INTROGRESSION OF *Sub1* GENE INTO LOCAL POPULAR VARIETIES AND NEWLY DEVELOPED ELITE BREEDING LINES IN THE MEKONG DELTA ADAPT TO THE CLIMATE CHANGE

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ABSTRACT

The development of high yielding rice varieties tolerant to submergence (flash flood) has been conducted in collaboration with IRRI and ACIAR. Flash flood is considered as major constraints in the rainfed lowland ecosystem of the Mekong Delta in wet season. Our goals focus on the advantages of molecular biology and genetics to develop rice varieties that have higher and more stable yield in saline and acid-sulphate areas as well as in areas affected by submergence. Base on the molecular biology research, it will assist in identifying genes that tolerate to different abiotic stresses. Furthermore, the basic information on the genetics of additional mechanisms and QTLs can help us combine them through conventional and marker-assisted breeding in high-yielding cultivars to achieve higher levels of tolerance. The integration of conventional and modern methods should be paid attentions, including the application of recent scientific achievements in order to improve the scientific basis for the further development of Vietnamese rice production, to build up genetic stocks and improve phenotypic evaluation methods. Marker-assisted selection (MAS) has proven a powerful tool in rice breeding programs and has been using extensively in submergence tolerance of locally-adapted rice varieties and elite lines. A further development of MAS is marker-assisted backcrossing (MABC). This technique is used for submergence tolerance (SUB1 gene).

***Keywords**: marker-assisted breeding, marker-assisted backcrossing, submergence (flash flooding), quatitive traits loci (QTLs)*

INTRODUCTION

Mekong River Delta is a one of the most important granary for the global rice supply, any deterioration of the production levels would threaten food security in a large scale. Higher water level in vast parts of the Mekong Delta if the sea level rises, resulting in more flooding. A vulnerability and risk need to be analysed to agricultural ecosystems and for flood forecasting and warning higher risks for rice production in the Mekong delta. Flash flood occurred in short period resulted in the hard afford in rice production, sometime it combined to salt intrusion due to sea level rising. Flash flood occurred more often during November and December in short period. Flash flood lasts for 7-10 days at the early stage of the rice plants, mostly after direct seeding. Factors cause flash flood to be considered as: a relatively low level of the rice field, high tides, heavy rains, typhoon affects. Farmers are not able to predict flash flood situation. Thus, it causes dead of the rice plants, usually at the early stage. To overcome this situation, farmers replant and filling the gaps with seedlings or seed broadcasting again. A major QTL, named *Saltol*, was mapped on rice chromosome 1 (Bonilla *et al.*, 2002). Recently, this locus has been fine-mapped and a marker-assisted backcrossing (MABC) system is developed and successfully used for its introgression into few popular varieties that sensitive to salt stress (Ismail *et al.*, unpublished). *Sub1*, a major QTL for submergence tolerance, is identified on chromosome 9 and recently cloned (Xu *et al.*, 2006). Molecular markers are developed and used in breeding numerous submergence tolerant versions of popular varieties; some of them are recently released in few countries in Asia. The advantage of the *Sub1* locus has been assessed in field experiments demonstrating a 2–3-fold yield increase (1.0 to 3.5 t/ha yield advantage) over intolerant varieties following submergence (Singh *et al.*, 2009). The MABC system used for the introgression of these two QTLs involves using background markers to completely recover the genetic constitution of the recurrent parents except for the new tolerance QTL (Septiningsih *et al.*, 2009). Progress is also made in understanding the genetics and physiology of tolerance to flooding during seed germination in rice. Donors were identified and characterized, major QTLs were identified for fine-mapping that used in breeding through MABC (Ismail *et al.*, 2009; Angaji *et al.*, 2009).

MATERIALS AND METHODS

Plant materials

Two BC_2F_1 populations of OM1490/IR64 *Sub1* and OMCS2000/IR64-*Sub1* for submergence tolerance were grown in the field. Screening for submergence tolerance was done by CLRRI protocol (Lang *et al.*, 2011).

DNA isolation and PCR amplification

Protocol for DNA extraction and PCR amplification was done according to the procedure by Zheng *et al.* (1995) and modified by Lang (2002).

Statistical analysis

Analysis of variance (ANOVA) of plant traits were carried out for each of the two stress treatments. Then the least significant differences were notice at the level of 0.05. The statistical analysis was done by using software CropStat version 7.2.

QTL analysis

Single-marker QTL analysis using linear regression was employed (Tanksley 1993). The marker alleles link to submergence was coded 1; and in contrast, coded 0 for conducting regression analysis. Markers-QTLs association for each trait, single-point (single marker) analysis of QGene computer program (Nelson 1997) were employed.

RESULTS AND DISCUSSIONS

Effect of submergence on survival and growth

Plant survival after 14-d submergence

Significant differences in percentage survival of plants was observed between tolerant and intolerant genotypes. Under flooding, all genotypes experienced reduction in survival, especially the intolerant genotypes. Percentage survival was lowest in OM1490 and highest in IR64 *Sub1* after 14 days submergence. IR64 *Sub1* expressed the highest survival of 79.5%. The low light intensity in the greenhouse and the high temperature of floodwater might have contributed to the relatively low percentage survival even in the tolerant genotypes. Lower light intensity and warmer temperatures are known leading to reduce biomass and increase mortality.

Shoot and root growth

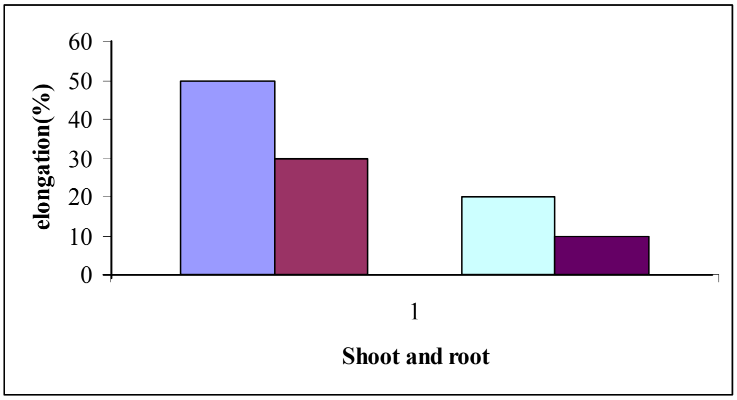

Figure 1. Effect of submergence on shoot and root elongation of IR64-*Sub1* and OM1490. Values are averages of three replication and the vertical bars represent ± SE.

IR64-sub1 in blue and OM1490 in brown bar represented percentage of shoot elongation and IR64-sub1 and OM1490 in green and purple bar represented percentage of root elongation respectively

Under submerged conditions, shoot elongation occurred in all genotypes but to a lesser extent in the tolerant genotypes than in the sensitive genotypes. IR64-*Sub1* and OM1490 showed higher elongation rate coupled with lower survival. Conversely, the sensitive genotypes showed decreased root elongation as compared to tolerant genotype (IR64-*Sub1*). Tolerant genotypes showed suppressing of shoot elongation may be for energy conservation under floodwater, but still maintained significantly higher root growth than the intolerant genotypes during submergence.

Growth and physiology - dry matter accumulation

Submergence affected on growth and dry matter accumulation of all genotypes. Before submergence, shoot dry weight of IR64-*Sub1* was higher than of OM1490. After 14- days submergence, the shoot dry weights of IR64-*Sub1* increased significantly whereas those of other genotypes generally decreased (Fig. 2). On the other hand, root dry weight, decreased significantly in all genotypes during submergence (Fig. 3).

Evaluating BC_1F_1 population

Results of phenotypic survival day for submergence tolerance or susceptibility using the data received from the seedlings of population 1 and 2 are presented in Fig 4. A mong of 189 plants in population 1 (OMCS2000 / IR64 *Sub1*), 42 lines were classified as highly tolerant (HT) (visual score of 55 -80), 23 lines fell into the tolerant group (T) (visual score of 40-50%), 29 lines were moderately tolerant (MT) (visual score of 30-40%), 53 lines were susceptible (S) (visual score of 20-30%), and 19 lines were highly susceptible (HS) (visual score of 0-10%). The frequency distribution of population 1 was continuous but skewed towards the susceptible parent (OMCS2000).

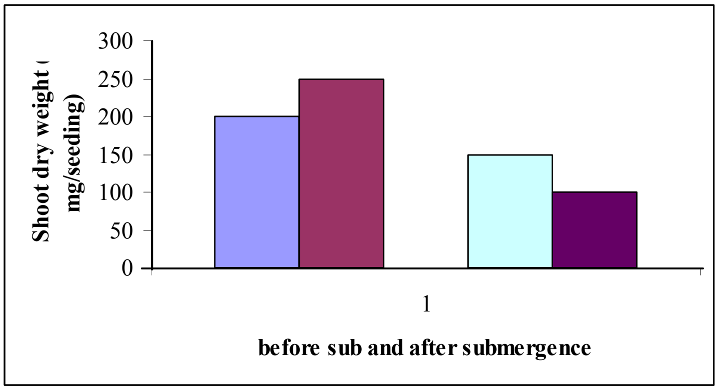

Figure 2. Shoot dry weight of IR64 *Sub1* and OM1490 rice genotypes before and after 14-d submergence. Values are averages of three replications and the vertical bars represent ± SE.

In population 2, there was variation among the recombinant inbred lines to submergence tolerance (Fig. 4). In total of 148 lines evaluated in population 2, 24 lines were highly tolerant, 35 lines were tolerant, 57 lines moderately tolerant, 37 lines were susceptible and 18 lines were highly susceptible. The frequency distribution of the population was continuous but skewed towards susceptibility (OM1490).

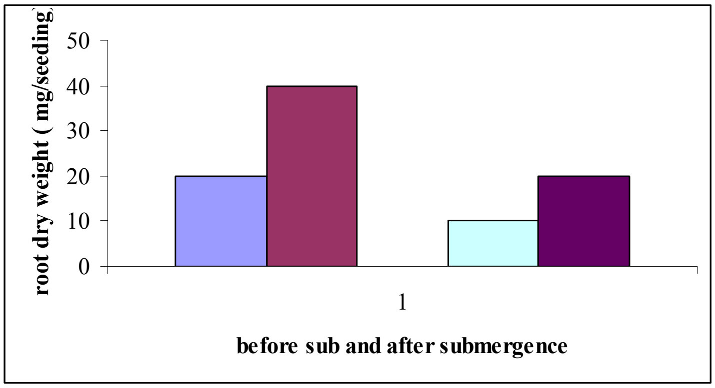

Figure 3. Root dry weight of IR64 *Sub1* and OM1490 rice genotypes before and after 14-d submergence.

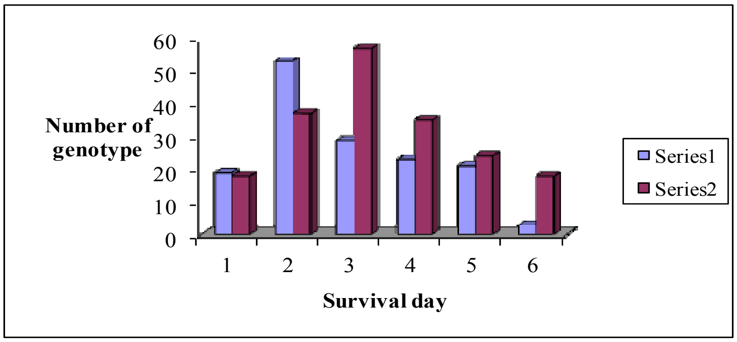

Figure 4. Frequency of percentage survival for submergence tolerance of BC_1F_1 lines at the seedling stage of two populations

Phenotypic variation within the BC_2F_1 population derived from OM1490/IR64-*Sub1* and OMCS2000/IR64 *Sub1*

OM1490, IR64-*Sub1*, IR42 as the control varieties together with 100 BC_1F_2 lines were evaluated for survival score. There was significant recombination of submergence tolerance in the population. Large differences in survival under flooded conditions are noticed in the populations, ranging from 0.0%-79.5% (Lang *et al*., 2011).

Among the 100 BC_2F_1 family of the cross OMCS2000 / IR64-*Sub 1*, there was wide variation observed for survival under flooded condition. The frequency distribution of 0.5%-85.0% survival in flood reaction among the BC_2F_1 was observed. This showed a good recombination for submergence tolerance in the population. Out of 100 BC_2F_1 progenies from the selected plants, about 100 plants were selected based on their possession of the *Sub1* locus and maximum recipient genome, with a similar size of *Sub1* introgression as the first version of OM1490/IR64 *Sub 1*.

Phenotypic variation within the BC_2F_2 population derived from OM1490 / IR64-*Sub1*

Three of control varieties as OM1490, IR64-*Sub1*, IR42 together with 100 BC_2F_2 progenies were evaluated for genotypes. The data noticed that they were higher genetic diversity showing in grain yield and of other traits. There was significant recombination for submergence tolerance in the population (Fig. 5). Large differences in survival under flooded conditions were noticed in the populations, ranging from 0-99% (Fig. 6-10). Among the 168 BC_2F_2 progenies of the cross OMCS2000 / IR64-*Sub 1*, there was wide variation observed for percentage of survival under flooded conditions. The frequency distribution of percentage of survival in flooded reaction among the BC_2F_2 was showed to be continuous. This indicated a good recombination for submergence tolerance in the population. Out of 100 BC_2F_2 progenies from the selected plants, about 100 plants were selected based on their possession of the *Sub1* locus and maximum recipient genome, with a similar size of *Sub1* introgression as the first version of OM1490 / IR64 *Sub 1*.

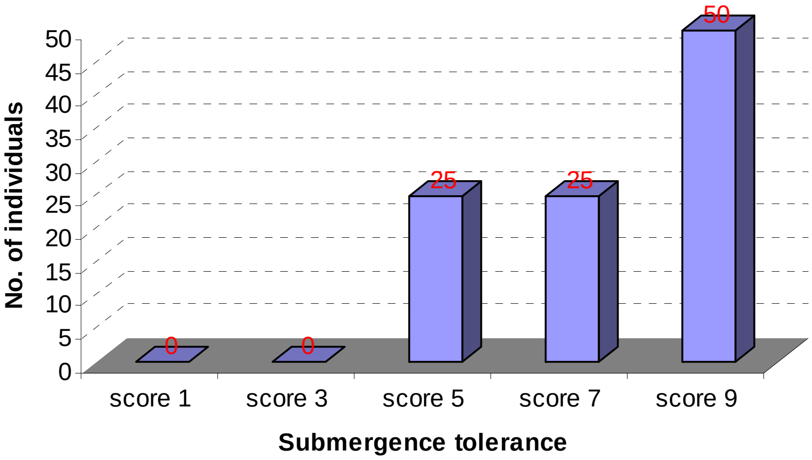

Figure 5. Frequency distribution of submergence tolerance in BC_2F_2 population of OM1490 / IR64-Sub1.

Frequency distribution of the varieties with respect to maturity, panicles per plant, number of filled grains, number of unfilled grains, 1000-grain weight, grain yield, biomass, harvest index and survival day after submergence stress show the diversity of two populations. These quantitative characters were found to be significant at 1% level. All measurements were not too far from normal distribution (Fig. 6-10). The abnormal distributions in two populations are indicated in figure 7 and 10. Distribution of varieties for the number of filled grains slightly skewed to the right with only a few varieties near the maximum value (Fig. 6) while distribution of varieties for the number of unfilled grains was slightly skewed to the left (Fig. 6 and 9) in two populations. Panicles / hill of two populations exhibited the normal distribution, the curve slightly skewed to the left (Fig. 6 and 9). The traits as 1000-grain weight, grain yield and panicles per plant represented by unimodal distributions with the curve skewed to the left. Such distribution is favourable particularly with number of unfilled grains because lower number of unfilled grains that gives higher yield. This is an important objective for most plant breeders in improving present day varieties. Grain yield nearly exhibited normal distribution, the curve slightly skewed to the left with only a few lines reached to the maximum value in BC_2F_2 population (Fig. 8 and 10). Regarding to maturity, almost half of the investigated genotypes exhibited short growth duration of 90-95 days. This fully meets the demand of the Mekong Delta's rice production. Phenotypic measurement is very important in tagging QTL, because quantitative traits are largely affected by environment like submergence tolerance. Phenotypic frequency distributions support the quantitative inheritance of submergence tolerance gene. There are large differences in those traits. Rice genotypes that exhibit limited elongation during submergence are usually more tolerant of submergence (Setter and Laureles, 1996; Ram *et al*., 2002; Jackson and Ram, 2003; Sarkar *et al*., 2006), and this agrees with our findings in the present study.

DNA survey among parents

Electrophoretic analysis of PCR products derived from OM1490 and IR64-*Sub1* using four primers were screened for DNA polymorphism between the parents. Two markers as RM219 and RM105 (Fig. 11) well exhibited polymorphism between the parents.

Selection of elite rice varieties

A total of 105 SSR markers randomly distributed on 12 chromosomes of rice were chosen to scan for the polymorphism between parents. Of these 30 markers were found polymorphic and suitable for background selection in two populations (OM1490/IR64 *Sub 1* and OMCS 2000/IR64 *Sub1*). Approximately 90% of the SSR markers were polymorphic between the parents, OM1490 and IR64 *Sub1*. The BC_1F_1 population was evaluated using 51 SSRs for OM1490/Pokkali and 54 SSRs exhibited polymorphism in case of OMCS2000/Pokkali. In two telomeric regions of the genome, the long arm of chromosome 1 showed extensive polymorphism with the markers. In a selected BC_1 population, the expected segregation ratio was 47.49% homozygotes (OM1490) and 52.51 % heterozygotes (OM1490/IR64-*Sub1*). This has the same in an allele frequency of population. For population 2, the ratio in this population was 50.03% and 49.87% homozygous and heterozygous ones, respectively.

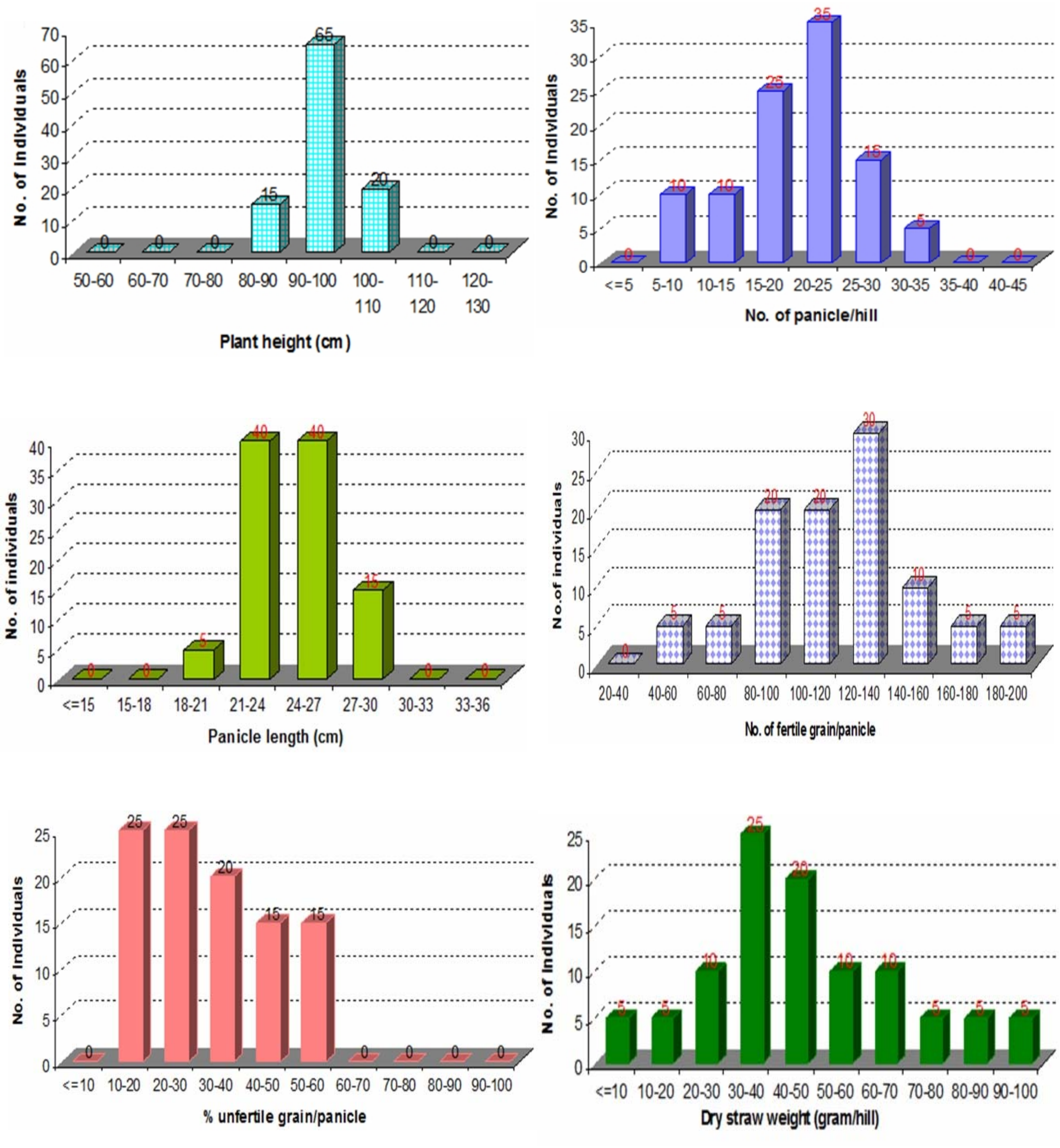

Figure 6. Frequency distributions of measured parameters in BC_2F_2 population of OM1490/IR64-*Sub1* (n=100).

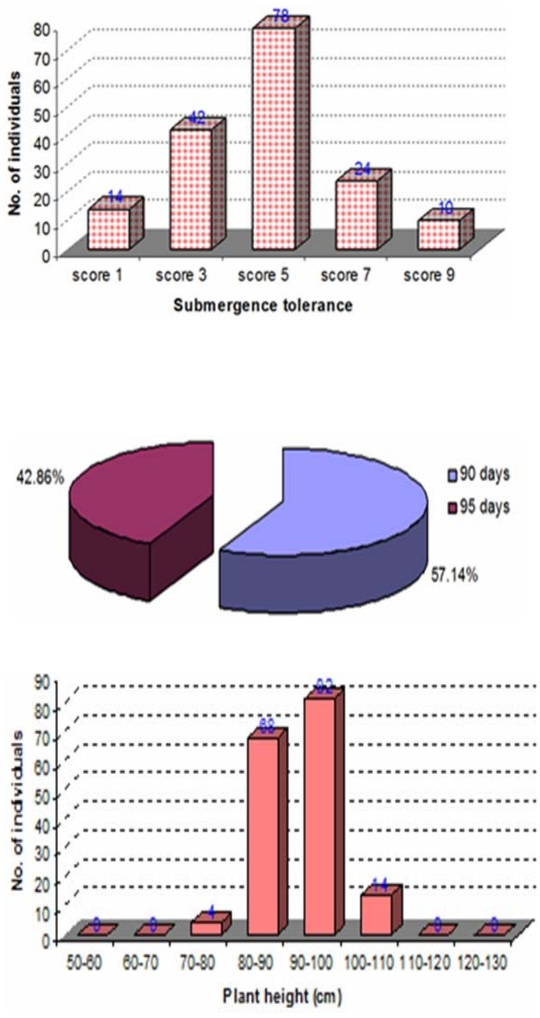

Figure 7. Frequency distributions for submergence tolerance score, days to flowering and plant height in BC_2F_2 population of OMCS2000 / IR64-SUB1 (n=168).

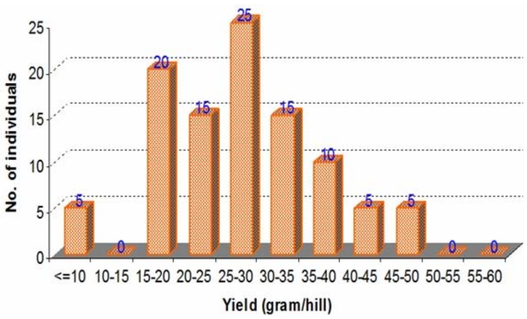

Figure 8. Grian yield (g/hill) of OM1490 / IR64-*Sub1* population before and after 14-d submergence

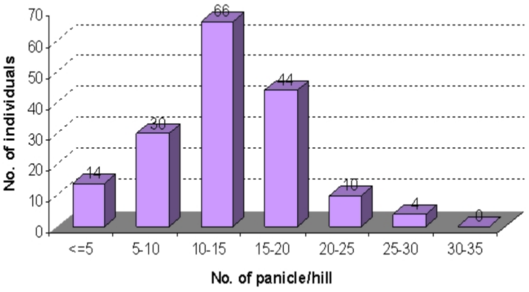

Figure 9. Frequency distribution for panicles/hill in BC_2F_2 population of OMCS2000 / IR64-SUB1 (n=168).

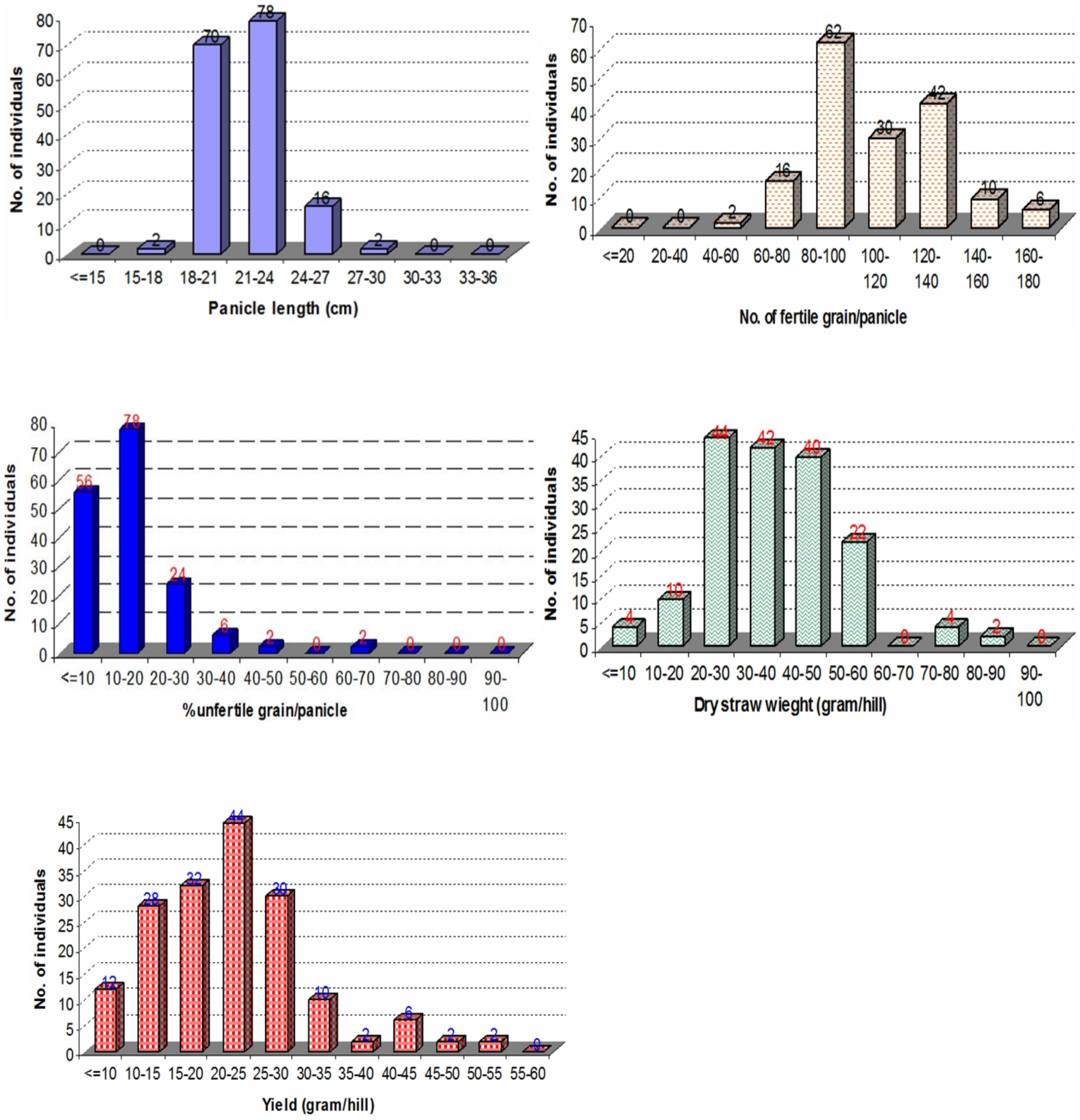

Figure 10. Frequency distribution for panicle length, fertile grains/panicle, sterile florets/panicle, dry weight of straw (grams/hill) and yield (grams/hill in BC_2F_2 population of OMCS2000 / IR64-SUB1 (n=168).

Quantitative trait loci (QTL) analysis

Single marker analysis revealed that a weak association with submergence tolerance at the seedling stage (survival days) in populations 1 (Table 1). Three QTLs were obtained at this segment submergence in rice. RM105, RM219 and RM23662 were found to be associated with survival days ($P<0.05$, 0.01) under submergence stress. In population 1, the data revealed that there are QTLs for submergence tolerance as measured by survival days in this region on chromosome 9. The phenotypic variation explained by the three QTLs was 24.4, 18.64 and 3.06% of the total variance in BC_1 of population 1. In population 2 (OMCS2000/IR64 *Sub1*), QTL related to submergence tolerance at the seedling stage (survival days) was found to be weak association. The phenotypic variation obtained in this validation studies ranged between 2.6-3.15% at locus S12055.

Table 1. QTL identified for submergence tolerance trait at seedling stage in population 1 (OM 1490/IR64 *Sub 1*)

Traits	**QTL**	**Markers**	**R^2**	**P value**
Survival days	QTL1	RM105	24.81	0.000
	QTL1	RM219	18.64	0.000
	QTL1	RM23662	3.03	0.049

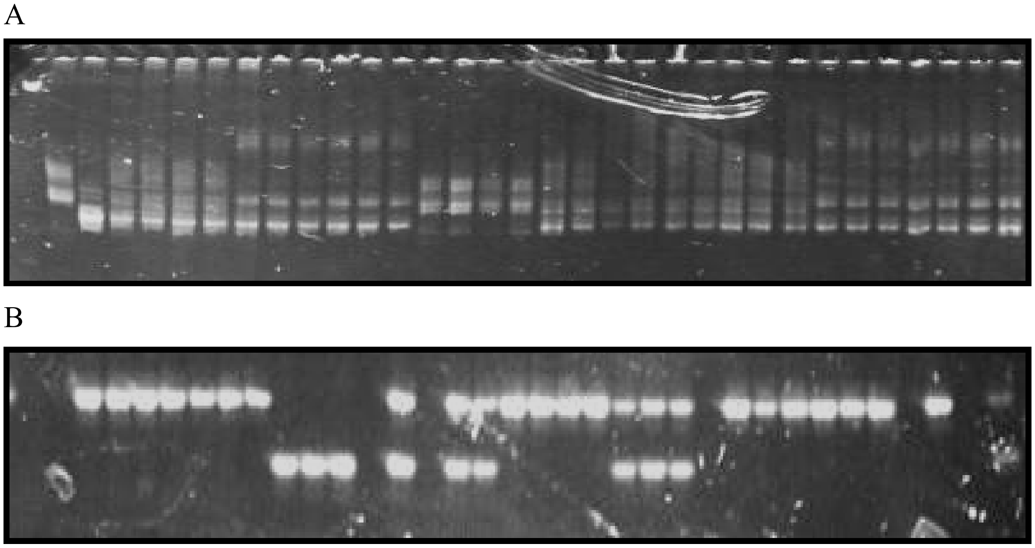

Figure 11. DNA bands amplified from leaves of BC_1F_1 population (OM1490/IR64-*Sub1*) using microsatellite markers (A) RM219 and (B) RM105 in an 8% polyacrylamide gel. Ladder = 100 bp

Analysis of the BC_2F_1 population

Based on the phenotypic evaluation of submergence gene and genotypic information derived from RM23662 and RM105 markers for the BC_2F_1 population, the two extremes of the population were identified for selective genotyping. A total of 190 lines identified by DNA markers, which showed polymorphism in parental survey were selected to evaluate the polymorphism between the BC_2F_1 progenies. The marker RM23662 showed polymorphism within the BC_2F_1 of OM1490/IR64-*Sub1*. It could distinguish submergence tolerant lines from intolerant ones. This polymorphism, therefore can be used as a marker to distinguish between the submergence gene and its allelomorph, which cannot tolerate submergence. The bands corresponding to an allele from parent OM1490 and IR64-*Sub1* were 240 bp and 230 bp respectively. In the early applications of MAB for developing submergence-tolerant varieties, the RM219 was used as diagnostic marker. Submergence tolerance of BC_2F_1 hybrids of OM1490/IR64-Sub1 was compared with that of the parents and IR42. The heterozygous plants were significantly less tolerant than the plants homozygous for the tolerant allele (Fig. 8). In addition, the expression of the OM1490 / IR64-*Sub1* allele in the heterozygotes was less than its expression in the homozygotes (Fig. 8). The results indicate that the *Sub1* tolerance locus is closely associated with the expression level and the dosage effect of the homozygous condition confers greater survival in flooded conditions. Genotyping is currently in progress for OMCS2000 / IR64-*Sub1* population.

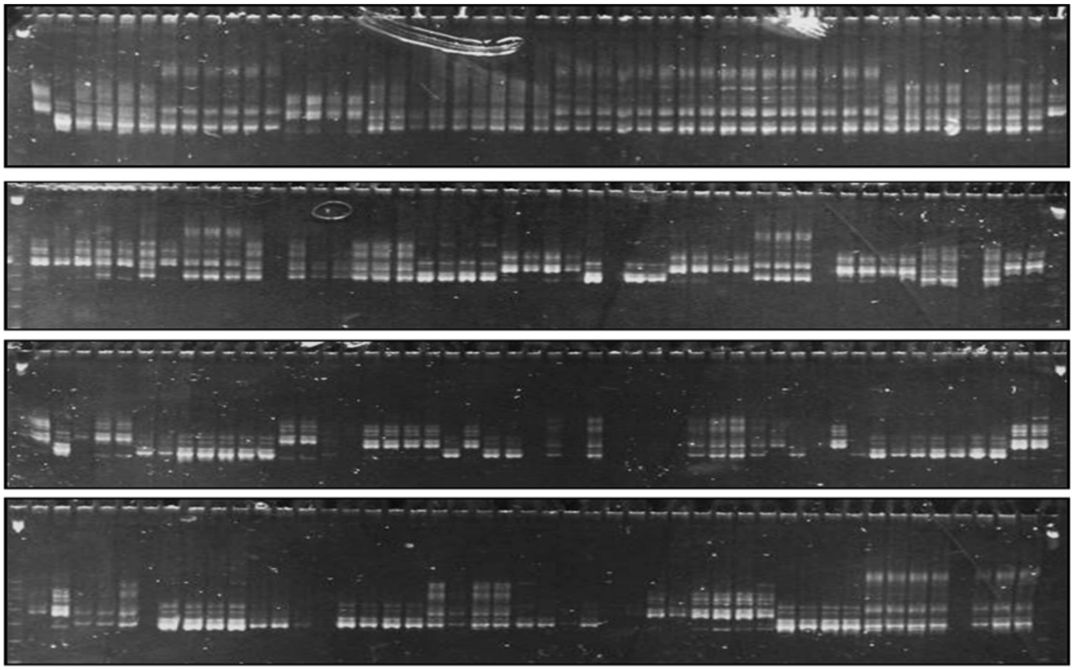

Figure 1. PCR products amplified from DNA of BC_2F_2 population (OM1490 / IR64-Sub 1) rice genotypes using microsatellite marker RM 219 on a 5% polyacrylamide gel. Ladder = 100 bp. The left lane is the ladder, then parents, progenies 1-48 on the top-most gel, lines 49-96 on gel 2, lines 97-144 in gel 3, and lines 145-190 on the bottom gel.

CONCLUSION

The tolerant genotype IR64-Sub1 has slower shoot elongation when submergence as compared to the sensitive genotypes OMCS2000 and IR64. This show elongation is associated with higher percentage survival. Some lines are highly tolerant to submergence, while others are very sensitive. This can be interpreted that under certain submergence condition, plant growth, dry shoot weight, and dry root weight are more severely affected by environment. The QTLs related to submergence tolerance excluded the major gene are in chromosome 9. Three microsatellite loci are tightly detected minor genes for submegence tolerance *e.g.* RM105, RM219 and RM23662 are found to be associated with survival days ($P<0.05$, 0.01) under submergence stress in population 1, which reveals that there are QTLs for submergence tolerance as measured by survival days in this region of chromosome 9. In population 2, single marker analysis reveals that it is weak association with submergence tolerance at the seedling stage (survival days).

Simultaneous or sequential transfer of marker-linked QTLs at different developmental stages may lead to the development of cultivars with submegence tolerance throughout the ontogeny of the plant. This study has provided much more detailed information on the relative importance of genomic segment and has increased our understanding of the genetic basis of submergence tolerance. Further studies are needed to confirm the estimation of fine mapping in marker-assisted selection for submergence tolerance.

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KHAI THÁC GEN *Sub1* ĐƯA VÀO CÁC GIỐNG LÚA BẢN ĐỊA VÀ NHỮNG DÒNG CON LAI ƯU TÚ ĐÁP ỨNG VỚI BIẾN ĐỔI KHÍ HẬU Ở ĐBSCL

Sự phát triển của các giống lúa năng suất cao chống chịu điều kiện ngập hoàn toàn, đang được tiến hành với sự hợp tác giữa IRRI và Úc. Lũ lụt là trở ngại chính tại các hệ sinh thái tại các vùng đất trũng dưới điều kiện mưa tự nhiên tại vùng ĐBSCL. Người ta tập trung vào nghiên cứu tạo ra được tác động nhanh chóng nhằm khai thác hiệu quả những tiến bộ trong sinh học phân tử và di truyền học, để phát triển các giống lúa có năng suất cao và ổn định tại các vùng đất bị xâm nhập mặn, đất phèn, cũng như các khu vực bị ảnh hưởng do điều kiện ngập. Trên cơ sở nghiên cứu sinh học phân tử, kết quả này sẽ hỗ trợ trong việc xác định gen chống chịu kèm theo những điều kiện phi sinh học khác nhau. Hơn nữa thông tin cơ bản về di truyền học của các cơ chế bổ sung và các QTLs có thể giúp đỡ nhà chọn giống kết hợp phương pháp truyền thống với sự hỗ trợ của marker phân tử nhằm tạo ra giống lúa cao sản có khả năng chống chịu stress phi sinh học tốt hơn. Chọn lọc có sự trợ giúp của marker (MAS) chứng minh rằng đây là là một công cụ mạnh mẽ trong chương trình chọn giống lúa, được sử dụng rộng rãi trong cải thiện khả năng chống chịu ngập. Bên cạnh đó, phương pháp hồi giao có sự trợ giúp của chỉ thị phân tử (MABC) cũng được khai thác có hiệu quả trong nghiên cứu này