DNA marker data may be useful for breeding, such as cultivar identity, assessment of genetic diversity and parent selection, and confirmation of hybrids. Traditionally, these tasks have been done based on visual selection and analysing data based on morphological characteristics (Bertrand C *et al*., 2008). In practice, seed of different strains is often mixed due to the difficulties of handling a large number of seed samples used within and between crop breeding programmes. Markers can be used to confirm the true identity of individual plants. The maintenance of high levels of genetic purity is essential in cereal hybrid production in order to exploit heterosis. In hybrid rice, SSR and STS markers were used to confirm genetic purity, which was considerably simpler than the standard 'grow-out tests' that involve growing the plant to maturity and assessing morphological and floral characteristics (Yashitola *et al*., 2002).

MATERIAL AND METHODS

In the study, The twelve lines in F7 generation from 7 crossing were used (showed in table 1).

Table 1. Material of 12 lines from 7 crossing

Order	Lines	Crossing
1	**F7 -13**	IR75499-84-1-B//IR64Sub1
2	F7-15-1	V3M-167-2-B/IR64Sub1
3	F7-15-2	V3M-167-2-B/IR64Sub1
4	F7-22	IR75499-73-1-B/IR64Sub1
5	F7-23	IR78913-B-19-B-B-B/IR64Sub1
6	F7-28-1	IR65191-3B-2-2-2-2/IR64Sub1
7	F7-28-2	IR65191-3B-2-2-2-2/IR64Sub1
8	F7-29-1	BP227D-MR-2-12/IR64Sub1
9	F7-29-2	BP227D-MR-2-12/IR64Sub1
10	F7-30-1	OM6162/ Swarna Sub1
11	F7-30-2	OM6162/ Swarna Sub1
12	F7-30-3	OM6162/ Swarna Sub1
13	**OM6162 (Check variety)**	
14	**IR64Sub1 (Check variety)**	

Data collection

The eight agronomic traits including days to heading, plant height, panicle length, number of panicles per plant, percent sterility, grains per plant, 1000-grain weight and yield per plant in each experiment was observed, the data were recorded as following parameters:

1. **Days to heading** was evaluated as the average number of days from seeding until 10% of the panicles had headed.
2. **Plant height** was measured as the average height of 10 plants in centimetre from the soil surface to the tip of the tallest panicle (awns excluded).
3. **Panicle length** was measured as the average number of centimeters from the panicle neck to the panicle tip (excluding the awns) based on an evaluation of all panicles from the 10 plants.
4. **Panicles per plant** was calculated as the average number of panicles on the n plants (panicles having less than five seeds were not counted).
5. **Percent sterility** was calculated by taking number of empty spikelets divided by the total number of spikelets per panicle evaluated for each panicle on all ten plants.
6. **Grains per plant** was measured as the average number of filled grains per plant calculated for ten plants.

7. **1000-grain weight** was the average weight of 1000 filled spikelets, measured in grams, averaged over three samples taken from bulk harvested grain from the ten plants.
8. **Yield per plant** was the average weight in gram of bulked harvested grain per plant averaged over three samples taken from bulk-harvested grain from the 10 plants.

DNA was extracted from fresh leaves of each plant following method of Lang *et al*. (2002). PCR protocol was as described by Lang (2002) with minor modifications. PCR products were separated on 3% agarose gel**.**

RESULT AND DISCUSSION

Selection of short-term lines

To find the target breeding tolerant to drought, submergence and short-maturity, the rice varieties were grown in green house at CLRRI, and the growth period observed for each stage of growing plant

Following single cross hybridization, varieties were grown for several generations and selected based on rate of segregation. The fourteen lines remained from the seven hybrid combinations. The lines were multiplied to assess the yield and yield components for each variety. The data of yield and the yield components of 12 lines from 7 combinations were shown in Table 2. Grain yield in rice is a complex trait multiplicatively determined by its three component traits: number of panicles, number of grains per panicle, and grain weight, all of which are typical quantitative traits (Yong zhong *et al*., 2010).

Only three lines had high yield and days of maturity was less than 100 days. These were line numbers 5 (F7-23), 6 (F7-28-1) and 7 (F7-28-2). The number of grains per panicle is usually highly proportional to the spikelet number. To understand the making of the number of grains per panicle, it is essential to understand the basic biological processes of panicle development, as well as the differentiation of meristems into spikelets. From an agronomic perspective, the number of spikelets per panicle can be attributed to two components: the duration of panicle differentiation and the rate of spikelet differentiation (Huang Y *et al.,* 2006). In this result, we found that there were three lines named F7-15-1, F715-2 and F7-30-3 had higher number of grains per panicle (214,205 and 196 respectively).

Table 2. Yield and yield component of 12 hybrid lines from the F7 population

Code of line	Growth duration	Number of panicle/ m^2	Number of grain/ panicle	Number of filled-grain/ panicle	Number of unfilled-grain/ panicle	Rate of unfilled grain(%)	Shape of grain	Yield (g/tilling)
F7 -13	105	264g	155f	73o	82a	52.9a	Long	7.57u
F7-15-1	102	231h	214a	140e	74b	34.6b	Oval	14.28k
F7-15-2	100	363c	205a	125g	80a	39b	Long	15.74gh
F7-22	102	165k	167e	117i	50b	29.9d	Oval	11.86opq
F7-23	95	198i	135h	115j	20j	14.8p	Long	25.21a
F7-28-1	97	264g	135h	95m	40e	29.6d	Long	20.30b
F7-28-2	95	198i	176d	130f	46c	26.1g	Oval	14.08kl
F7-29-1	100	165k	173d	140e	33h	19.1o	Long	15.86h
F7-29-2	100	231h	161e	126g	35g	21.7kl	Long	16.69ef
F7-30-1	105	198i	193b	138f	55b	28.5e	Long	15.50h
F7-30-2	105	231h	127i	97m	30i	23.6h	Long	15.19hij
F7-30-3	107	231h	196b	158c	38f	19.4no	Long	16.85def
OM6162 Check	110	166j	119j	76o	43e	36.1b	Oval	16.45fg
IR64Sub1 (ChecK)	100	296f	145g	120h	25j	17.2o	Long	17.43cde

The situation of pests and disease were not shown in this study, brown plant hopper (BPH) did not appear seriously. In Winter-Spring 2012 disease appeared in mild level. In summary, 30 hybrid combinations were segregated over generations and screened for submergence and drought traits. From these 12 lines from F7 generation that tolerance to submergence and drought were selected.

However, besides the presence of two genes resistance to drought and submergence, short duration and high productivity genes are also required. The analysis showed that the 3 lines carrying the short maturity gene were the single cross lines (F7-23), (F7-28-1) and (F7-28-2). These lines need further research and continued selection in later stages.

The individuals were selected from the single cross combination of F7 population

In the F7 generation, submergence and drought resistant genes were identified by molecular markers on 12 lines. For self-pollinated crops, an important aim may be to fix alleles in their homozygous state as early as possible. For example, in bulk and single-seed descent breeding methods, screening is often performed at the F7 generation when most loci are homozygous. By using co-dominant DNA markers, it is possible to fix specific alleles in their homozygous state as early as the F2 generation. However, this may require large population sizes; thus, in practical terms, a small number of loci may be fixed at each generation (Koebner & Summers 2003). An alternative strategy is to 'enrich' rather than fix alleles—by selecting homozygotes and heterozygotes for a target locus—within a population in order to reduce the size of the breeding populations required (Bonnett *et al*., 2005).

The results of this method using 23 markers with the program GGT showed in Figure 1

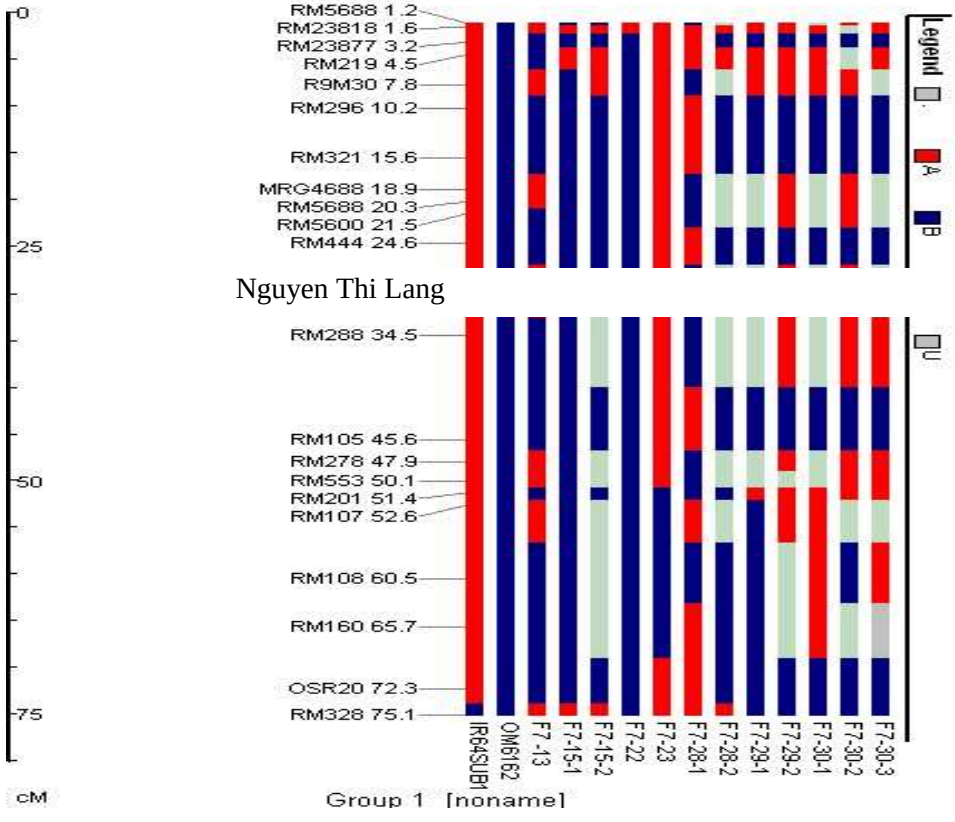

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Figure 1: Graphical genotypes of a F7 population on chromosome 9

The results recorded in 12 lines carrying the submergence gene assessed through genotype analysis by using RM105 marker This found a high rate of polymorphism on 3 lines carrying submergence gene with size of 220bp, namely lines No 1 (IR64sub1, No.8 (F7-23) and No. 9 (F7-28-1). The bands with the size of 200bp were the same with OM6162 that mean susceptible with submergence.

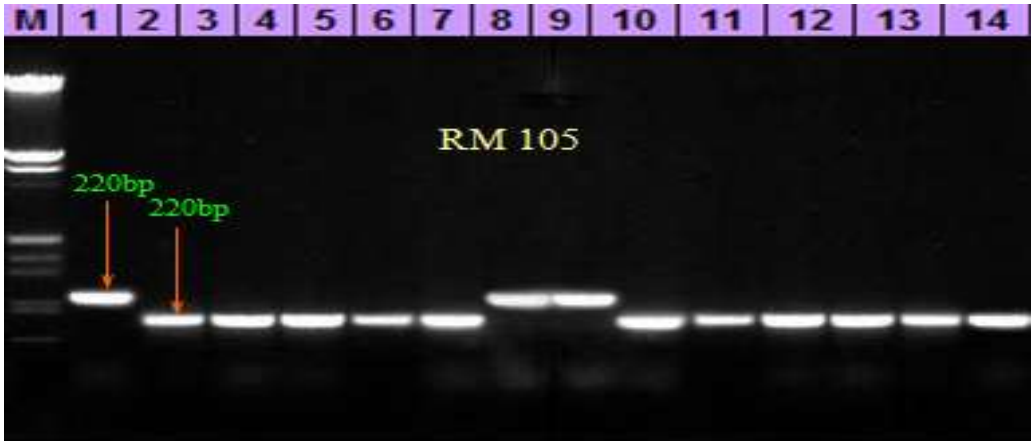

Figure 2: Product of PCR were marked by RM105 of the individuals in the population F7

Note: No. 1 (IR64Sub1); No. 2 (OM6162); No. 3 (F7-13); No. 4 (F7-15-1); No. 5 (F7-15-2); No. 6 (F7-22); No. 7 (F7-23), No. 8 (F7-28-1); No. 9 (F7-28-2); No. 10 (F7-29-1); No. 11 (F7-29-2); No. 12 (F7-30-1); No. 13 (F7-30-2); No. 14 (F7-30-3).

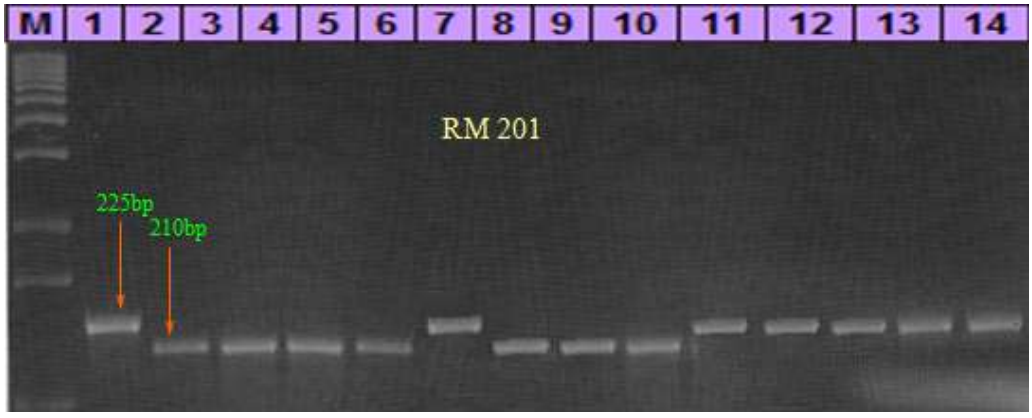

Figure 3: Product of PCR with RM201 of the individuals in the population F7

Note: No. 1 (IR64Sub1); No. 2 (OM6162); No. 3 (F7-13); No. 4 (F7-15-1); No. 5 (F7-15-2); No. 6 (F7-22); No. 7 (F7-23), No. 8 (F7-28-1); No. 9 (F7-28-2); No. 10 (F7-29-1); No. 11 (F7-29-2); No. 12 (F7-30-1); No. 13 (F7-30-2); No. 14 (F7-30-3).

With RM201 marker, the analysis recorded in two alleles. An allele with size of 225bp carried drought-tolerance genes, that were band number 6 (F7-22), 10 (F7-30-1), 11 (F7-30-2), 12 (F7-30-3), 13 (F7-30-2) and 14 (F7-30-3). An allele with size of 210bp carrying drought susceptible gene was on the band with locations of the same size for 2, 3, 4, 5, 7, 8 and 9.

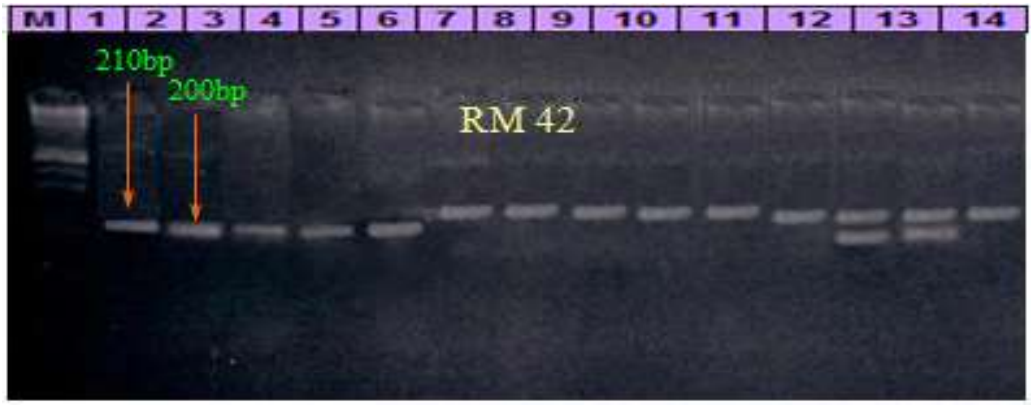

Figure 4: Product of PCR with RM42 of the individuals in the population F7

Note: No. 1 (IR64Sub1); No. 2 (OM6162); No. 3 (F7-13); No. 4 (F7-15-1); No. 5 (F7-15-2); No. 6 (F7-22); No. 7 (F7-23), No. 8 (F7-28-1); No. 9 (F7-28-2); No. 10 (F7-29-1); No. 11 (F7-29-2); No. 12 (F7-30-1); No. 13 (F7-30-2); No. 14 (F7-30-3).

With RM42 marker, there were two individuals carried a heterozygous gene (line number 12 and 13) and seven individuals expressed resistant gene (1, 6, 7, 8, 9, 10, 14) and 4 individuals were infection gene (2, 3, 4, 5) showed in Figure 4.

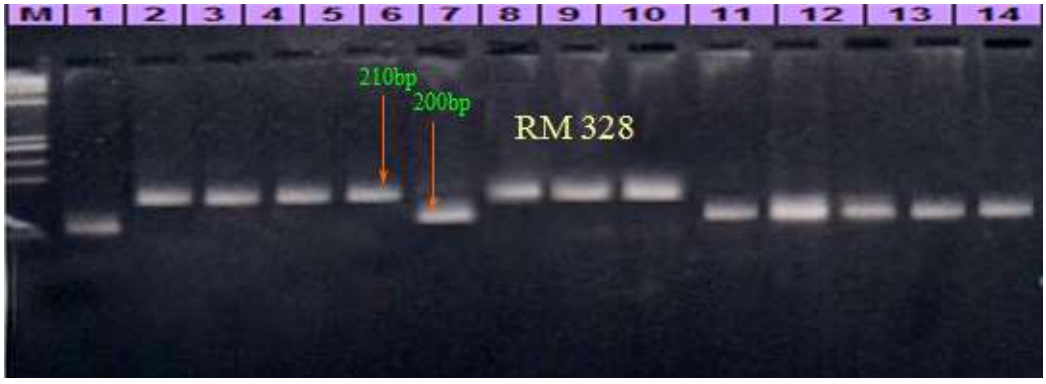

Figure 5: Product of PCR with RM328 of the individuals in the population F7.

Note: No. 1 (IR64Sub1); No. 2 (OM6162); No. 3 (F7-13); No. 4 (F7-15-1); No. 5 (F7-15-2); No. 6 (F7-22); No. 7 (F7-23), No. 8 (F7-28-1); No. 9 (F7-28-2); No .10 (F7-29-1); No. 11 (F7-29-2); No. 12 (F7-30-1); No. 13 (F7-30-2); No. 14 (F7-30-3).

Analysis with RM328 showed two alleles with size of 210bp and 200bp, in which the allele with size of 210bp expressed a resistant gene and the allele with size of 200bp expressed a susceptible gene. There were seven bands with the same size of 200bp, therefore these individuals expressed suscceptible gene. And there were 7 individuals which size of band was 210bp carrying resistance genes.

Assessment of quality of the promising lines

From the lines of F7 generation were selected after screening for both drought and submergence resistance, result in 12 lines were selected. Various qualities of the 12 lines from F7 are showed in Table 3. Only 4 lines have low amylose content less than 20%, that are two lines from crosses IR65191-3B-2-2-2-2/IR64 Sub1 and two lines from crosses OM6162/Swarna Sub1 which were F7-13, F7-28-1, F7-30-1 and F7-30-2.

Table 3. Analysis of quality trait on the single cross

N_0	Name of variety	Long grain (mm)	Width grain (mm)	Chalkiness (level)	Amylose content (%)	Gel consistency (cm)	Gelatini-zation temperature (level)	Protein content (%)	Aroma (level)	Phytic acide content (level)
1	**F7 -13**	**7,3a-e**	**3,1a-c**	**1**	**18,2h**	**77,5b**	**3**	8,5d-f	0	2
2	F7-15-1	7,5a-e	3,1b-d	0	24,5a	65,5ij	3	8,7b-d	0	2
3	F7-15-2	7,7a-c	3,2bc	1	23,5a-c	65,8h-j	3	7,4kl	0	2
4	F7-22	7,2d-f	3,5a	0	21,3ef	69,5f-i	3	9,1a	0	2
5	F7-23	7,4b-f	3,2bc	1	22,3c-e	70,2e-h	3	7,8ij	0	2
6	F7-28-1	7,0f	3,2bc	9	**16,2i**	90,0a	7	8,6c-e	0	2
7	F7-28-2	7,3c-f	3,1b-d	0	20,5fg	74,2b-e	3	8,9ab	0	2
8	F7-29-1	7,4b-f	3,0c-e	0	21,2ef	71,2d-g	3	8,7b-d	0	2
9	F7-29-2	7,6a-d	3,0c-e	0	21,2ef	72,3c-g	3	8,5de	0	2
10	F7-30-1	7,8ab	3,3ab	0	**18,2h**	77,4b	5	8,8bc	1	2
11	F7-30-2	7,9a	3,2bc	0	**18,6h**	77,6b	5	8,7b-d	1	2
12	F7-30-3	7,4b-f	3,1b-d	0	23,2a-d	75,6b-d	3	7,8ij	0	2
13	IR64Sub1 (Submergence Resistant check varietiy)	7,5a-e	3,2bc	0	24,1ab	60,6kl	3	8,5de	0	2
14	OM6162 (Drought Resistant check variety)	7,6a-d	3,1b-d	0	22,1de	71,2d-g	3	8,7b-d	0	2

CONCLUSION

In short, among 7 hybrid combinations 12 promising lines were retested with 4 molecular markers corresponding to flanking linkage marker for a drought tolerance gene and a submergence tolerance gene. Results of 23 markers have polymorphism and found good homozygote and hetezygote lines with high productivity.

The analysis of crosses showed that hybrids had good tolerance to drought conditions. However, the variation was very great.

Four lines have low amylose content below 20% and two slight scented lines. These lines need to be continued for further using in breeding programme.

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REFERENCE

Bertrand C. Y. Collard and David J. Mackill. 2008. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Phil. Trans. R. Soc. B 363, 557–572.

Bonnett, D. G., Rebetzke, G. J. & Spielmeyer, W. 2005. Strategies for efficient implementation of molecular markers in wheat breeding. Molecular Breeding. **15**, 75–85.

Koebner, R. M. D. & Summers, R. W., 2003. 21st century wheat breeding: plot selection or plate detection? Trends Biotech. **21**, 59–63.

Mackill D.J., 2006. Breeding for resistance to abiotic stresses in rice: the value of

quantitative trait loci. In: Lamkey KR, Lee M (eds) Plant breeding: The Arnel R Hallauer international symposium. Blackwell Pub, Ames, IA, pp 201–212

Huang Y, Zhang L, Zhang J, Yuan D, Xu C, Li X, Zhou D, Wang S, Zhang Q., 2006. Heterosis and polymorphisms of gene expression in an elite rice hybrid as revealed by a microarray analysis of 9198 unique ESTs. *Plant Molecular Biology.* **62**:579–91

Nguyen Thi Lang (2002), Basic Methods in biotechnology research, Agricultural Publisher, Ho Chi Minh City.

N.T. Lang, C.T. Nha, P.T.T. Ha and B. C. Buu, 2013a. Quantitative trait loci (QTLs) associated with drought tolerance in rice (*Oryza sativa*). L.SABRAO Journal of Breeding and Genetics **45** (3) 409-421

Nguyen Thi Lang, Nguyen Van Hieu, Tran Thi Nhien, Bui Phuoc Tam, Vo Thi Tra My, Bui Chi Buu, Romeo V. Labios, Abdelbagi Ismail, Russell Reinke and Reiner Wassmann, 2013b. Enricing gene pool to enhance rice productivy under submergence and medium stagnant water stresses in Mekong Delta. Omonrice 19. *Agricultural Publishing House*. 19:89-96.

Nguyen Thi Lang, Pham Thi Thiu Ha, Chau Thanh Nha, Nguyen Van Hieu, Doan Van Hon, Abdelbagi Ismail, Russell Reinke and Bui Chi Buu, 2013. Introgression of sub1 gene into local popular vaerities and newly developed elite breeding lines in the Mekong Delta adap to the climate change. Omonrice 19. Agricultural Publishing House 19:27-29.

Yashitola, J., Thirumurugan, T., Sundaram, R. M., Naseerullah, M. K., Ramesha, M. S., Sarma, N. P. & Sonti, R. V., 2002 Assessment of purity of rice hybrids using microsatellite and STS markers. *Crop Science*. **42,** 1369–1373.

Yongzhong Xing and Qifa Zhang, 2010. Genetic and Molecular Bases of Rice Yield. ANRV410-PP61-11 ARI 22 January 2010 10:37

TÓM TẮT

Chọn dòng lúa triển vọng của thế hệ F7 có kết hợp hai tính trạng chịu mặn và chịu ngập thông qua sử dụng dấu chuẩn phân tử

Từ 7 tổ hợp lai được lựa chọn và đánh giá hai chỉ tiêu phối hợp khô hạn và ngập kết quả chọn được 12 dòng ở thế hệ F7. Để tiếp tục chọn lựa các dòng thuần, 4 chỉ thị phân tử trên các nhiễm sắc thể số 9 được đánh dấu và lựa chọn để tìm các dòng thuần. Qua đánh dấu cho thấy có các dòng cho đa hình rất tốt với các chỉ thị RM105, RM201, RM38 và RM42.

Ngoài ra trong phân tích đánh giá năng suất kết hợp các dòng ngắn ngày có tiềm năng năng suất cao bao gồm các dòng như dòng số 5, 6 và số 7. Các dòng này được tiếp tục đánh giá về phẩm chất, kết quả ghi nhận các dòng sau đây có hàm lượng amylose dưới 20% là dòng số 1 (F7-13) từ cặp lai IR75499-84-1-B//IR64Sub1 (18,2%), dòng số 6 (F7-28-1) từ cặp lai IR65191-3B-2-2-2-2/IR64Sub1 (16.2%), dòng số 10 (F7-30-1) từ cặp lai OM6162/ Swarna Sub1 (18,2%) và dòng số 11 (F7-30-2) từ tổ hợp lai OM6162/ Swarna Sub1 (18,6%). Ngoài ra có hai dòng thơm là dòng 10 và dòng số 11 từ tổ hợp lai OM6162/ Swarna Sub1. Các dòng này cần tiếp tục đưa ra so sánh và khảo nghiệm trong các vụ khác nhau để đưa vào sản xuất.

Từ khóa: *Amylose, mùi thơm, chống chịu khô hạn, chịu ngập, chỉ thị phân tử.*