Indonesian Journal of Agricultural Science
Vol. 18 No. 1 June 2017: 7–16
DOI: http//dx.doi.org/10.21082/ijas.v.18.n1.2017.p.7–16

PHENOTYPIC PERFORMANCE OF CIHERANG SUB1 NEAR ISOGENIC LINE AS AN ADAPTIVE VARIETY FOR FLOODING CONDITIONS

Penampilan Fenotipik Galur Isogenik Ciherang Sub1 sebagai Varietas Tahan Genangan

Yudhistira Nugraha[a]*, Nurul Hidayatun[b], Trisnaningsih[a], Dini Yuliani[a], Shinta Ardiyanti[a] and Triny Suryani Kadir[a]

[a]*Indonesian Center for Rice Research*
Jalan Raya No. 9 Sukamandi, Subang 41256, West Java, Indonesia
[b]*Indonesian Center for Agricultural Biotechnology and Genetic Resource Research and Development*
Jalan Tentara Pelajar No 3A, Bogor 16111, West Java, Indonesia
**Corresponding author: yudhistira.nugraha@gmail.com*

Submitted 21 May 2016; Revised 3 April 2017; Accepted 10 April 2017

ABSTRACT

Marker assisted back crossing (MABC) is a molecular tool that can help breeders in reducing backcrossed generation. However, effectiveness of this method still needs further approval using actual phenotypic performances. The International Rice Research Institute had developed Ciherang near isogenic line (NIL) of submergence tolerance, Sub1. The study aimed to evaluate phenotypic performances of Ciherang Sub1 NIL in the greenhouse and field conditions. The study was conducted in ten locations using five submergence-tolerant varieties and a control treatment under normal conditions. The results showed that the average grain yields and some agronomic traits of Ciherang Sub1 were not significantly different compared with those of Ciherang (recurrent parent). However, under 10- and 15-days of submergence. Ciherang Sub1 was significantly different to Ciherang. The survival rate of Ciherang Sub1 was higher than Ciherang after 14-days submerged in the greenhouse tank experiment. Response of Ciherang Sub1 to brown planthopper biotype 1, 2 and 3, *Xanthomonas oryzae* pathotype III, IV and VIII, and rice tungro virus inocula from Subang, Magelang and Lanrang were also comparable with its recurrent parent. Quality and physico-chemical properties of rice (milled rice) of Ciherang Sub1 were not different with those of Ciherang. Similarity level of phenotypic traits of Ciherang Sub1 compared to Ciherang was more than 87.5%. This finding proved that a single backcross method can produce progeny identic with its parent. This MABC line can be recommended to farmers in flood-prone area where the Ciherang is preferred.

[**Key words**: Ciherang Sub1, Euclidean, grain yield, near isogenic lines, rice, submergence]

ABSTRAK

Marker assisted back crossing *(MABC) merupakan teknik molekuler yang dapat membantu pemulia dalam mengurangi generasi yang dibutuhkan dalam pemuliaan silang balik. Namun, efektivitas metode tersebut masih perlu dibuktikan melalui kinerja fenotipe yang sesungguhnya.* International Rice Research Institute *(IRRI) telah merakit varietas padi toleran rendaman menggunakan metode MABC dengan latar belakang genetik varietas padi yang populer di Indonesia, yakni Ciherang. Penelitian ini bertujuan untuk mengevaluasi penampilan fenotipe galur isogenik Ciherang Sub1 di rumah kaca dan di lapangan. Uji daya hasil pada kondisi normal di 10 lokasi menggunakan lima varietas toleran rendaman dan satu varietas pembanding. Hasil penelitian menunjukkan rata-rata hasil dan sejumlah karakter agronomi tidak berbeda nyata. Namun, uji rendaman di lapangan selama 10 dan 15 hari menunjukkan Ciherang Sub1 berbeda nyata dengan Ciherang. Demikian pula pada pengujian di rumah kaca pada fase bibit selama 14 hari rendaman, Ciherang Sub1 memiliki persentase bibit hidup lebih tinggi dibandingkan dengan Ciherang. Respons Ciherang Sub1 terhadap cekaman biotik seperti wereng cokelat biotipe 1, 2, dan 3;* Xanthomonas oryzae *patotipe III, IV dan VIII; dan inokulum virus tungro dari Subang, Magelang dan Lanrang sama dengan tetuanya, yakni Ciherang. Penampilan kualitas fisik dan kimia beras Ciherang Sub1 juga sama dengan Ciherang. Kesamaan fenotipe antara Ciherang Sub1 dan Ciherang lebih dari 87,5%. Hasil penelitian ini menunjukkan bahwa silang balik gen target yang dilakukan satu kali dapat menghasilkan galur yang identik dengan tetuanya. Galur ini dapat direkomendasikan untuk ditanam di lahan yang bermasalah dengan banjir dan petaninya menyukai varietas Ciherang.*

[**Kata Kunci**: Ciherang Sub1, Euclidean, hasil gabah, galur isogenik, padi, rendaman]

INTRODUCTION

Recent advances in rice genomic research and completion of the rice genome sequence have made it possible to identify and map precisely several genes through linkage to DNA markers (Jena and Mackill 2008). Furthermore, the use of cost-effective DNA markers derived from the fine mapped position of the genes for important agronomic traits will provide opportunities for breeders to develop high-yielding, stress-resistant, and better-quality rice cultivars. DNA marker as a tool for selection was initially used for confirming the targeted gene in selected individual in pedigree or bulk population hence it is called as a marker assisted selection (MAS). Its usage had been expanded to back crossing selection, and it is termed as a marker assisted back crossing (MABC) (Collard and Mackill 2008). MABC may reduce the back cross generation, if the targeted gene and the genetic background of the recipient parent could be identified correctly. The recovery degree of the recurrent parent, however, may be offset by the smaller number of selected plants during the process of applying MABC.

Swarna-Sub1 and IR64-Sub1 were the first submergence-tolerant rice varieties developed using MABC approach in IRRI. Those two varieties performed similar agronomic traits, such as grain yield and grain quality with their recurrent parent (Singh et al. 2009). Further, those varieties showed higher grain yield advantage over their recurrent parent under submergence for 10 days or more during the vegetative stage (Sarkar et al. 2006; Neeraja et al. 2007; Septiningsih et al. 2009; Nugraha et al. 2013a). This indicated that there was a complete restoration of the recurrent parent genetic background. Those two varieties have been introduced in Indonesia and released as Inpara 4 and Inpara 5 in 2010 (Suprihatno et al. 2012) and the seed has been distributed to farmers in the flood-prone areas in Java and Sumatra (Ismail et al. 2013). However, result of a study in the flood-prone area of northern coast of West Java showed that the submergence-tolerant varieties were not adopted by farmers due to undesirable grain quality and susceptibility to pests and diseases (Manzanilla et al. 2011). Farmers preferred to plant submergence-sensitive variety, Ciherang, despite having to face a risk of devastated flooding during the wet season. Ciherang is not only preffered by farmers in this area but also occupies more than 45% of total rice planted area in Indonesia (Ruskandar 2010). It is important, therefore, to develop Ciherang-submergence tolerant variety to minimize crop losses due to unexpected inundation during rice growth and to accelerate its adoption by farmers who already familiar with Ciherang variety.

In the case of development of Ciherang Sub1, the SUB1 donor used in MABC was IR64 Sub1, the ancestor of Ciherang (IR64) which also has SUB1 (Ismail et al. 2013). This slightly close related genomic distance allows to apply ‘one back cross strategy’ by introgressing a major QTL/gene, such as Sub1, in a relatively short time, i.e. three seasons (Frisch and Melchinger 2001; Frisch and Melchinger 2005). The conventional breeding method requires 5–6 back cross generations to transfer more than 90% of recurrent parent genetic background (Vogel 2009). The MABC method facilitated the acceleration of releasing varieties within 1–1.5 years, depending on whether two or three generations of rice planting could be performed within a year.

IR09F436 (Ciherang Sub 1) was selected from F_2BC_2 generation which was developed using SC3 and ART5 marker for SUB1 locus (foreground selection) and 48 SSR markers for genetic background of the Ciherang genome (Septiningsih et al. 2014). Since the process had fewer polymorphic markers due to close relatedness of donor and recurrent parents, it successfully completes the conversion at the BC1F2 stages. This is faster than the standard BC_3F_2 usually employed, like the development of IR64 Sub1 and Swarna Sub1 (Septiningsih et al. 2009). After accomplishment of breeding process, the seed of Ciherang Sub1 was introduced in Indonesia in 2011. However, this breeding material requires field and greenhouse trials to test its phenotypic performances and similarity to its recurrent parent, Ciherang. The study result will also be able to confirm the effectiveness of a shorter process of backcrossing strategy in developing a new rice variety using MABC method. The objectives of this study were to evaluate the phenotypic performances of Ciherang Sub1 NIL in the greenhouse and field conditions. In this present study, we compared Ciherang Sub1 on the advanced yield trial in some locations with its recurrent parent, Ciherang. We observed the grain yield and agronomic characters, their response to major pest and diseases, and their grain/cooking quality.

MATERIALS AND METHODS

Multi-Location Yield Trials

The experiments to evaluate yield potential and agronomic traits were carried out in the wet season of 2010/2011 and in the dry season of 2011 (Table 1) in ten locations representing rice production center with different soil types and elevations. Six rice genotypes consisted of five submergence-tolerant genotypes (Ciherang Sub1, PSBRc82 Sub1, Inpara 3, Inpara 5 and B13138-MR-2-7), and a sensitive-check variety, Ciherang, were evaluated. In each location, a randomized complete block design was used with three replications. Plot size was 4 m x 5 m and plant spacing was 25 cm x 25 cm, one plant per hill. Seeds were sown in the seedling bed for 21 days and then transplanted to the field. Nitrogen, phosphorus, potash and zinc nutrients were applied at a rate of 90:30:30:5 $kg.ha^{-1}$ as basal fertilizers. Second and third applications of nitrogen were conducted at 40 days after transplanting (DAT) and 60 DAT, respectively. Crop management followed the standard rice cultural practices. Grain yield was measured at maturity from 10 m^2 subplots, with area under the missing hills was subtracted from harvest area. The yield was adjusted to a moisture content of 14% fresh weight and converted to $t.ha^{-1}$.

Table 1. Characteristics of locations used for advanced yield trial of submergence-tolerant rice line.

Location	Season	Coordinate point	Elevation above sea level (m)	Soil type
Kesugihan-2, Cilacap	WS 2010/2011	S 7° 19' 45.98" E 108° 43' 35.54"	16	Alluvial
Tanjung Lubuk, OKI	WS 2010/2011	S 3° 33' 45.23" E 104° 47' 32.65"	15	Organosol
Kayu Agung, Palembang	WS 2010/2011	S 3° 28' 59.75" E 104° 48' 14.68"	11	Organosol
Solokanjeruk, Bandung	WS 2010/2011	S 7° 01' 039" E 107° 43' 965"	687	Andosol
Cimalaka, Sumedang	WS 2010/2011	S 6° 49' 759" E 107° 58' 676"	419	Regosol
Jatitujuh, Majalengka	WS 2010/2011	S 6° 38' 59.80" E 108° 13' 35.36"	23	Latosol
Sukamandi, Subang	WS 2010/2011	S 6° 21' 02.07" E 107° 39' 04.52"	14	Aluvial
Cilamaya, Karawang	DS 2011	S 6° 20' 17.36" E 107° 33' 09.85"	22	Aluvial
Anjatan, Indramayu	DS 2011	S 6° 18' 20" E 107° 56' 10"	10	Aluvial
Kesugihan-1, Cilacap	DS 2011	S 7° 19' 45.98" E 108° 43' 5.54"	16	Aluvial

WS = wet season; DS = dry season

Submergence Trial in the Field

The experiments to evaluate the response of rice genotypes under submerged conditions were carried out at Sukamandi Experimental Station of the Indonesian Center for Rice Research (ICRR) during the wet season of 2010/2011. The same genotypes as those tested in the grain yield trials were evaluated for submergence tolerance. The six genotypes were tested using a randomized complete block design, in three replicates. Plot size was 1 m x 5 m, plant spacing was 25 cm x 25 cm, one seedling per hill. Three ponds of 1000 m^2 area, 1.5 m depth and surrounded with concrete cement in each side were used. The three ponds were set up for application of different submergences, e.i. (1) complete submergence for 5 days, (2) complete submergence for 10 days, and (3) complete submergence for 15 days. Crop management followed the standard rice cultural practices.

The submergence treatment was started at 14 days after transplanting. Irrigation was applied from noon to allow sufficient time for rice to accumulate carbohydrate through photosynthesis in the morning. Desired water depth was maintained at 1.2 m by adding water regularly in the ponds. Algae were minimized by removing from the water surface using small fish nets.

Data were collected for nondestructive samples, such as percentage of survival, days to flowering and plant height. The yield attributes were determined by random sampling of 10 hills from each plot. Panicles were hand-threshed and the filled and unfilled spikelets were separated after drying under the sun. The subsamples were then oven-dried at 70^o C till constant weights to determine 1000-grain weight and spikelet number per panicle. Grain yield was measured at maturity from 1 m^2 subplots, with area under the missing hills was subtracted from harvest area. The yield was then adjusted to a moisture content of 14% fresh weight and converted to $t.ha^{-1}$.

Submergence Trial in the Greenhouse

Submergence trial in the greenhouse was carried out in the dry season of 2011 in Muara Experimental Station of ICRR. The submergence test followed the direct seeded method (Mazerado,A.M. and Vergara B.S.(1982)). The seeds of the same genotypes with the first experiment were sown in 12 cm x 24 cm x 30 cm trays filled with soil. Each genotype had one row followed the tolerant check, FR13A and sensitive check, IR42 in four replicates. At the 14-day-old seedlings the trays were transferred to the tank filled with water of 1 m depth. The water was maintained at desired depth by adding water regularly. After 14-day submergence, the water was removed. The shoot elongation was measured after the water was receded and compared with the genotypic measurements before submergence treatments. Survival rate was observed at seven days allowing plant to recover. The survival rate was determined by counting the ratio of survived plants after submerged to total plants used before submergence treatment.

Evaluation of Resistance to Pest and Disease

Ciherang and Ciherang Sub1 were used for evaluation of their resistance to biotic stress in the greenhouse experiment. Brown planthopper (BPH) test was carried out at Sukamandi Experimental Station of ICRR. Three BPH biotypes (biotype 1, 2 and 3) were bred in different varieties TN-1, IR26 and IR42, respectively following the method developed by Panda et al. (1982). Twenty five seedlings per genotype were planted in 200 cm x 75 cm x 20 cm wood box. Infestation used eight instars per seedling. Scoring was made when the different varieties died by following the Standard Evaluation System for Rice, SES (IRRI 2002).

Resistance to bacterial leaf blight was studied at the booting stage (50 days after planting) using three cell suspensions of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) pathotype III, IV and VIII at a concentration of 10^8 cells. ml^{-1}. The rice genotypes were planted in the field at 20 cm x 20 cm plant spacing, 20 hills per row. The tolerant checks IRBB5 and IRBB7 and susceptible checks IR64 and TN1 were included. Inoculation was done by cutting the leaves at 5 cm from the tips. Disease severity was observed by measuring the length of symptoms at 15 days after inoculation (DAI). Disease severity was determined by counting the ratio between the length of symptoms to the length of leaves inoculated based on SES IRRI for rice (IRRI 2002).

Resistance to rice tungro virus was evaluated following the International Rice Tungro Nursery (IRTN). Seedlings were planted in a row in the 70 cm x 30 cm x 30 cm plastic box. Tukad Petanu (resistant check) and TN1 (susceptible check) were planted in every 10 rows. Tungro viruses were tested by feeding acquisition of *Nepothettix virescens* to tungro inocula from Subang, Lanrang and Magelang for 24 hours. The viruliferous *N. virencens* were then released to the ten-day old seedlings for 24 hours to inoculate tungro virus. The test plants were observed and determined the scale of symptom severity at 14 days after inoculation based on SES for Rice (IRRI 2002).

Evaluation of Physical and Chemical Quality of Rice

Physical and chemical characteristics of Ciherang and Ciherang Sub1 milled rice were evaluated in the post-harvest laboratory of ICRR using the method developed by Juliano (2003). The physical characteristics consisted of grain width and length, degree of whiteness, clearness, chalkiness, milling recovery, and head rice recovery. Meanwhile the chemical characteristics consisted of amylose content, gelatinization temperature, alkali value and gel consistency.

Statistical Analysis

The data resulted from this study were tabulated and computed using Microsoft EXCEL 2007© software. Comparisons among genotypes were analyzed using a least significant difference with SAS 9.0© (SAS Institute Inc 2009). Morphological and agronomic characters, reaction to pest and disease, and physical-chemical quality of the grain were used to analyze the genetic divergence among genotypes. Multivariate analysis was applied to study the similarity level among genotypes tested. The parameters used for analyzing the similarity were plant height, tiller number, flowering date, grain number, filled grain number, fertility, 1000-grain weight, grain yield, reaction to brown planthopper, bacterial leaf blight, and tungro virus, and physical-chemical characteristics of milled rice consisted of grain width and length, whiteness, clearness, chalkiness, milling recovery, head rice recovery, amylose content, gelatinization temperature, alkali value and gel consistency. All parameters were counted for all possible pairwise comparisons between genotypes. Matrices of Euclidean similarity coefficients based on morphological data set were analyzed using Mini Tab V.5 (Minitab Inc. 2010).

RESULTS AND DISCUSSION

Grain Yield under Normal Conditions

Grain yields among genotypes in all locations were significantly different, except for Majalengka. The effects of interaction between genotypes and environment on grain yield were also statistically significant, indicating that there was a high variation of genotype responses to different environmental conditions (Table 2). The average grain yield in ten locations for Ciherang Sub1 (6.18 $t.ha^{-1}$) was not significantly different from that of Ciherang (5.89 $t.ha^{-1}$). Genes that control grain yield are polygenic and affected by environment, therefore the grain yield genes of Ciherang might have been well recovered in the Ciherang Sub1. This was also confirmed by the performances of agronomic traits of Ciherang Sub1 tested in ten locations which were not significantly different from those of Ciherang (Table 3).

Grain yields of Ciherang and Ciherang Sub1 were not significantly different, but average grain yield of Ciherang Sub1 was slightly higher than that of Ciherang at seven out of ten locations. A similar phenomenon was reported by Singh et al. (2009) and Nugraha et al. (2013a) where Swarna Sub1 and IR64 Sub1 were insignificantly different with its parent under normal conditions. It had been reported that introgression of SUB1 gene resulted additional effects that the SUB1 lines demonstrated more tolerant to drought (Fukao and Xiong 2013) and to shading (Fukao et al. 2012). The SUB1 locus confers submergence tolerance in rice and the SUB1 was classified as a family gene called an ethylene response factor (ERF) like gene (Xu et al. 2006). The SUB1 genes are members of group VII in the ERF gene family (Nakano 2006) and are more closely related than any other rice ERF genes (Gutterson and Reuber 2004). The gene regulates ethylene, a common phyto-hormone produced by plant in stress conditions (Fukao et al. 2011). This result confirmed that introduction of SUB1 locus into rice varieties gave beneficial effect,

Table 2. Grain yields of five rice genotypes tested in ten locations for advanced yield trial.

Genotypes	Grain yield (t.ha^{-1})										
	1	2	3	4	5	6	7	8	9	10	Means
Ciherang Sub1	6.16	4.27	6.96	3.72	8.41	6.61	4.64	5.95	7.88	8.28	6.29
Ciherang	5.39	4.01	7.16	3.05	8.30	6.65	4.97	5.40	7.81	8.15	6.09
PSBRC82-SUB1	5.42	4.14	5.99	3.07	7.95	5.90	4.72	5.27	6.86	7.44	5.68
B13138-7-MR-2	4.60	4.07	6.52	2.81	8.34	6.00	4.62	5.93	7.51	7.77	5.82
Inpara 3	4.18	4.23	5.58	2.77	6.34	6.45	3.71	5.04	7.14	7.78	5.32
Inpara 5	4.94	2.97	7.95	3.13	7.66	5.95	4.83	4.90	6.19	6.84	5.54
Means	5.11	3.95	6.69	3.09	7.83	6.26	4.58	5.42	7.23	7.71	5.79
G						2.63					
Rep (Loc)						0.37					
Loc						10.8					
G x Loc						3.58					
CV (%)						5.69					
LSD (0.05)						0.51					

Notes: 1 = Cilacap 1; 2 = Karang Ampel, Indramayu; 3 = Tanjung Lubuk, Ogan Komering Ilir; 4 = Kayu Agung; 5 = Bandung; 6 = Sumedang; 7 = Majalengka; 8 = Sukamandi; 9 = Cilacap 2; 10 = Karawang.

*) Significantly different at 5% level. G = Genotypes, Loc = Location, Rep = Replication.

Table 3. Yield components and agronomic data of Ciherang Sub1, Ciherang and other Sub1 varieties under control conditions in ten different sites in Indonesia under normal conditions.

Genotypes	Yield component						
	PH	TN	DF	FG	FR	UFG	1000-W
Ciherang-Sub1	101.7	16	79	110	82	24	26.7
Ciherang	100.3	16	77	105	81	21	26.7
PSBRC-SUB1	95.3	18	78	96	82	22	26.0
B13138-7-MR-2-KA	105.0	15	80	106	78	33	25.6
Inpara3	100.8	15	80	102	78	26	25.6
Inpara5 (IR64 Sub1)	94.2	19	75	92	81	18	26.0
G	**	**	**	ns	ns	*	**
Rep (L)	**	ns	ns	ns	ns	ns	**
L	**	**	**	**	**	**	**
G x L	**	**	**	**	**	**	**
Means	99.5	17	78	102	80	24	26.1
CV (%)	4.4	13.2	1.8	11.6	6.4	4.9	4.4
LSD 0.05	1.7	0.8	0.5	4.8	1.8	0.4	1.7

Data were collected from ten different sites in Indonesia, with four replications in each site: Cilacap in Central Java (2010 WS and 2011 DS); Indramayu, Bandung, Sumedang, Majalengka, Sukamandi, Karawang in West Java; Tanjung Lubuk and Kayu Agung, Ogan Komering Ilir in South Sumatra in 2011 DS.

GY = grain yield, PH = plant height, TN = productive tiller number, DF = number of days to 50% flowering, FG = number of filled grains, FR = fertility, UFG = number of unfilled grains, 1,000-W = 1,000 grain weight.

** and * Significantly different at 0.05 and 0.01 level, respectively; ns = not significantly different.

not only improving plant tolerance to submergence but also to other abiotic stress that might appear during the experiment in the field resulting in the increasing grain yield under normal conditions.

Genotypic Performance under Submergence Conditions

Ciherang Sub1 and Ciherang along with the two other checks were also planted at Sukamandi Experimental Station of ICRR during the dry season of 2011 under 5-d, 10-d and 15-d submergence. Plant survivals among all genotypes were not significantly different under 5-d submergence (Figure 1). Variations of plant survivals were observed under 10-d and 15-d submergence treatments, where the tolerant varieties survived better compared to Ciherang. The survival rate of Ciherang was 60% when submerged for 10 days, while Ciherang Sub1 could maintain its survival at 88%. The survival rate under 15-d submergence for Ciherang was 40%, while that of Ciherang Sub1 was 75%. Although the survival rates of Ciherang and Ciherang Sub1 decreased

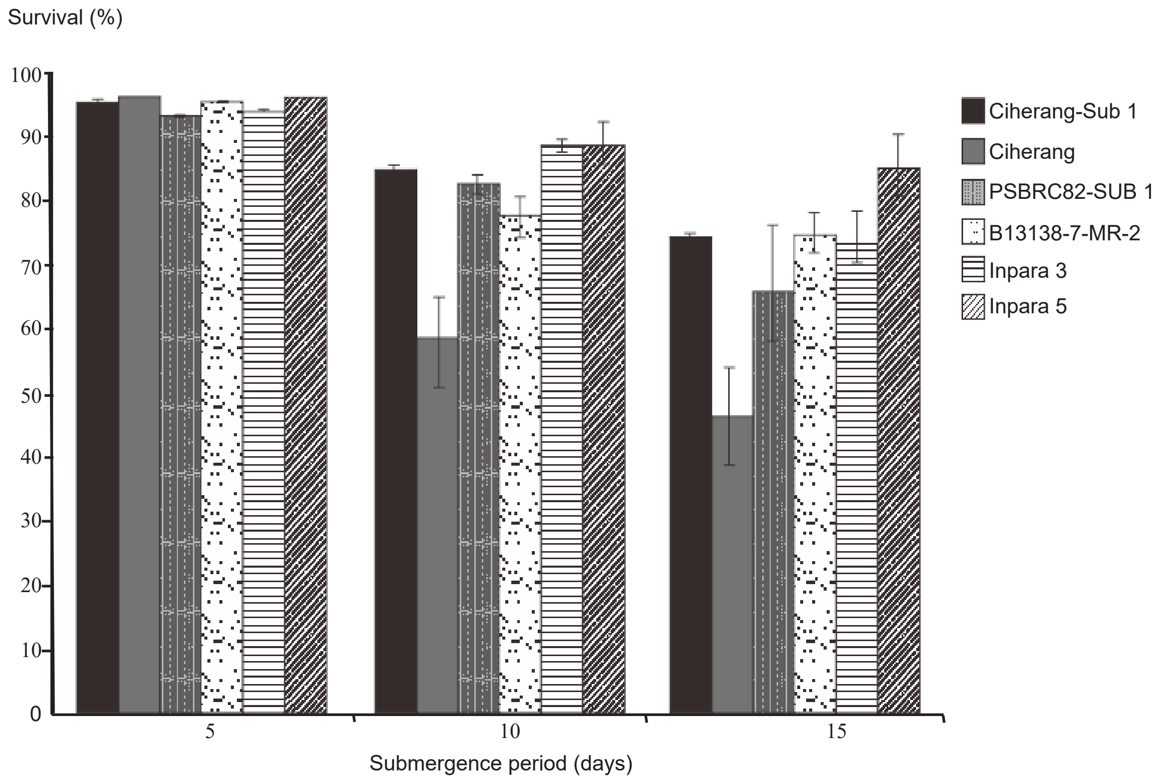


Fig. 1. Survival of some rice genotypes under 5, 10 and 15 days submergence in Sukamandi, wet season of 2010/2011. Error bar is standard error of the means of three replications.

under 15-d submergence, Ciherang Sub1 containing SUB1 gene survived better than Ciherang.

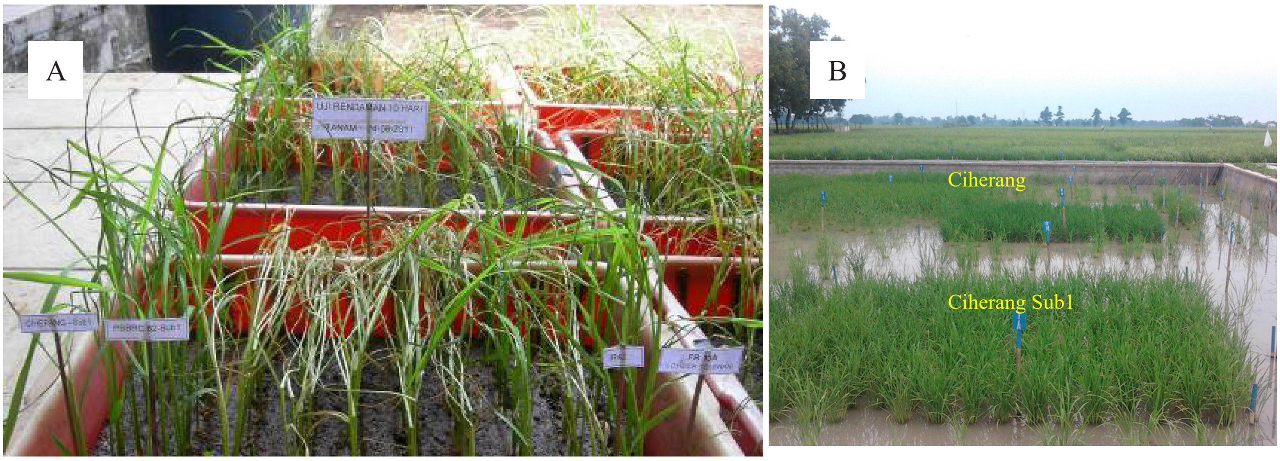
Plant survival affected grain yield only under severe submergence for 15 days. Submergence treatment for 5 days resulted insignificant effect compared to normal condition on plant survival and grain yield. Submergence for 10 days decreased grain yield of Ciherang to 3.9 t.ha^{-1} compared to that at normal conditions in Sukamandi (Table 2), but its grain yield was not significantly different to that of tolerant varieties (Table 4). This was attributed to the survival plants resulting more tillers to compensate spacious population due to some plants died during submergence as revealed by Ciherang producing 18 and 20 tillers under 10-d and 15-d submergence, respectively. However, under severe submergence for 15 days, genotypes revealed significant differences in grain yields, where Ciherang yielded only 2.0 t.ha^{-1} while Ciherang Sub1 produced grains almost double to 3.9 t.ha^{-1} followed other submergence-tolerant variety, Inpara 5. Reduction in grain yield under submerged conditions was attributed to the degree of plant injury, which was dependent on the level of submergence tolerance.

The result of greenhouse experiments confirmed the results in the field trials where Ciherang Sub1 survived 100%, while Ciherang survived only 60% under 14-d submergence treatment (Table 5). Survival of Ciherang Sub1 was also comparable to that of source gene of SUB1A-1, FR13A and other submergence-tolerant varieties (Figure 2). In the water tank experiment, Ciherang demonstrated slightly better survival rate than the sensitive check IR42. This result was also confirmed by the submergence field experiments demonstrating moderate tolerance (50% survival rate) of Ciherang under 10-d submergence treatment (Figure 1).

The SUB1 gene was reported encoding two or three ethylene-responsive factors, namely SUB1A, SUB1B and SUB1C. The SUB1A was subsequently identified as the major determinant for submergence tolerance, while SUB1B and SUB1C alleles did not show important roles in plant tolerance to submergence (Xu et al. 2006). Recent study reported that submergence-tolerant rice accessions possess the SUB1A-1 allele, whereas accessions that contain less highly expressed SUB1A-2 allele are submergence intolerant (Septiningsih et al. 2009). The slightly better survival rate of Ciherang than that of the sensitive check was probably because the genotypes had one of the three alleles of the SUB1 locus and expressed SUB1A-2 allele with small effect as it did in IR64, while

Table 4. Agronomic data and grain yield of Ciherang Sub1, Ciherang and other Sub1 varieties under 5-days submergence (5-d), 10-days submergence (10-d) and 15-days submergence (15-d) of field plot, in Sukamandi in 2011 dry season.

Genotypes	Plant height (cm)			Tiller number (pieces)			Days to 50 % flowering (day)			Grain yield (t.ha^{-1})		
	5-d	10-d	15-d	5-d	10-d	15-d	5-d	10-d	15-d	5-d	10-d	15-d
Ciherang-Sub1	97	99	98	17	17	14	74	77	81	5.6	4.2	3.9
Ciherang	96	98	95	16	18	20	74	76	89	5.2	3.9	2.0
PSBRC-SUB1	97	99	91	17	17	14	75	72	79	5.1	3.5	3.1
B13138-7-MR-2-KA-1	106	106	101	13	13	13	73	76	80	5.5	3.9	3.2
Inpara 3	106	106	105	14	14	12	74	78	81	4.5	4.4	2.8
Inpara 5 (IR64 Sub1)	91	91	84	17	17	14	69	71	76	5.0	3.8	4.1
CV (%)	3.4	3.4	5.1	12.6	12.6	6.2	1.8	1.8	1.6	6.7	4.9	13.4
LSD	4.8	4.9	6.9	2.8	2.8	1.2	1.9	1.9	1.9	0.5	0.3	0.6



Ciherang Sub1 PSBRC82 Sub1 IR42 Sensitive check Ciherang Sensitive B 13138-7 Inpara5 FR13A Tolerant

Fig. 2. High seedling recovery and less elongation of Ciherang Sub1 (foremost left) after 14 days submerged in greenhouse trays, similar with Sub1 locus donor FR13A (foremost right) (A). High survival of Ciherang Sub1 after 15 days submerged in the field compared to its recurrent parent, Ciherang (B).

Table 5. Shoot length of rice genotypes as affected by 14 days submergence in greenhouse test, Bogor, 2011 dry season.

Genotypes	Shoot length (cm)			Survival (%)	Score[1)]
	Before submerged	After submerged	Differences		
Ciherang Sub I	23.7 ± 2.2	29.4 ± 4.6	5.7 ± 2.4	100	1
Ciherang	22.5 ± 2.5	42.8 ± 7.3	20.3 ± 5.2	63	7
PSBRC 82 Sub I	23.1 ± 1.9	30.2 ± 4.3	7.1 ± 2.4	98	3
B13138-7-MR-2-KA-1	21.9 ± 2.5	28.6 ± 3.6	6.7 ± 1.1	100	1
Inpara 3	24.2 ± 2.3	34.1 ± 4.9	10.9 ± 2.6	93	5
Inpara 5	22.5 ± 1.5	26.3 ± 6.7	3.8 ± 5.2	100	1
IR42 (sensitive check)	23.6 ± 1.4	45.8 ± 10.2	22.2 ± 9.2	10	9
FR13A (tolerant check)	29.4 ± 2.8	35.2 ± 4.5	5.8 ± 1.7	100	1

[1)]1 = tolerant; 3 = moderately tolerant; 5 = moderately sensitive; 7 = sensitive; 9 = very sensitive.
The data are averages of three replications.

in the pedigree tree of Ciherang, one of its ancestors is IR64 (Suprihatno et al. 2012). The tolerant genotypes showed less shoot elongation compared to those of the sensitive genotypes (Table 5). Ciherang shoot elongation was similar to the sensitive check, IR42, which in turn their shoot length was four-fold higher than that of Ciherang Sub1. Other submergence-tolerant varieties showed less shoot elongation comparable to the tolerant check, FR13A. Under complete submergence, shoot elongation was not necessary for survival indicator which would tend to be lodging after water recede. Shoot elongation consumed more energy and took stored assimilate (Jackson and Ram 2003). The remaining carbohydrate after submergence will presumably be important for growth recovery after de-submergence (Singh et al. 2001; Ram et al. 2002; Nugraha et al. 2013b). Hence, genotypes which have less shoot elongation would recover faster and produce higher yield compared to elongated-type rice varieties during submergence.

Response to Pests and Diseases

Ciherang Sub1 and Ciherang varieties revealed the same response to major pests and diseases tested (Table 6). Both rice genotypes showed moderately susceptible to BPH biotypes 1 and 2, but were susceptible to BPH biotypes 3. The response to bacterial leaf blight (BLB) for both Ciherang and Ciherang Sub1 was moderately resistant to strain III but was moderately susceptible to strain IV and VIII, their response to rice tungro virus was susceptible on three different inoculation experiments. This indicated that there was no effect of the introgression of SUB1 locus into Ciherang variety, with regard to pest and disease reactions.

Grain Quality

Introgression of SUB1 locus to Ciherang variety did not alter the physical and chemical quality of the grain (Table 7). The grain type for both Ciherang Sub1 and Ciherang was long-slender, which met the preference of rice consumers and traders in Indonesia. Other physical grain qualities of both genotypes were also similar. The chemical properties of grain quality which related to the cooking quality of Ciherang Sub1 and Ciherang showed no any apparent differences. This suggests that Ciherang Sub1 would be accepted by farmers and consumers, because its performance is similar to high yielding popular variety, Ciherang.

Similarities Between Ciherang NIL and Its Parents

The genetic distance measured using Euclidean similarities analysis showed that Ciherang Sub1 had close position (95% similarity) with Ciherang (Figure 3). Inpara 5 or IR64 Sub1 which was the ancestor of both varieties were in the third position or had 65.9% similarity. This result indicated that although the process of backcrossing was done only twice, more than 87.5% phenotypic similarity of the parent was present in the offspring. This result also indicated that instead of recovering its parent genetic background, plant performance in the field was also homogenous (Figure 2B). Theoretically, the second backcross generation would be transferred 75% of the recurrent parent genetic background (Hospital 2005). In the process of MABC, from the F_1BC_1 generation only 2-3 plants were selected

Table 6. Response of Ciherang Sub1 and Ciherang to major pest and diseases.

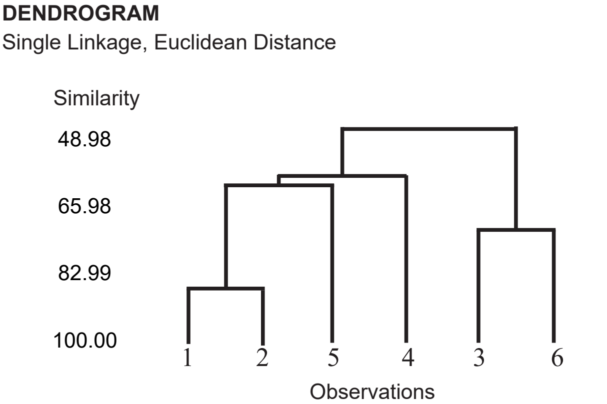
Pest and diseases	Ciherang Sub1		Ciherang	
	Score	Criteria	Score	Criteria
Brown planthopper				
Biotype 1	5.0	MS	5.5	MS
Biotype 2	5.0	MS	5.5	MS
Biotype 3	6.5	S	7.0	S
Bacterial leaf blight				
Pathotype III	4	MR	3	MR
Pathotype IV	6	MS	6	MS
Pathotype VIII	5	MS	5	MS
Tungro				
Subang inoculum	7	S	7	S
Lanrang inoculum	7	S	7	S
Magelang inoculum	7	S	7	S

MR = moderately resistant; MS = moderately susceptible, S = susceptible.
The data are averages of three replications.

Table 7. Grain quality of Ciherang Sub1 and Ciherang.

Grain quality	Ciherang Sub1		Ciherang	
	Score	Criteria	Score	Criteria
Physical properties				
Length (mm)	7.36	Long	7.40	Long
Width (mm)	2.12		2.20	
Ratio L/W	3.47	Cylinders	3.36	Cylinders
Whiteness (%)	33.40	White	33.60	White
Clearness	1.06	Clear	1.01	Clear
Milling recovery (%)	60		61	
Dehull rice	78.76		78.43	
Milling rice	70.26		68.34	
Head rice	95.64		90.15	
Chalkiness	0.06	Small	0.27	Small
Chemical properties				
Amylose (%)	22.40	Medium	23.13	Medium
Gel consistency (mm)	50	Medium	44	Medium
Alkali value (score)	1		1	
Gelatination (C°)	>74	High	>74	High

The data are averages of two replications.



1 = Ciherang Sub 1; 2 = Ciherang; 3 = PSBRC 82 Sub 1; 4 = B13138-7-MR-2-KA-1; 5 = Inpara 3; 6 = Inpara 5

Fig. 3. Similarity of six rice genotypes based on phenotypic performance of 21 characters of yield, yield components, agronomic characters, pest and disease resistance, and grain quality using Euclidean analysis.

having a homozygote allele of targeted gene from donor parent and all homozygote background alleles from recurrent parent. This process was then continued in the successive generation, F1BC2, to obtain the homozygote targeted locus and homozygotes in all loci of genetic background.

In development of Ciherang Sub1, a set of 285 SSR markers was used to survey polymorphism between the recurrent and donor parents, Ciherang and IR64 Sub1, respectively. However, since the two are closely related (IR64 is one of the parents of Ciherang), only 29 markers were found polymorphic and used for genotyping the population of 48 SSR markers (Hidayatun et al. 2011), which was less than those used in the development of Swarna Sub1 using 200 SSR markers. This was because the donor parent used was IR64 Sub1, which had similarity genetic background to that of Ciherang as confirmed by the genetic Euclidean analysis (Figure 3). This indicated that the backcross process would be accelerated if the donor and the recipient parents were similar. More recently molecular markers tools have been established using high-throughput and low-cost next generation sequencing (NGS) platforms, so that much of the genotyping work can now be easily outsourced in a cost-effective manner. These NGS platforms are being extensively utilized for *de novo* development of markers and also for genotyping (Edwards and Gupta 2013). In addition, a high throughput genotyping system using single nucleotide polymorphism (SNP) markers has been discovered and has commonly been applied in a number of crop species, including rice (Thomson 2014).

Several Sub1-varieties were previously released in Indonesia, including Inpara 3, Inpara 4 (Swarna-Sub1) and Inpara 5 (IR64-Sub1). However, the development of Ciherang Sub1 could provide more options for farmers to choose their favorite varieties and minimize crop losses due to unexpected flooding. In 2012, Ciherang Sub1 was officially released in Indonesia and named Inpari 30 Ciherang Sub1 (Ministry of Agriculture of Republic of Indonesia Decree no: 2292.1/KPTs/SR120/6/2012).

CONCLUSION

Morphological performance, agronomic characters, grain yield and yield components of Ciherang and Ciherang Sub1 were similar based on the results of advanced yield trials in ten locations. Introgression of SUB1 locus to the genome of Ciherang variety did not alter the response of the variety to pests and diseases, such as brown planthopper, bacterial leaf blight, and rice tungro virus. There was also no notable changes in physical and chemical grain qualities of Ciherang Sub 1 and its recurrent parent. Ciherang Sub1 demonstrated grain yield advantage compared to that of Ciherang when it was subjected to submergence for 15 days during the vegetative stage. The morphological similarity between Ciherang Sub1 and Ciherang was also confirmed through phenotypic analysis which revealed 87.5% similarity based on similarity analysis.

ACKNOWLEDGEMENT

We thank Dr. David J. Mackill and Dr. Endang Septiningsih for the seeds and allowing Ciherang Sub1 to be tested in Indonesia. The work reported here was supported by the ICRR budget of 2010-2011 fiscal year.

REFERENCES

Collard, B.C.Y. & Mackill, D.J. (2008) Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*. [Online] 363 (1491), 557–572. Available from: doi:10.1098/rstb.2007.2170.

Frisch, M. & Melchinger, A.E. (2001) Marker-assisted backcrossing for simultaneous introgression of two genes. *Crop Science*. [Online] 41 (6), 1716–1725. Available from: doi:10.2135/cropsci2001.1716.

Frisch, M. & Melchinger, A.E. (2005) Selection theory for marker assisted backcrossing. *Genetics*. [Online] 170 (2), 909–917. Available from: doi:10.1534/genetics.104.035451.

Edwards, D & Gupta, P. (2013). *Sequence Based DNA Markers and Genotyping for Cereal Genomics and Breeding*. pp: 57-76 In: Cereal Genomics II. Elsevier. Amsterdam, ND. [Online] Available from doi:10.1007/978-94-007-6401-9_3

Fukao, T. & Xiong, L. (2013) Genetic mechanisms conferring adaptation to submergence and drought in rice: Simple or complex? *Current Opinion in Plant Biology*. [Online] 16 (2), 196–204. Available from: doi:10.1016/j.pbi.2013.02.003.

Fukao, T., Yeung, E. & Bailey-Serres, J. (2012) The submergence tolerance gene, SUB1A, delays leaf senescence under prolonged darkness through hormonal regulation in rice. *Plant Physiology***.** **[**Online] 160, 1795–1807. Available from: doi:10.1104/pp.112.207738.

Fukao, T., Yeung, E. & Bailey-Serres, J. (2011) The submergence tolerance regulator SUB1A mediates crosstalk between submergence and drought tolerance in rice. *The Plant cell***.** [Online] 23 (1), 412–427. Available from: doi:10.1105/tpc.110.080325.

Gutterson, N. & Reuber, T.L. (2004) Regulation of disease resistance pathways by AP2/ERF transcription factors. *Current Opinion in Plant Biology***.** [Online] 7 (4), 465–471. Available from: doi:10.1016/j.pbi.2004.04.007.

Hidayatun N., Alvaro, P., Septiningsih, E.M., & Mackill, D.J. (2011) Pengembangan varietas toleran rendaman ciherang Sub1 melalui pendekatan *marker assisted backcrossing* (MABC).In: *Prosiding Seminar Nasional Padi 2010***.** Buku 1. Sukamandi, Balai Besar Penelitian Tanaman Padi, pp.109–117.

Hospital, F. (2005) Selection in backcross programmes. *Philosophical Transactions of the Royal Society B: Biological Sciences***.** [Online] 360 (1459), 1503–1511. Available from: doi:10.1098/rstb.2005.1670.

IRRI (2002) *Standard Evaluation System for Rice***.** Los Banos, International Rice Research Institute.

Ismail, A.M. Singh, U.S., Singh, S., Dar, M.D. & Mackill, D.J. (2013) The contribution of submergence-tolerant (Sub1) rice varieties to food security in flood-prone rainfed lowland areas in Asia. *Field Crops Research***.** [Online] 152, 83–93. Available from: doi:10.1016/j.fcr.2013.01.007.

Jackson, M.B. & Ram, P.C. (2003) Physiological and molecular basis of susceptibility and tolerance of rice plants to complete submergence. *Annals of Botany***.** [Online] 91 (SPEC. ISS. JAN.), pp.227–241. Available from: doi:10.1093/aob/mcf242.

Jena, K.K. & Mackill, D.J. (2008) Molecular markers and their use in marker-assisted selection in rice. *Crop Science***.** [Online] 48 (4), 1266–1276. Available from: doi:10.2135/cropsci2008.02.0082.

Juliano, B. (2003) *Rice Chemistry and Quality***.** [Online] Manila The Phillippines, Phillippines Rice Research Institute. Available from: doi:10.1002.

Manzanilla, D.O. Paris, T.R., Vergara, G.V., Ismail, A.M., Pandeya, S., Labios, R.V., Tatlonghari, G.T., Acdac, R.D., Chi, T.T.N., Duoangsila, K., Siliphouthone, I.,.Manikmas, M.O.A., & Mackill, D.J. (2011) Submergence risks and farmers ' preferences : Implications for breeding Sub1 rice in Southeast Asia. *Agricultural Systems***.** [Online] 104 (4), 335–347. Available from: doi:10.1016/j.agsy.2010.12.005.

Mazerado, A.M. & Vergara, B.S (1982). *Physiological differences in rice varieties tolerant and susceptible to complete submergence*. In Proceeding of the International Deepwater Rice Workshop. Manila: International Rice Research Institute 327-341

Minitab Inc. (2010) *Minitab, 2010. Minitab Assistant White Paper***.** USA, Minitab Assistant White Paper.

Nakano, T. (2006) Genome-wide analysis of the ERF gene family in arabidopsis and rice. *Plant Physiology***.** [Online] 140 (2), 411–432. Available from: doi:10.1104/pp.105.073783.

Neeraja, C.N., Maghirang-Rodriguez, R., Pamplona, A., Heuer, S., Collard, B.C.Y., Septiningsih, E.M., Vergara, G., Sanchez, D., Xu, K., Ismail, A.M., & Mackill, D.J. (2007) A marker-assisted backcross approach for developing submergence-tolerant rice cultivars. *TAG. Theoretical and Applied Genetics.* [Online] 115 (6), 767–776. Available from: doi:10.1007/s00122-007-0607-0.

Nugraha, Y., Vergara, G.V., Mackill, D.J., & Ismail A.M. (2013a) Genetic parameters of some characters and their correlation with rice grain yield in relation to the plant adaptability to semi-deep stagnant flooding condition. *Penelitian Pertanian Tanaman Pangan*. [Online] 32 (2), 74–82 Available from http://ejurnal.litbang.pertanian.go.id/index.php/jpptp/article/view/2882.

Nugraha, Y., Vergara, G.V., Mackill, D.J., & Ismail A.M (2013b) Response of Sub1 introgression lines of rice to various flooding conditions. *Indonesian Journal of Agricultural Science***.** [Online] 14 (1), 15–22. Available from: doi:10.21082/ijas. v14n1.2013.p15-26.

Panda, N., Heinrichs, E.A. & Box, P.O. (1982) Levels of tolerance and antibiosis in rice varieties having moderate resistance to the brown planthopper, Nilaparvata lugens (Stål) (Hemiptera: Delphacidae). *Population English Edition***. 1**2 (2), 1204–1214.

Ram, P.C., Singh, B.B., Singh, A.K., Ram, P., Singh, P.N., Singh, H.P., Boamfa, I., Harren, F., Santosa, E., Jackson, M.B., Setter, T.L., Reuss, L.J., Wade, L.J., Singh, V.P., Singh, R.K. (2002) Submergence tolerance in rainfed lowland rice: Physiological basis and prospects for cultivar improvement through marker-aided breeding. *Field Crops Research***.** [Online] 76 (2–3), 131–152. Available from: doi:10.1016/S0378-4290(02)00035-7.

Ruskandar, A. (2010) Persepsi petani dan identifikasi faktor penentu pengembangan dan adopsi varietas padi hibrida. *Iptek Tanaman Pangan*. [Online] 5 (2), 113–125. Available from http://ejurnal.litbang.pertanian.go.id/index.php/ippan/article/view/2602

Sarkar, R.K., Reddy J.N., Sharma, S.G., & Ismail, A.M. (2006) Physiological basis of submergence tolerance in rice and implications for crop improvement. *Current Science***.** 91 (7), 899–905. Available from http://www.jstor.org/stable/24094287

SAS Institute Inc (2009) *SAS/STAT 9.2 User's Guide.* SAS Institute Inc., Cary, NC. [Online] p. 8640. Available from: doi:10.1111/j.1532-5415.2004.52225.x.

Septiningsih, E.M., Pamplona A.M., Sanchez, D., Neeraja, C.V., Vergara, G.V., Heuer, S., Ismail, A.M., & Mackill, D.J. (2009) Development of submergence- **t**olerant rice cultivars: The Sub1 locus and beyond. *Annals of Botany***.** [Online] 103 (2), 151–160. Available from: doi:10.1093/ aob/mcn206.

Septiningsih, E.M., Hidayatun, N., Sanchez, D.L., Nugraha, Y., Carandang, J., Pamplona, A.M., Collard, B.Y.C., Ismail, A.M., & Mackill, D.J. (2014) Accelerating the development of new submergence tolerant rice varieties: The case of Ciherang- Sub1 and PSB Rc18-Sub1. Euphytica. [Online] 202 (2), 259–268.Available from: doi:10.1007/s10681-014-1287-x.

Singh, S., Mackill, D.J. & Ismail, A.M. (2009) Responses of SUB1 rice introgression lines to submergence in the field: Yield and grain quality. *Field Crops Research***.** [Online] 113 (1), 12–23. Available from: doi:10.1016/j.fcr.2009.04.003.

Suprihatno, B. Darajat, A.A., Satoto, Baehaki, S.E., Suprihanto, Setyono, A., Indrasari, S.D., Wardana, I.P., & Sembiring, H. (2012) *Deskripsi Varietas Padi*. Sukamandi, Balai Besar Penelitian Tanaman Padi.

Thomson, M.J. (2014) High-Throughput SNP Genotyping to Accelerate Crop Improvement. *Plant Breeding and Biotechnology* [Online] 2(3), 195-212. Available from DOI: https://doi.org/10.9787/PBB.2014.2.3.195

Vogel, K.E. (2009) Backcross breeding. *Methods in Molecular Biology***.** [Online] 526, 161–169. Available from: doi:10.1007/978- 1-59745-494-0-14.

Xu, K., Xu, X., Fukao, T., Canlas, P., Maghirang-Rodriguez, R. ., Heuer, S., Ismail, A.M., Bailey-Serres, J., P.C. Ronald, P.C. and Mackill, D.J. (2006) Sub1A is an ethylene-response-factor-like gene that confers submergence tolerance to rice. *Nature***.** [Online] 442 (7103), 705–708. Available from: doi:10.1038/nature04920.