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Phenotypic and molecular marker analysis of F_1 population derived from crossing of gogo-dryland x paddy-field rice varieties

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Demand of rice as a staple food in Indonesia continues to increase, but the capacity to produce more rice paddy is limited. Meanwhile, the use of *Gogo*-dryland rice could help to meet the demand, but the contribution of *Gogo*-dryland rice was lower than Paddy-field rice. Therefore, need to develop *Gogo*-dryland rice which have drought tolerance and high yield potential. The objectives of the study were to evaluate the performance of F_1 hybrid derived from the crossing of *Gogo*-dryland rice (Situ Bagendit and Towuti) x Paddy-field rice (Ciherang and Cibogo) and to identify pure hybrid of F_1 using simple sequence repeat (SSR). The research was conducted in screen house and plant biotechnology laboratory of Brawijaya University, Malang, Indonesia. Ninety six of F_1 plants derived from four combined crosses were evaluated in morphological and agronomic characters. To identify pure hybrid, four primers *i.e.* RM219, RM260, RM525, and RM318 were used. The result showed that there was significant difference in both morphological and agronomic characters between F_1 and their respective female parents except in plant height variable. The F_1 hybrids with Situ Bagendit as female parent have similar band with the male parents (Paddy-field rice). In contrast, Towuti as female parent produced F_1 hybrids which have similar band with the female parent itself. The F_1 hybrids which similar with male parents can be considered as the true *hybrid*. The information generated from this study can be a valuable information to develop new rice cultivar with drought tolerance and high yield potential.

Keywords: Gogo-dryland Rice, Paddy-field Rice, F_1 Hybrid, Simple Sequence Repeat (SSR)

INTRODUCTION

Rice (*Oryza sativa* L.) is a dominant and important staple food in Indonesia. The demand of rice keep on increasing in line with population growth whilst the capacity to increase the production of rice is limited. The efforts to increase rice production continuously are facing some challenges in consequence of the high rate of population growth and the reduction of some productive wetland area (Panuju et al., 2013). In 2013, the area of paddy-field rice in Indonesia decreased compare with the year of 2012. The paddy-field rice area in 2012 was known about 8,132,345 hectares and decreased to 8,112,103 hectares in 2013 (Center for Agriculture Data and Information System, 2014). The reduction of wetland area has direct impact on national rice production. So that the role of gogo-dryland rice to support rice production and strengthen food security in Indonesia need to increase more.

Gogo-dryland rice refers to rice grown on both flat and slopping fields that are prepared and seeded under dry land conditions and dependent on rainfall for moisture. Indonesia has more than

35 million hectares of dry land that are suitable for development of food crops which include marginal dry land for developing gogo-dryland rice. But the productivity of gogo-dryland rice is low compared with the production of paddy-field rice (Sadimantara et al., 2016). According to Sadimantara and Muhidin (2012), the average productivity of gogo-dryland rice in 2011 was 3.091 ton ha^{-1}, much lower than paddy-field rice which reached the average of 5.179 ton ha^{-1}. Rice production could be two-to three-fold higher in paddy-field rice with irrigation system than production in rain-fed system particularly in dry land area (Kim et al., 2017). Xia et al., (2014), stated that gogo-dryland rice may accumulate more drought-resistance genetic variance than paddy-field rice. From this information we know that gogo-dryland rice is more adaptable with dry condition but with low yield. Contrariwise, paddy-field rice is more susceptible with dry condition but have high yield. Thus, the development of drought-tolerant rice varieties with high yielding potential is considered as an important strategy to maintain sustainable national rice production.

One of the efforts to develop drought-tolerant rice varieties with high yielding potential is by crossing the gogo-dryland rice with paddy-field rice. This is an option and one solution to improve gogo-dryland rice production to meet national rice production demand. The combination of conventional breeding through crossing and molecular breeding by using molecular marker could make the progress of breeding program more efficient. Rao et al., (2014), stated that molecular breeding refers to the development of plant breeding program by integrating the means of modern biotechnology into conventional breeding methods, so it could compensate the deficiencies of conventional breeding which often takes a long time.

A Molecular marker is a sequence of DNA that is readily detected and whose inheritance could be easily monitored. Amongst another DNA markers available, microsatellite or SSR (simple sequence repeat) markers are considered to be appropriate for assessment of genetic diversity, genetic purity, and genetic identification because of their ability to detect large numbers of discrete alleles repeatedly, accurately and efficiently (Sonkar et al., 2016). Microsatellite markers have been ideal for identification and purity checking of true hybrids and rice varieties. Microsatellites are PCR-based markers, they are abundant, co-dominant, highly reproducible and interspersed throughout the genome (Temnykh et al., 2000).

The purposes of this study were to evaluate the phenotypic performance of F_1 hybrid derived from the crossing of gogo-dryland rice varieties (Situ Bagendit and Towuti) and paddy-field rice varieties (Ciherang and Cibogo) compared to their respective female parents, and also to identify pure hybrid of F_1 using microsatellite markers.

MATERIALS AND METHODS

The field research was carried out in screen house of Faculty of Agriculture, University of Brawijaya, Malang, Indonesia. The molecular marker analysis was accomplished in Laboratory of Plant Biotechnology, Faculty of Agriculture, University of Brawijaya, Malang, Indonesia. The study was conducted on September 2017 until February 2018.

Parental lines and crosses

Four varieties consisted of two gogo-dryland varieties i.e. Situ Bagendit and Towuti as female parents and two paddy-field rice varieties i.e. Ciherang and Cibogo as male parents were selected in previous research. Each of the varieties were crossed and four cross combination were generated.

Field evaluation and data collection

Ninety six of F_1 hybrids i.e. F_1 SBCH (Situ Bagendit × Ciherang), F_1 SBCB (Situ Bagendit × Cibogo), F_1 TWCH (Towuti × Ciherang), and F_1 TWCB (Towuti × Cibogo), along with their respective parents i.e. Situ Bagendit and Towuti both as female parents and as comparisons and Ciherang and Cibogo as male parents, were raised and arranged in a single plot design. Each of F_1 hybrids and their respective parents are consisted of 24 plants, so that 192 plants in total. Data collection was accomplished by single plant method, so that every single plants were tagged for recording data. Three morphological characters i.e. plant height (cm), leaf number, and panicle length (cm), and also six agronomic characters i.e. number of tiller, number of productive tiller, heading date (days after transplanting, dat), harvest date (dat), weight of 100 grain (g), and grain yield (g) were observed.

DNA isolation

To perform the molecular analysis, genomic DNA was isolated from fresh young leaves of 14 days old after transplanting following the protocol of GeneJet Plant Genomic DNA Purification Mini Kit.

SSR markers and PCR amplification

Four simple sequence repeat (SSR) markers related to drought tolerance traits / QTLs were used. The primers names, repeat motifs (primer sequences), chromosome number and related trait / QTLs were shown in Table 1.

Polymerase chain reaction was performed according to the description of ThermoScientificDreamTaq Green PCR Master Mix (2X) product, containing a total 25 µl volume, 1 µg DNA template, 1.0 µM forward primer, 1.0 µM reverse primer, and added nano-pure water until the volume is 50 µl. PCR reactions were performed in the following cycle profile; initial denaturation at 94°C for 5 min and then 35 cycles of 45 sec denaturation at 94°C, 30 sec annealing at 55°C and 1 min extension at 72°C and 10 min at 72°C for the final product extension.

Electrophoresis and visualization of amplified products

PCR amplified products were subjected to electrophoresis in 1.0% agarose gels in 1x TAE buffer at 50V at 45 min using Mupid®-EXu submarine electrophoresis unit. The electronic image of the ethidium bromide stained gel was visualized and documented in a gel documentation system using Bio-Rad® Molecular Imager.

Field data analysis

All data obtained from the research were subjected for descriptive statistic (range, mean, standard deviation) and were analyzed using SPSS 17.0. Significant differences were evaluated using student's t-test.

SSR data analysis

The amplified bands were scored for each SSR marker based on the presence (1) or absence (0) of bands, generating a binary data matrix for each marker system. For each primer used in SSR analysis, the total number of bands scored, the number of polymorphic bands, and the percentage of polymorphism were determined. To measure the informativeness of the markers, polymorphic information content (PIC) values were calculated with the following formula (Anderson et al., 1993):

$$PICi = 1 - \sum_{j=1}^{n} P^2\, ij$$

Where, n is the number of marker alleles for marker *I* and P*ij*is the frequency of the *j* th allele for marker *i*. The PIC value provides an estimate of the discriminating power of the markers.

RESULTS AND DISCUSSION

Morphological Characters

Means value of plant height were presented in Table 2, showed that the crossing of Situ Bagendit and paddy-field rice varieties (Ciherang and Cibogo) generated F_1 SBCH and F_1 SBCB that have lower plant height (96.04 and 96.08 cm, respectively) compared with the female parent (97.90 cm). But, F_1 TWCH and F_1 TWCB that derived from the crossing of Towuti with both Ciherang and Cibogo resulted in higher plant height (93.88 and 95.25 cm, respectively) compared with the female parent (93.15 cm) as shown in Figure 1. Nevertheless, plant height character was not significantly different between F_1 hybrids and their respective female parents. Similar result have been reported by Limbongan et al., (2008), that there is no significant difference in plant height character between F_1 hybrids and their comparison.

Plant height is a major trait affecting yield potential in rice (Zhang et al., 2017). There is complete phenotypic and partial genomic inheritance for plant height and some related characters (Mohamed and Hanna, 1964; Adiredjo and Grieu, 2018).Plant height is generally considered to be controlled by both qualitative and quantitative genes. There are more than 1,000 QTLs related to rice plant height character that have been detected and are distributed on 12 chromosomes (Wang et al., 2016).

The result of number of leaves observation shown in Table 2 which exhibited that all F_1 hybrids have lower number of leaves compared with their respective female parents. In despite of having lower number of leaves, there was any significant difference between F_1 hybrids with their respective female parents, which was found in F_1 SBCH, F_1 TWCH, and F_1 TWCB. F_1 SBCB was the only F_1 hybrid that was not significantly different with its female parent. Byrne (2005), explained that leaf development starts with the recruitment of founder cells from the shoot apical meristem, which is characterized by the down-regulation of *knotted1*-like homeobox genes. The number of leaves in rice determined rice's photosynthetic potential and played important roles in determining rice yield.

Table 1. Description of SSR markers used in the study

No.	Primers	Primer Sequences	Trait / QTL	CN	Size Of Bands (bp)	References
1	RM219	F: CGTCGGATGATGTAAAGCCT R: CATATCGGCATTCGCCTG	QRsf9	9	186-216	Yue et al., (2006)
2	RM260	F: ACTCCACTATGACCCAGAG R: GAACAATCCCTTCTACGATCG	MQTL12.1	12	103-130	Swamy et al., (2011)
3	RM525	F: GGCCCGTCCAAGAAATATTG R: CGGTGAGACAGAATCCTTACG	BRT,RN, RFW,RDW	2	122-152	Qu et al., (2008)
4	RM318	F: GTACGGAAAACATGGTAGGAAG R: TCGAGGGAAGGATCTGGTC	qssf2.1	2	134-154	Srividhya et al., (2011)

CN, chromosome number; QRsf9: QTL associated with spikelet fertility; MQTL12.1, QTL associated with grain yield; BRT, basal root thickness; RN, root number; RFW, root fresh weight; RDW, root dry weight; and qssf2.1, QTL associated with spikelet fertility;

Table 2. The mean data of morphological characters of F1 hybrids with their female parent

No.	Characters	SB	F1 SBCH	F1 SBCB	TW	F1 TWCH	F1 TWCB
1	Plant Height (cm)	97.90	96.04^{ns}	96.08^{ns}	93.15	93.88^{ns}	95.25^{ns}
2	Number of Leaves	119	98**	116 ^{ns}	154	128*	134*
3	Panicle Length (cm)	27.74	26.38**	24.51**	26.92	24.89**	25.16**

Description : SB, Situ Bagendit; TW, Towuti; ns, not significant; *, **, indicate significant difference between F1 hybrids and their female parent which determined by Student's t-test at P<0.05 and P<0.01, respectively.

(a) (b) (c) (d)

Figure 1. Plant height comparison between F_1 hybrids and their respective parents. a) F_1 SBCH, b) F_1 SBCB, c) F_1 TWCH, dan d) F_1 TWCB.

The average means value of panicle length were presented in Table 2, showed that all F_1 hybrids were very significantly different with their respective female parents despite of having lower panicle length as shown in Figure 2. This result was in contrast with Limbongan et al., (2008) and Sutaryo (2014), which reported that there is no significant difference between F_1 hybrid with their comparison for panicle length. Panicle length is an important trait for improving panicle architecture and grain yield. Aryana et al., (2017), stated that panicle length is one of the selection criteria of rice because it contributes directly to grain yield.

Agronomic characters

Tillering is one of the most important agronomic traits for grain production in rice because tiller number per plant determines panicle number, a key component of grain yield (Bian et al., 2013). The observation results of number of tiller were presented in Table 3. The observation result showed that all F_1 hybrids have lower tiller number compared with their respective female parents. Each of F_1 SBCH and F_1 SBCB have 19 of number of tiller whereas their female parent (Situ Bagendit) have 23 of tiller number.

(a) (b) (c) (d)

Figure 2.Panicle length comparison between F_1 hybrids and their respective parents. a) F_1 SBCH, b) F_1 SBCB, c) F_1 TWCH, dan d) F_1 TWCB.

Table 3. The mean data of agronomic characters of F1 hybrids with their female parent

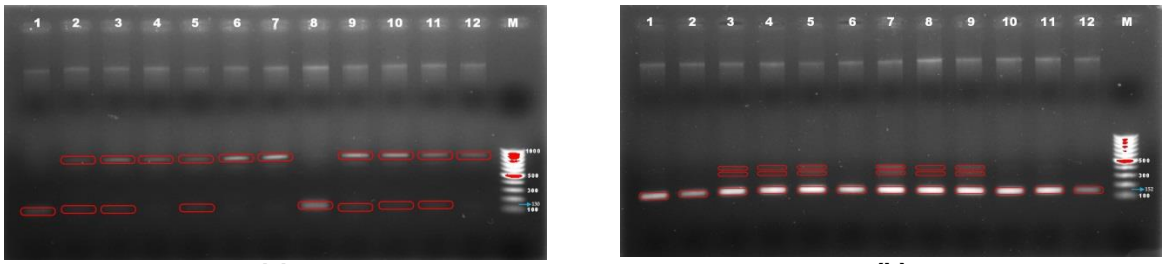
No.	Characters	SB	F1SBCH	F1SBCB	TW	F1TWCH	F1TWCB
1	Number of tiller	23	19*	19*	24	20*	21*
2	Number of productive tiller	13	9*	11*	16	10*	10*
3	Heading date (dat)	83	77*	79*	79	80^{ns}	79^{ns}
4	Harvest date (dat)	106	116^{ns}	116^{ns}	119	123*	121^{ns}
5	Weight of 100 grain (g)	3,22	2,94*	$3,24^{ns}$	3,48	3,16*	3,27*
6	Grain yield (g)	14,97	4,30*	7,63*	17,25	7,48*	9,41*

Description : SB, Situ Bagendit; TW, Towuti; dat, date after translanting; ns, not significant; *, **, indicate significant difference between F1 hybrids and their female parent which determined by Student's t-test at P<0.05 and P<0.01, respectively.

Tabel 4. Amplification result of each SSR markers used in the study.

No.	Names	RM219		RM260		RM525		RM318	
		Allel	Size (bp)	Allel	Size (bp)	Allel	Size (bp)	Allel	Size (bp)
1	Situ Bagendit	+	200	+	130	+	150	+	150
2	Towuti	+	200	++	130 dan 900	+	150	+	150
3	Ciherang	+	200	++	130 dan 900	+++	150, 300, dan 500	+	150
4	Cibogo	+	200	+	900	+++	150, 300, dan 500	+	150
5	F_1SBxCH	-	-	++	130 dan 900	+++	150, 300, dan 500	+	150
6	F_1SBxCH	+	250	+	900	+	150	+	150
7	F_1SBxCB	+	250	+	900	+++	150, 300, dan 500	+	150
8	F_1SBxCB	+	250	+	130	+++	150, 300, dan 500	+	150
9	F_1TWxCH	+	250	++	130 dan 900	+++	150, 300, dan 500	+	150
10	F_1TWxCH	+	250	++	130 dan 900	+	150	+	150
11	F_1TWxCB	+	250	++	130 dan 900	+	150	+	150
12	F_1TWxCB	+	250	+	900	+	150	+	150

Description : +, Alleles and number of allelles; and -, null allele .



(a) **(b)**

Figure 3.PCR-amplification result using 1.0% electrophoresis gel on RM260 (A) and RM525 (B). M: DNA marker; Lane 1, Situ Bagendit (gogo-dryland parent 1); Lane 2, Towuti (gogo-dryland parent 2); Lane 3, Ciherang (paddy-field parent 1); Lane 4, Cibogo (paddy-field parent 2); Lane 5-6, F_1 SBCH derived from Situ Bagendit× Ciherang; Lane 7-8, F_1 SBCB derived from Situ Bagendit× Cibogo; Lane 9-10, F_1 TWCH derived Towuti× Ciherang; and Lane 11-12, F_1 TWCB derived from Towuti× Cibogo.

Meanwhile, F_1 TWCH (20 tiller number) and F_1 TWCB (21 tiller number) was slightly have less number of tiller compared with Towuti (24 tiller number) as their female parent. The same result also found in observation of number of productive tiller, which showed that all F_1 hybrids have lower number of productive tiller compared with their respective female parents. Nonetheless, All F_1hybrids were known significantly different in number of tiller and number of productive tiller compared with their comparison. This result was in contrast with Limbongan et al., (2008), which reported that number of tiller character is not significantly different between F_1 hybrids and their comparison.

The productive tillers are the tillers that capable of producing panicles. Number of productive tillers directly affects the number of panicles that are produced. The more productive tillers, the higher the number of grains produced. Tiller development is regulated by a complex network of genetic, hormonal, and environmental factors (Hussien et al., 2014). Rice tiller were divided into primary which are produced first, then followed by secondary and tertiary tillers. Tertiary tillers are often produced in high tillering varieties only. Tillers that grow late in the season will not produce any grain and will lower the overall harvest index (Hussien et al., 2014).

The mean data of heading date shown in Table 3. It showed that heading date in F_1 SBCH (77 dat) and F_1 SBCB (79 dat) were lower than in Situ Bagendit (83 dat). These F_1 hybrids were significantly different with their female parent. Otherwise, heading date in F_1 TWCH (80 dat) and F_1 TWCB (79 dat) were known higher or the same with Towuti (79 dat), and these F_1 hybrids were not significantly different with their female parent. In observation of harvest date, the opposite happened. Compared with their respective female parents, F_1 hybrids of F_1 SBCH, F_1 SBCB, and F_1 TWCB were not significantly different but F_1 TWCH were. Bollich (1957) stated that generally the heading date in F_1 generation is intermediate but nearer to their early parent.

According to Murayama and Sarker (2002), most F_1 hybrids are significantly lower than those in their respective parents in heading date and maturity. The results of the study showed that heading date in F_1 hybrid were relatively lower than their respective female parents, but relatively higher in harvest date. Lower heading date and harvest date are usually desirable, because these traits will cause the hybrids to mature earlier as compared to their parents.

Based on the observation of weight of 100 grain as showed in Table 3, noted that all F_1 hybrids i.e. F_1 SBCH (2.94 g), F_1 TWCH (3.16 g), and F_1 TWCB (3.27 g) were slightly lower than their comparison, except in F_1 SBCH (3.24 g) that was relatively higher. From the result also known that F_1 SBCB was the only F_1 hybrid that was not significantly different with its comparison while the other three were significantly different. Meanwhile, the result of grain yield observation showed that all F_1 hybrids have much lower grain yield than their respective female parents as their comparison. F_1 SBCH (4.30 g), F_1 SBCB (7.63 g), F_1 TWCH (7.48 g), and F_1 TWCB (9.41 g) were much lower than Situ Bagendit (14.97 g) and Towuti (17.25 g). The result of this study was in contrast with Sutaryo (2014), which reported that weight of 1000 grain and grain yield are not significantly different between F_1 hybrids and their comparison. The low grain yield in F_1 hybrids arguably have something related with spikelet

sterility. Murayama and Sarker (2002), explained that F_1 hybrids generally show variable degrees of sterility hence F_1 hybrids often show a lower harvest index than their parent cultivars. Moreover, Suwarto et al., (2013), stated that grain yield is a complex character and is the result of interaction of many variables due to different gene association.

SSR markers analysis

Polymorphism level of among F_1 hybrids and their respective parents were evaluated by calculating allelic number and PIC values for each of the four SSR primers used. The results revealed that three out of four primers i.e.RM219, RM260 and RM525 showed polymorphism among the samples studied indicating the robust nature of microsatellites in revealing polymorphism whilst RM318 produced monomorphism. A total of 65 alleles were detected at the loci of four microsatellites markers across twelve samples with 41 alleles were polymorphic (63.07%) and 24 were monomorphic (36.92%). The amplicon size of all twelve samples that evaluated for each marker alleles varied from 130-900 bp (Table 4).

According to Williams et al., (1990), polymorphic represents images of DNA bands that appear at certain sizes, but in other samples no DNA bands are found in these sizes. In contrast, monomorphic bands are the bands that contained in some samples, so they have no variation. The monomorphic bands will appear in the evaluated samples with the same size. The bands are supposed to encode the constitutive trait or associated with house-keeping gene. The high monomorphic bands often lead to a narrowing of genetic diversity (Mulyaningsih and Indrayani, 2014).

PIC value

PIC value refers to the value of a marker for detecting polymorphism within a population, depending on the number of detectable alleles and the distribution of their frequency (Ramadan et al., 2015).

The polymorphic information content (PIC) was employed for each locus to assess the information of each marker and its discriminatory ability and it is a reflection of allele diversity and frequency among twelve samples that are evaluated. As it shown in Table 5, the PIC values ranged from 0.00 to 0.777 with an average of 0.491. The highest PIC value was obtained for RM219 (0.777) followed by respectively RM525 (0.75), RM260 (0.437), and RM318 (0.00). PIC value revealed that RM219 was considered as the best marker for the twelve tested samples. This result was consistent with Ramadan et al., (2015) who reported greatly variation in PIC values for all tested SSR loci (from 0.21 to 0.79 with an average of 0.48). Higher averages of PIC values was reported by Afiukwa et al., (2016) with the average of 0.77. According to Carsono et al., (2014), PIC values were divided into three categories, i.e.PIC > 0.50 is highly informative, 0.50 < PIC < 0.25 is informative, and PIC < 0.25 is slightly informative. Based on the result of the study, RM219 and RM525 were highly informative and RM260 was informative.

Analysis of purity of F1 hybrids detection

To test the conformity of hybrid, one must be able to distinguish the true hybrid resulting from cross between male and female parents and one coming from self-pollination of female parent.

In the present study, F_1 SBCH, F_1 SBCB, F_1 TWCH, F_1 TWCB, and their respective parents were characterized on the basis of SSR profile. Based on the complimentary banding pattern between the hybrid and their parents, the two out of four SSR primers i.e.RM260 and RM525 were identified as the effective primers to distinguish F_1 hybrids from their parental lines as shown in Figure 3. The primer RM260 amplified specific alleles of size both in 130 and 900 bp in F_1 SBCH1, F_1 SBCH2, F_1 SBCB1, and their respective male parents (Ciherang and Cibogo) whilst F_1 SBCB2 and its female parent (Situ Bagendit) only amplified in a specific allele of size 130 bp. Meanwhile, F_1 TWCH1, F_1 TWCH2, F_1 TWCB1, and F_1 TWCB2 which derived from the crossing of Towuti and paddy-field rice varieties (Ciherang and Cibogo) amplified in the same allele of size 130 and 900 bp. The presence of both female and male parents allele were observed as a resultant of crossing between two parents (F_1 hybrids). It confirmed the crossing and hybridity between the two parents (Macha et al., 2011). Pallavi et al., (2010), also stated that the hybrid which have marker of both male and female parents is usually considered as genuine hybrid.

Table 5. Percentage of polymorphism and PIC value of SSR markers used in the study

No.	Primers	Fragment			Polymorphism (%)	PIC
		Total	Monomorphic	Polymorphic		
1	RM219	11	0	11	100	0.777
2	RM260	18	0	18	100	0.437
3	RM525	24	12	12	50	0.75
4	RM318	12	12	0	0	0

Different from RM260, in primer RM525 resulted in amplifying allele of size 150, 300, and 400 bp in F_1 SBCH1, F_1 SBCB1, F_1 SBCB2, and both of their respective male parents (Ciherang and Cibogo) whereas Situ Bagendit as female parent and F_1 SBCH2 only amplified in specific allele of size 150 bp. The different result showed in F_1 hybrids obtained from the crossing of Towuti as female parent and both Ciherang and Cibogo as male parents where only F_1 TWCH1 and Ciherang (male parent) amplified at 150, 300, and 400 bp, whilst the other F_1 TWCH2, F_1 TWCB1, and F_1 TWCB2 had the same amplicon at 150 bp like their female parent. Liu et al., (2007), noted that the band patterns of some true hybrids exhibited the absence of the female and existence of the male parent-specific marker.

F_1 hybrids that have similar band with their female parents rather than their male parents could be suspected as a result of selfed female parent so that could be considered as false hybrid.

CONCLUSION

In conclusion, there were any significant difference both in morphological and agronomic characters except in plant height variable among the F_1 hybrids and their respective female parents. In purity of F_1 hybrid identification test showed that the F_1 hybrids obtained from the crossing of Situ Bagendit with paddy-field rice varieties (Ciherang and Cibogo) tended to generate true hybrids rather than the crossing of Towuti with paddy-field rice varieties. The study also suggest that there is a need to evaluate more in drought resistance variables and agronomic characters that related to the yield components in F_2 and next generation as another step to develop drought-tolerant rice varieties with high yielding potential.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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AUTHOR CONTRIBUTIONS

ZDH, ALA and NRA conceived, designed and performed the experiments. ZDH and ALA analysed the data. ALA, RES, DAM contributed reagents/materials/analysis tools. ZDH and ALA wrote the paper. ALA coordinated the research project.All authors read and approved the final version.

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