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Phenotypic Evaluation and Genetic Profiling of Shallot Genotypes Adapted to Peatland of South Kalimantan Using Simple Sequence Repeat (SSR) Markers

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ABSTRACT

The escalating demand of shallot has forced the increase of domestic production, including through extension of harvest area by utilization of arable land on peatland. This study was aimed to analyze genetic profile of shallot adapted in peatland areas using SSR markers. Twenty-one shallot genotypes were tested in the field and eighteen primers dispersed throughout the genome was applied to analyze genetic diversity of the peatland-adapted shallots. Phenotypic evaluation revealed that shallot yield potential ranged from 6.66 to 14.21 t/ha. Of these, seven shallot clones (11 NA, 1111 TA, 12 NA, 12 NC, 20 NA and 22 N) had good yield potential and comparable with those of five released shallot varieties. Nine out of 12 shallot clones were moderately resistant to *Alternaria porii*. Clustering analysis showed that shallot genotypes were clustered into two main groups, Clustered I and II which consisted of 13 and 8 genotypes, respectively. The closest genetic relatedness was observed between 8NC and 8NA (0.85), while the farthest ones was between 11NA and Kramat2 (0.51). This result implies that cross combination between 11NA and Kramat2 is valuable and suitable for breeding programs aimed at improving shallot potential yield in the future.

INTRODUCTION

Shallot (*Allium cepa* group *Aggregatum*) is an important vegetable and classified as strategic and prime commodity for Indonesia which continuously gains government attention in order to be developed through the productivity increase and expansion of plant area (Irianto, Yakup, Harun, & Susilawati, 2017). Shallot is used in various culinary in household and food industry either as spices or condiments. The increase of shallot demand as the result of population growth should be followed by the increase in shallot production. Indonesia is expected to become a self-sufficient state and the leading exporting country for shallots in ASEAN by 2045. Indonesia targets 40.000 tons of exports. In order to achieve these targets, the addition of shallot production land area of 34.307 ha or 1.183 ha/year is required (Directorate General of Horticulture, 2017). To increase the production of shallots can be pursued by the addition of land production and land management that already exist with as much as possible, through land improvement efforts so that the productivity of land will increase (Rahayu, Mujiyo, & Arini, (2018). Prior to expansion of plant areas, identification of adapted shallot cultivars under marginal environment like peatland area is also considered as a best way to increase shallot production in Indonesia.

Peatland is categorized as marginal land and it consists of natural accumulation of partially or fully decomposed materials and organic materials in wet condition (Noor, 2012). It is accounted for

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14.91 million ha in Indonesia, and usually the type of peatland that can be used for plant cultivation are those fully decomposed land with fiber content less than 17 % (Subagyo, 2002). Previous study reported that some shallot varieties, i.e. cv. Manjung and Sumenep have been observed to be well adapted in peatlands of West Kalimantan. These cultivars have the productivity of 6.34 to 8.02 t/ha and were relatively tolerant to *Alternaria porii* (Purbiati, Umar, & Supriyanto, 2010). Those peatland-adapted shallot cultivars could potentially be utilized as genetic resources for crop improvement. Shallot cv. Sembrani located in Centre Kalimantan produced 18.7 tons/ha that was higher than cv. Bima Brebes (7.2 t/ha) and cv. Mentes (1.1 t/ha).

Genetic diversity in plant genetic resources (PGR) provides opportunity for plant breeders to develop new and improved cultivars with desirable characteristics, which include both farmer-preferred traits, such as yield potential and large seed, and breeders preferred traits, such as pest and disease resistance and photosensitivity (Govindaraj, Vetriventhan, & Srinivasan, 2015). The genetic diversity study in the shallot breeding program would also be beneficial to obtain new shallot varieties suitable for low income farmers in marginal production environments such as in peatland areas. To meet this effort, a number of shallot breeding lines have been developed and selected based on their adaptability in peatland areas in South Kalimantan.

Molecular marker is a tool to help breeders and geneticists to analyze plant genome. Molecular marker can be interpreted as an effort to determine plant characteristics at the gene level. It is used to monitor variation on DNA sequence in the species and also to manipulate genetic variation by introducing desired characters. Molecular marker can be an alternative system to be used in gene mapping, tracing genetic relationship, marker for selection for qualitative as well as quantitative characteristics (Araki et al., 2010; Arifin, Ozaki, & Okubo, 2000; Bachmann et al., 2001; Cunha, Hoogerheide, Zucchi, Monteiro, & Pinheiro, 2012; Masuzaki et al., 2006; Song et al., 2004). This approach especially is required to trace clone or accession selected for parents in hybridization that can create a wide variability and expected characteristics of both parents. In comparison to the management of plant maintenance in the field, the use of molecular marker systems are continually sought for practical applications in germplasm conservation, management and enhancement, due to this method is considerably rapid, reliable, informative and relatively simple (Widaningsih, Purwanto, Nandariyah, & Reflinur, 2014).

Simple Sequence Repeat (SSRs) have been the most widely used markers for genotyping plants over the past 20 years because they are highly informative, codominant, multi-allele genetic markers that are experimentally reproducible and transferable among related species (Mason, 2015). A number of studies have reported the use of SSR markers for germplasm characterization in a multiplicity of crop species (Weising, Atkinson, & Gardner, 1995). SSRs markers are widely distributed throughout the genome, especially in the euchromatin of eukaryotes, and coding and non-coding nuclear and organellar DNA (Phumichai, Phumichai, & Wongkaew, 2015). In Allium crops, SSR markers have been broadly used as an ideal DNA marker for several purposes, such as constructing a linkage map (Tsukazaki et al., 2008), to the variety identification (Tsukazaki et al., 2010) and genetic purity tests (Kim et al., 2012), and to the genetic diversity study and genomic synteny analysis (Khar, Lawande, & Negi, 2011). Several SSR markers has been successfully selected and applicable as a molecular tool in the heterogeneity and variety traceability tests (Tsukazaki et al., 2006). In addition, Kim et al. (2012) reported that a total of 20 selected SSR markers screened from a total of 1,049 SSR primers were identified as DNA markers suitable for genetic purity. Based on genotyping on a total of 14 bulb onion using the 20 selected SSR markers, the genetic purity of three breeding lines were clearly obtained. Prior to linkage map construction, a number of SSRs markers derived from bunching and bulb onions genomes has been successfully assigned into eight basic chromosomes of *Allium cepa* (Tsukazaki et al., 2008). Several study has reported that SSR markers have been preferred to be used in the genetic diversity of *Allium cepa*. McCallum *et al*. (2008) reported onion genetic diversity tested using 56 EST-SSR markers and four genomic SSR markers. The objective of this study was to identify the genetic profiling of shallot varieties which adapted in peat-land area of South Kalimantan using microsatellite or simple sequence repeats (SSR) markers.

MATERIALS AND METHODS

Plant Materials

Twenty-one shallot genotypes were used as plant genetic materials in the present study (Table 1). Of these, a total of 12 shallot genotypes including clones 8 NC, 8 NA, 11 NA, 11I1 TA, 11I12 TA, 12 NA, 12 NC, 12I1 TA, 16 NC, 20 NA, 22 N, and 22I1 TA have advanced shallot clones in F12 generation and the remaining nine accessions were the commercial shallot varieties. The twelve shallot clones were derived from cross combinations of the six commercial shallot varieties, such as Sembrani, Kramat 1, Kramat 2, Tiron, Maja, and Manjung. Pedigree of 12 shallot clones were presented in Table 1. Three commercial varieties, such as Sumenep, Kuning, and Batu Putih used as control plants in present study. However, in the phenotypic observation, except for screening the purple blotch disease resistance, one of the commercial shallot variety, Batu Putih was excluded due to many individual plants damaged during field experiment. These shallot genetic materials were evaluated for their adaptability in peatland. Evaluation for adaptation of shallot genotypes used in present study has been conducted at peat land of Landasan Ulin, South Kalimantan from June to August 2016. The experiment was laid out in a randomized block design in a randomly block design with 4 replications. The plot size was 1 x 6 m and each plot consisted of 200 individual plant with a 20 x 15 cm of spacing between plants. Field management was carried out following normal agronomic practices. Fertilizers were properly applied at the rate of 250 kg NPK (16:16:16) per ha before transplanting and followed by the application of liquid fertilizer of NPK (16:16:16) at the rate of 200 ml per plant at 3, 5, and 7 week after planting.

Phenotype Observation

Three phenotype traits consisted of morphological characters, disease resistance to purple blotch disease (*Alternaria porri*), and agronomic traits were observed from the twenty-one shallot accessions. Morphological characters such as plant height (cm), leaf lenght (cm), leaf diameter (mm), tiller number, and leaf number per tiller were observed at flowering initiation stage. Evaluation of shallot accessions against A. porri were carried out under controlled condition by spraying the spore suspension of *A. porri* (10^6 spores/ml). During disease evaluation, those shallot accessions were kept in the greenhouse of IVEGRI (Indonesian Vegetable Research Institute), Lembang, West Java. Response of shallot accessions against *Alternaria porri* was recorded based on the disease severity (Sharma, 1986) which categorized in different disease reaction groups, including 0 = no disease symptom, 1 = a few tip spots with 10 % infected leaf area, 2 = purplish brown patches with 20 %, infected leaf area, 3 = multiple merging patches with 40 % infected leaf area, 4 = streaking leaves with 75 % infected leaf area, and 5 = complete drying and breakage of leaves. Some importance agronomic traits such as bulb performances including number of bulb per hills, bulb weight per hills (g), bulb diameter (mm), bulb height (mm), weight per bulb (g) and shallot yield potential (t/ha) were observed after harvesting. Harvesting time was carried out at the time plants senescence.

Table 1. Shallot genotypes used in present study

No	Genotype[a]	Pedigree[b]
1.	8NC	Sembrani x Kramat1
2.	8NA	Sembrani x Kramat2
3.	11NA	Tiron x Sembrani
4.	11 1TA	Tiron x Sembrani
5.	11 2TA	Tiron x Sembrani
6.	12NA	Tiron x Kramat1
7.	12NC	Tiron x Kramat2
8.	12 1TA	Tiron x Kramat2
9.	16NC	Tiron x Maja
10.	20NA	Kramat2 x Maja
11.	22N	Kramat2 x Manjung
12.	22 1TA	Kramat2 x Manjung
13.	Sembrani	Parental lines
14.	Kramat1	Parental lines
15.	Kramat2	Parental lines
16.	Tiron	Parental lines
17.	Maja	Parental lines
18.	Manjung	Parental lines
19.	Sumenep	Check variety
20.	BatuPutih	Check variety
21.	Kuning	Check variety

Remarks: [a] = Shallot genotypes No. 1 to 12 referred to advanced and selected promisingly shallot clones (bulb generation-12 or BG12 generation which developed through the bulb development derived from the obtained F1 seeds); genotype no. 13 to 18 referred to commercial shallot variety used as parental lines of the 12 new released varieties; and the shallot genotypes listed from No.19 to 21 referred to the control varieties). [b] = No. 1 to 12 referred to the cross combinations in generating the 12 elite shallot clones.

Table 2. List of SSR primers used in present study

Primer	Sequences		
	Chromosome	Forward	Reverse
ACM024	2	Ccccattttcttcattttctca	Tgctgttgctgttgttgttg
ACM071	5	Tctcatttcaactttctacctatcc	Ctgacatttgctcgactgga
ACE010	5	Atgtaccacatggatgaaaaacaca	ggtagctgaagcaaatcaagcaa
ACE039	4b	Atggtcgtccatttcttattgaagt	cagttgttacatgtggtatcagagc
ACE044	3a	Atgtacgcagaatctcgtcctttt	ttcaaattcttttgctgatgggttc
ACE101	2b	Tttaacaggtctccctcttctcccc	agggggtactttcatcactccgtt
ACE111	2a	ttgacctaacaaatatagtcccacaaa	ttgacctaacaaatatagtcccacaaa
ACM154	1a	Cgatgaatacaccgatgacg	Cgatgaatacaccgatgacg
ACE113	7b	tcgagaacaagcatccaacaattcca	tcgagaacaagcatccaacaattcca
ACM096	6a	agttccaagcaccaaaagaagcac	agttccaagcaccaaaagaagcac
AFAA02D08	1a	tatttttacagcaacggagcagca	tatttttacagcaacggagcagca
AFB20G05	6a	Ttccacaccgtaatccaactcctt	attaaaatgagcgttgttggctcg
AFRA111F11	2a	ccgccattagtgagagactacgtg	gggcaaacggttacatatagccaa
AFS015	1a	atctcactgtccttgtacctgaaag	catcttgactttgtgatatttgtgc
AFS104	3a	Tcaaggaaacacgtatgccact	Tcgccctttagattcatttcc
AFS145	3b	acccttgggataagtggtttattga	ggtcatgagtaattcaccgaacatt
AFS149	4a	aaccaattgattacctctcatctgc	tgcggaccttccatagtctgtataa
AFS039	6a	cgggtaataacggatatcataaaca	cagttgttacatgtggtatcagagc

Molecular Analysis

Total genomic DNA of each genotype was isolated using the small-scale extraction method that was previously described by Doyle & Doyle (1990). The protocol has been modified to include PVP (*Polyvinylpyrrolidone*) and sodium bisulfite in DNA extraction buffer (2 % (w/v) CTAB (*Cetyltrimethylammonium bromide*), 100 mM Tris-HCl pH 8.0, 1.4 M NaCl, 20 mM EDTA pH 8.0, 1 % (w/v) PVP, and 0.3 % (w/v) sodium bisulfite). The concentration and quality of isolated DNA were determined spectrophotometrically by measuring at 260 nm with a NanoPhotometer™ (Implen, Munich, Germany). DNA purity assessment was carried out with the 260:280 ratio. The concentration of DNA samples was equalized among all samples to a final concentration of 20 ng/µL before performing PCR analysis.

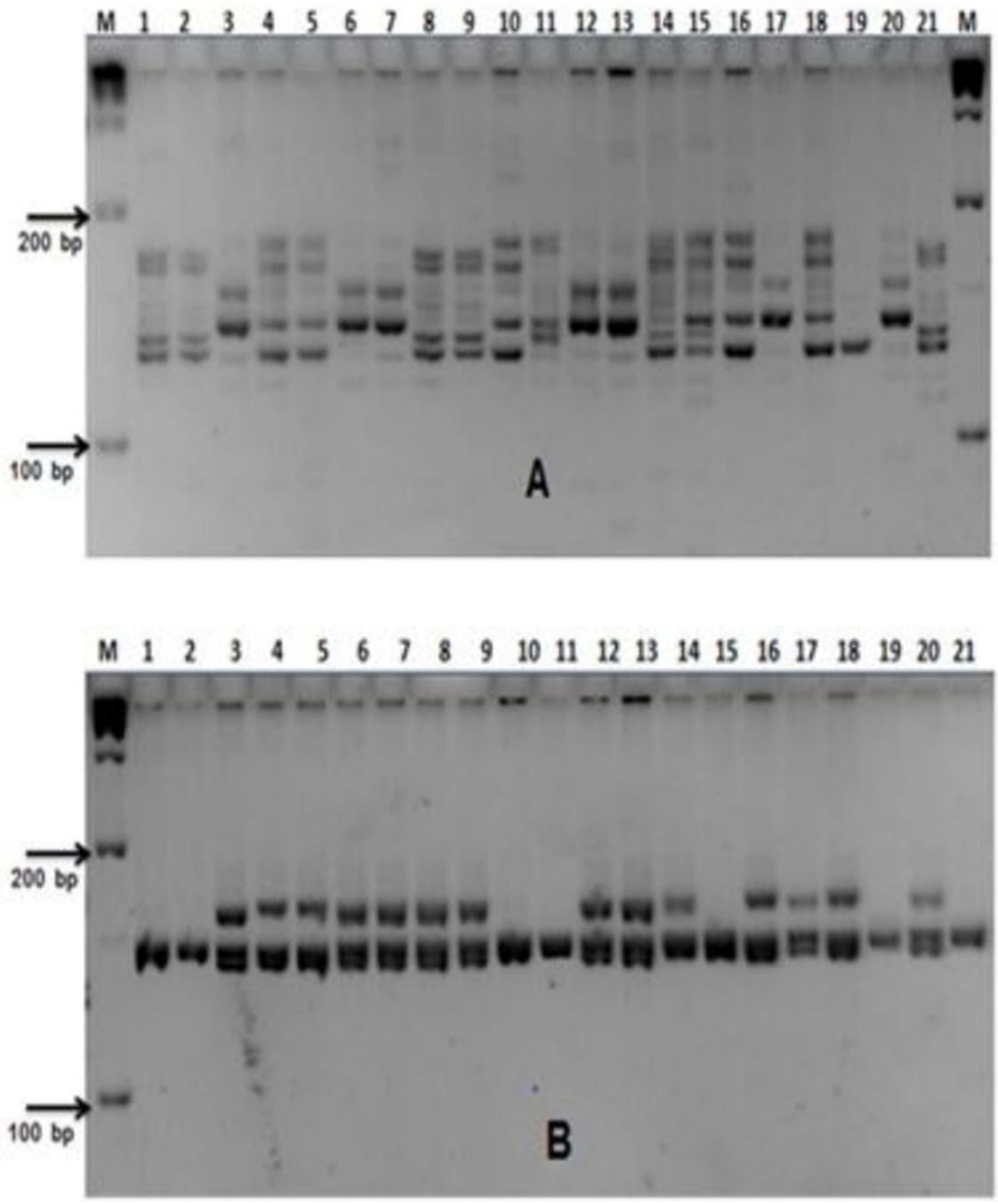
Each DNA sample was genotyped using 18 SSR primers dispersed throughout the genome. The detail of SSR primers was shown in Table 2. PCR reactions were conducted in a total volume of 20 µl and contain 20 ng of genomic DNA, 0.25 µM of each primer (forward and reverse), 0.125 mM of each dNTP, 0.16 units of *taq polymerase*, and 1X PCR buffer. The PCR amplification was performed using in a 96-well plates in a tetrad thermal cycler (*DNA Engine Tetrad*, *MJ Research*). The amplification conditions consisted of an initial denaturation step of 5 minutes at 94 °C, followed by 35 cycles of 45 seconds at 94 °C, 30 seconds at 52-56 °C (depending upon temperature melting of primers), 45 seconds at 72 °C with a final extension at 72 °C for 10 minutes.

The amplified products were separated on a non-denaturing 8 % polyacrylamide gel electrophoresis (Model MGV, CBG Scientific Co.) in 1X TBE buffer (for 2 hours at 90 volt) stained with ethidium bromide. The banding patterns were visualized under UV light using a digital system and image scanning Gel Documentation EQ system (Biorad). To estimate size of the SSR banding profile, a 100 bp ladder was loaded along with PCR products (Fig. 1).

Data Analysis

To measure the informativeness of the SSR markers, the polymorphism information content (PIC) values defined by Botstein, White, Skolnick, & Davis (1980) for each SSR, gene diversity, defined as the probability that two randomly chosen alleles from the population are different (Weir, 1996), and total number of alleles at each SSR locus were calculated using the PowerMarker software (Liu & Muse, 2005). The presence or absence of each single fragment amplified by microsatellite primers for different varieties was coded by 1 or 0, respectively and scored for a binary data matrix. Genetic similarity estimation was estimated following Nei & Li (1979) by the formula: gsij = 2Nij/(Ni+Nj), where Nij is the number of bands present in varieties i and

j, Ni (resp. Nj) is the number of bands present in variety I (resp. j). Markers with missing observations for variety i and/or j not included in the calculation of gsij. The genetic similarity matrix, Unweighted Pair Group Method of Arithmetic Average (UPGMA) i.e. cluster analysis were used to assess pattern of diversity among the wheat varieties. All calculations were performed using the NTSYS-pc version 2.1 software (Rohlf, 2002).



Remarks: (A) Primer ACM 024, and (B) ACM071

Fig. 1. Band patterns of 21 shallot genotypes adapted in peatland using SSR markers

RESULTS AND DISCUSSION

Phenotypic Evaluation

The phenotypic performances of the shallot accessions tested in peatland were presented in Table 3. Of the total 21 shallot accessions, Clone 11 NA showed the highest values for several morphological characters, such as plant height (47.51 cm), leaf length (40.74 cm), and leaf number per tiller (7.8), while Sumenep variety showed the lowest ones for the plant height (29.26 cm), leaf length (24.32 cm) and leaf number per tiller (3.76). On the other hand, Clone 11 NA showed the lowest tiller number (2.48) and Sumenep the highest ones (9.65). The widest value of the leaf diameter was observed in Clone 1112TA (9.77 mm).

The evaluation of shallot genotypes to A. porri revealed that the tested cultivars responded differently to the pathogen (Table 4). Among the tested genotypes, 15 accessions were categorized as moderately resistant, 4 accessions were fallen under susceptible, 1 was under highly susceptible and 1 was classified as highly resistant. None of the tested cultivars was considered as immune. This findings were in accordance with Nanda, Chand, Mandal, Tripathy, & Joshi (2016) when evaluating 43 shallot accessions.

The resistant cultivar (cv. Sumenep) had the lowest disease intensity (6 %) among the tested cultivars. The respected cultivar, however, have never produced flower under local circumstances. These conditions led to the barrier to gene transfer through hybridization. The alternative technique for crop improvement might still be conducted then using other potential methods, including biotechnology approaches.

Phenotypic evaluation of 20 shallot accessions for bulb characters and yield components was presented in Table 5. Number of bulb produced per hills ranged from 2.46 (clone 11 NA) to 15.25 (Sumenep). Bulb weight per hills observed ranged from 20.80 g to 44.41 g with an average of 32.70 g. The highest value was observed in Sembrani and clone 8 NC while the lowest was found in clone 16 NC. Bulb diameter of the tested shallot accessions ranged from 11.72 mm (8 NC) to 17.95 mm (Sembrani) with an average value of 15.04 mm. Bulb height ranged from 20.42 mm (Kuning) to 31.34 mm (Sembrani) with an average value of 26.01 mm. The two lines showed better performances in relation to yield component traits. Despite clone 11 NA had the lowest mean value for the bulb number per hills, its bulb diameter was the highest ones amongst others. Whereas, the clone 8 NC produced the highest bulb weight per hills and this is comparable to the bulb weight produced by Sembrani accession.

Table 3. Variability of morphological traits in twenty clones/varieties of shallot

No.	Clones/ Varieties	Plant Height (cm)*	Leaves Length (cm)*	Leaves Diameter (mm)*	Tiller Number*	Leaves Number per tiller*
1	8NC	43.59^{ab}	37.50^{ab}	5.00^{b}	8.65^{ab}	4.29^{cd}
2	8 NA	40.98^{bc}	34.65^{bc}	4.40^{b}	8.23^{ab}	4.13^{cd}
3	11 NA	47.51^{a}	40.74^{a}	6.94a^{b}	2.48^{h}	7.18^{a}
4	11I1TA	33.63^{fg}	28.05^{ef}	4.35^{b}	4.19^{gh}	4.59^{bc}
5	11I2TA	33.12^{gh}	27.15^{ef}	9.77^{a}	5.15^{ef}	4.44^{cd}
6	12NA	36.71^{de}	31.12^{de}	4.42^{b}	5.71^{cd}	4.68^{bc}
7	12NC	37.53^{de}	31.25^{de}	5.00^{b}	5.10^{ef}	4.87^{bc}
8	12I1TA	36.45^{ef}	30.89^{de}	4.30^{b}	4.73^{fg}	4.41^{cd}
9	16NC	38.23^{cd}	31.52^{de}	4.31^{b}	4.90^{fg}	5.10^{bc}
10	20NA	40.41^{bc}	34.03^{bc}	4.28^{b}	5.40^{de}	4.63^{bc}
11	22N	45.71^{ab}	39.46^{ab}	5.14^{b}	4.21^{gh}	5.61^{b}
12	22I1TA	32.66^{gh}	27.00^{ef}	3.94^{b}	4.33^{gh}	4.62^{bc}
13	Sembrani	45.95^{ab}	38.53^{ab}	5.67^{ab}	5.01^{ef}	5.26^{bc}
14	Kramat 1	40.55^{bc}	34.13^{bc}	4.43^{b}	8.77^{ab}	3.71^{e}
15	Kramat 2	40.51^{bc}	34.25^{bc}	4.45^{b}	5.98^{cd}	4.77^{bc}
16	Tiron	41.41^{bc}	35.60^{ab}	4.59^{b}	6.85^{bc}	5.17^{bc}
17	Maja	42.46^{ab}	35.30^{bc}	4.49^{b}	7.17^{bc}	4.32^{cd}
18	Manjung	40.98^{ab}	34.82^{bc}	4.39^{b}	7.65^{ab}	5.22^{bc}
19	Sumenep	29.26^{h}	24.32^{f}	5.76^{ab}	9.65^{a}	3.76^{de}
20	Kuning	38.83^{cd}	33.39^{cd}	5.43^{ab}	7.44^{bc}	4.72^{bc}
	GA	39.32	33.19	5.05	6.08	4.77
	CV	8.82	10.00	57.06	22.28	14.22

Remarks: * = Values followed by the same letter in columns under every character head are not significantly different at P = 0.05 according to Duncan's Multiple Range Test; GA = general average; CV = coefficient variance

Table 4. Evaluation of shallot genotypes for purple blotch resistance under controlled condition

No.	Clones/Varieties	Disease Intensity	Reaction
1.	8NC	18	Moderately Resistant
2.	8NA	22	Moderately Resistant
3.	11NA	20	Moderately Resistant
4.	11I1NA	20	Moderately Resistant
5.	11I12NA	24	Moderately Resistant
6.	12NA	22	Moderately Resistant
7.	12NC	20	Moderately Resistant
8.	12I1TA	20	Moderately Resistant
9.	16NC	22	Moderately Resistant
10.	20NA	28	Susceptible
11.	22N	28	Susceptible
12.	22I1TA	24	Moderately Resistant
13.	Sembrani	22	Moderately Resistant
14.	Kramat1	30	Susceptible
15.	Kramat2	22	Moderately Resistant
16.	Tiron	20	Moderately Resistant
17.	Maja	22	Moderately Resistant
18.	Manjung	36	Susceptible
19.	Sumenep	6	Highly Resistant
20.	Batu Putih	22	Moderately Resistant
21	Kuning	40	Highly Susceptible

Table 5. Variability of morphological traits in twenty clones/varieties of shallot

No.	Clones/ Varieties	Bulb produced per hill	Bulb weight per hill (g)	Bulb diameter (mm)	Bulb height (mm)	Weight per bulb (g)*	Yield per plot (kg)*	Yield potential (t/ha)*
1	8NC	8.00^{de}	44.33^{a}	11.71^{d}	26.07^{ab}	5.54^{cd}	11.53^{a}	14.19^{a}
2	8 NA	7.30^{de}	40.47^{ab}	12.15^{cd}	23.56^{bc}	5.90 ^{cd}	10.52^{ab}	12.95^{ab}
3	11 NA	2.46^{j}	35.54^{ab}	20.07^{a}	29.25^{ab}	14.61^{a}	9.24^{ab}	11.37^{ab}
4	11I1TA	4.73^{gh}	34.52^{ab}	15.54^{ab}	30.31^{ab}	7.58^{bc}	8.98^{ab}	11.05^{ab}
5	11I2TA	5.06^{gh}	23.96^{bc}	13.89^{bc}	28.77^{ab}	4.95^{cd}	6.23^{bc}	7.67^{bc}
6	12NA	6.38^{ef}	35.44^{ab}	15.71^{ab}	24.23^{ab}	5.68^{cd}	9.22^{ab}	11.34^{ab}
7	12NC	4.93^{gh}	34.61^{ab}	14.80^{ab}	22.41^{cd}	7.28^{bc}	9.00^{ab}	11.07^{ab}
8	12I1TA	4.65^{gh}	20.85^{d}	14.96^{ab}	24.90^{ab}	4.51^{cd}	5.42^{cd}	6.67^{d}
9	16NC	4.47^{hi}	20.80^{d}	13.27^{bc}	26.47^{ab}	4.70^{cd}	5.41^{cd}	6.66^{d}
10	20NA	5.33^{gh}	30.33^{ab}	17.39^{ab}	28.23^{ab}	5.91^{cd}	7.89^{ab}	9.71^{ab}
11	22N	4.02^{ij}	37.64^{ab}	19.26^{ab}	25.94^{ab}	9.21^{b}	9.79^{ab}	12.04^{ab}
12	22I1TA	4.25^{i}	22.96^{cd}	13.74^{bc}	24.76^{ab}	5.37^{cd}	5.97^{cd}	7.35^{cd}
13	Sembrani	4.52^{gh}	44.41^{a}	17.95^{ab}	31.34^{a}	9.29^{b}	11.55^{a}	14.21^{a}
14	Kramat 1	8.45^{cd}	33.09^{ab}	13.68^{bc}	25.44^{ab}	3.70^{ef}	8.60^{ab}	10.59^{ab}
15	Kramat 2	5.83^{fg}	25.10^{bc}	14.64^{ab}	24.48^{ab}	4.29^{de}	5.02^{d}	8.03^{bc}
16	Tiron	9.79^{bc}	36.36^{ab}	15.59^{ab}	22.69^{cd}	3.67^{ef}	7.27^{ab}	11.64^{ab}
17	Maja	6.18^{fg}	27.55^{ab}	14.11^{ab}	27.67^{ab}	4.26^{de}	5.51^{cd}	8.82^{ab}
18	Manjung	10.29^{b}	41.66^{ab}	13.36^{bc}	27.72^{ab}	4.04^{ef}	8.33^{ab}	13.33^{ab}
19	Sumenep	15.25^{a}	35.68^{ab}	15.70^{ab}	25.60^{ab}	2.31^{f}	7.14^{ab}	11.42^{ab}
20	Kuning	8.12^{cd}	28.66^{ab}	13.29^{bc}	20.42^{d}	3.64^{ef}	5.73^{cd}	9.17^{ab}
	GA	6.50	32.70	15.04	26.01	5.82	7.92	10.46
	CV	17.37	32.95	23.89	16.72	31.77	33.15	32.95

Remarks: * = Means followed by the same letter within a column are not significantly different at P = 0.05 according to Duncan's Multiple Range Test; GA = general average; CV = coefficient variance

In regards to yield per bulb (weight per bulb), the twenty shallot accession produced average weight per bulb value of 5.82 g. The lowest value was observed in Sumenep (2.31 g), while the highest ones was in clone 11 NA (14.61 g). Surprisingly, the weight per bulb produced by clone 11 NA was higher than that of produced by Sembrani (9.29 g) which has bigger bulb in size (Table 5). However, the highest mean value of yield per plot of bulb (bulb weight per plot) was observed in clone 8 NC (11.53 kg) and Sembrani (11.55 kg), while the lowest ones was observed in Kramat 2 accession (5.02 kg). Yield potential of shallot accessions observed in present study ranged from 6.66 to 14.21 t/ha with an average value of 10.46 t/ha. The lowest mean value of yield potential was observed in 16NC and 1211TA, while the highest ones was observed in Sembrani and clones 8 NC (Table 5). Besides clone 8 NC which derived from Sembrani and Kramat 1 crosses, a total of seven shallot clones including 11NA, 11I1TA, 12NA, 12NC, 20NA and 22N adapted in peatland areas also had good yield potential which showed comparable yield potential with five released shallot varieties, such as Kramat 1, Tiron, Manjung, Sumenep, and Kuning (Table 5). These results indicated that several shallot clones were potential as new promisingly adaptable shallot accessions grown in marginal land such as peat-land areas. Further characterization of those selected accessions at molecular level is considered as one of the important breeding activities to identify genetic relatedness among shallot accession. The genetic relationship among shallot accession can be used as a basic knowledge for crop improvement in the future shallot breeding program, such as in selecting the parental lines to identify the best heterosis values.

SSR Polymorphism Analysis and Allele Diversity

Eighteen microsatellite markers used in this study showed well amplified products and clearly polymorphic bands along the shallot genotypes. These markers generated reproducible, clear, distinct and polymorphic amplification products at one or more loci. Those polymorphic markers were further used for the statistical analysis using Power Marker (Table 3). The polymorphic loci showed unique fingerprints providing a total of 184 alleles for all 21 cultivars. The number of alleles per locus found in the present study varied from two (ACM096) to 29 (AFS104) with an average number of 10.2 allele per locus.

PIC value of individual markers ranged from 0.33 for AFAA02D08 to 0.88 for ACM071 and AFS149, with an average of 0.61 for all markers. The higher the PIC value of molecular markers in a population, the broader their genetic diversity (Gramazio et al., 2018). Thirteen out of 18 SSR markers showed a PIC value greater than 0.50 reflecting that these loci were among informative SSR loci that were more contributed in genetic diversity of the shallot genotypes. Therefore, both the number of allele per locus and the PIC values in a population represent genetic diversity index. SSR marker analyses revealed a gene diversity of 0.75. The lowest gene diversity value was found on AFAA02D08 (0.4) and the highest one on ACM071 and AFS149 (0.89).

The 13 informative SSR markers (ACM024, ACM071, ACE010, ACE039, ACE101, ACE111, ACM154, ACE113, AFRA111, AFS015, AFS104, AFS149, and AFS039) found in the present study are important for future breeding program, especially would be useful as a tool in discriminating shallot genotypes in Indonesia. Marker with high PIC and informative turned out to be a marker set for DNA fingerprinting analysis for identification and differentiation of varieties (Bredemeijer et al., 2002).

Heterozygosity pointed towards genetic diversity in the population which mean that the genetic variability is measured as the amount of potential heterozygosity. In case of the study of genetic diversity study of Lentil crop using SNP markers, it is reported that expected heterozygosity is a more accurate measure of polymorphism of SNP markers as this parameter measures distribution of alleles across the germplasm under examination (Lombardi et al., 2014). In present study, the heterozygosity value ranged from 0.05 (ACE044 and ACM096) to 1 (ACE111 and AFS104) with an average of 0.61 (Table 6). On the other hand, two out of 18 SSR markers was unable to detect heterozygous alleles on the shallot genotypes used in this study. Usatov et al. (2014) reported that highly heterotic hybrids (long-term study) using SSR markers revealed reliable correlation between genetic distances and seed yield heterosis. As a result, these highest two markers (ACE111 and AFS104) found in current study suggested potential markers for predicting hybrid performance or heterosis in hybrid materials.

Genetic Variability and Phylogenetic Relationship

Phylogenetic tree constructed based on molecular marker analysis of shallot accession using a total of 18 polymorphic SSR markers clearly elucidated the relationships among the 21 shallot cultivars (Fig. 2) and revealed that the genetic

similarity coefficient of shallot genotypes ranged from 0.60 to 0.85, indicating narrow genetic variability of the shallot collection. Based on clustering analysis using NTSYS software, shallot genotypes were clustered into two main groups with similarity coefficient of 0.67 (Fig. 2). Cluster I consisted of 13 shallot genotypes, while cluster II was 8 genotypes. Each cluster was then grouped into sub-cluster, named as sub-cluster A and B. Sub-cluster IA was the highest density comprising of 9 shallot genotypes.

Table 6. Descriptive statistics of SSR markers resulted in present study

Primer	Allele number	Range of Allele (bp)	Major allele frequency	Gene Diversity	PIC	Heterozigosity
ACM024	15	119-254	0.40	0.81	0.80	0.95
ACM071	11	178-246	0.14	0.89	0.88	0.67
ACE010	13	231-322	0.29	0.88	0.87	0.76
ACE039	6	154-176	0.30	0.77	0.74	0.40
ACE044	3	185-187	0.52	0.61	0.54	0
ACE101	7	274-307	0.29	0.81	0.78	0.95
ACE111	18	92-288	0.48	0.76	0.75	1.00
ACM154	13	162-287	0.38	0.83	0.82	0.95
ACE113	10	184-272	0.19	0.88	0.87	0.67
ACM096	2	201-205	0.63	0.47	0.34	0
AFAA02D08	3	79-83	0.74	0.40	0.33	0.05
AFB20G05	4	74-89	0.40	0.65	0.58	0.81
AFRA11F11	7	230-304	0.40	0.75	0.72	0.62
AFS015	11	369-459	0.31	0.81	0.79	0.43
AFS104	29	106-447	0.45	0.78	0.78	1.00
AFS145	6	189-220	0.48	0.69	0.65	0.09
AFS149	17	298-510	0.14	0.89	0.88	0.62
AFS039	9	202-302	0.19	0.86	0.85	0.95
Total	184					
Average	10.2		0.37	0.75	0.72	0.61

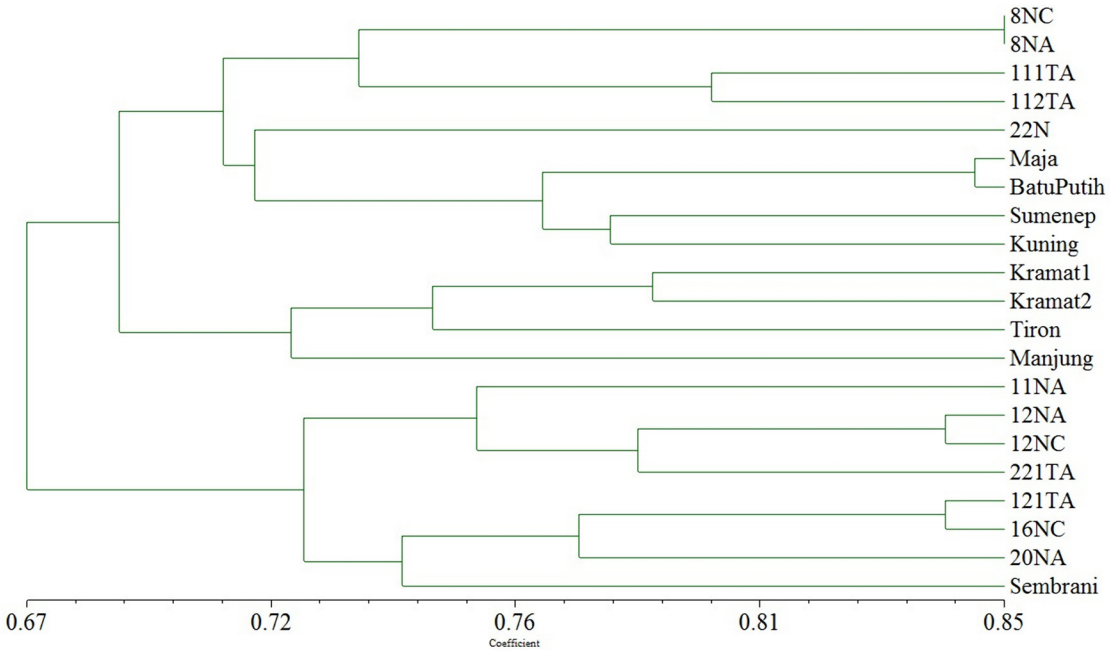


Fig. 2. Phylogenetic tree of twenty-one shallot genotypes adapted in peatland based on jaccard coefficient analyzed using NTSYS-pc 2.1

Table 7. Genetic similarity matrix of twenty-one shallot genotypes based on 18 SSR markers analysis

Sampel	8NC	8NA	11NA	1111TA	112TA	12NA	121TA	16NC	20NA	22N	221TA	Sembrani	Kramat1	Kramat2	Tiron	Maja	Manjung	Sumenep	Batu	Putih	Kuning
8NC	1.00																				
8NA	0.85	1.00																			
11NA	0.72	0.66	1.00																		
1111TA	0.75	0.70	0.77	1.00																	
112TA	0.72	0.76	0.69	0.80	1.00																
12NA	0.69	0.71	0.78	0.75	0.74	1.00															
12NC	0.70	0.66	0.78	0.71	0.71	0.84	1.00														
121TA	0.71	0.68	0.74	0.69	0.70	0.73	0.73	1.00													
16NC	0.66	0.66	0.67	0.66	0.70	0.76	0.73	0.84	1.00												
20NA	0.70	0.69	0.73	0.69	0.64	0.71	0.72	0.77	0.78	1.00											
22N	0.74	0.71	0.66	0.68	0.71	0.65	0.66	0.70	0.72	0.70	1.00										
221TA	0.68	0.68	0.71	0.71	0.70	0.78	0.79	0.74	0.79	0.76	0.70	1.00									
Sembrani	0.62	0.64	0.70	0.63	0.65	0.66	0.68	0.72	0.75	0.76	0.69	0.73	1.00								
Kramat1	0.69	0.68	0.63	0.63	0.70	0.66	0.66	0.69	0.73	0.67	0.71	0.70	0.67	1.00							
Kramat2	0.69	0.71	0.59	0.65	0.74	0.65	0.65	0.67	0.68	0.66	0.72	0.68	0.62	0.79	1.00						
Tiron	0.67	0.67	0.63	0.66	0.67	0.63	0.65	0.69	0.67	0.72	0.67	0.71	0.68	0.75	0.75	1.00					
Maja	0.69	0.73	0.62	0.70	0.71	0.69	0.66	0.69	0.68	0.67	0.70	0.65	0.69	0.71	0.72	0.69	1.00				
Manjung	0.69	0.76	0.63	0.69	0.73	0.69	0.65	0.66	0.66	0.70	0.70	0.72	0.64	0.71	0.75	0.71	0.72	1.00			
Sumenep	0.68	0.73	0.63	0.66	0.72	0.67	0.65	0.67	0.68	0.67	0.73	0.67	0.68	0.65	0.69	0.65	0.78	0.66	1.00		
Batu	0.70	0.75	0.62	0.67	0.73	0.65	0.60	0.63	0.64	0.67	0.70	0.64	0.62	0.65	0.70	0.65	0.84	0.77	0.75	1.00	
Putih																					
Kuning	0.72	0.76	0.67	0.69	0.71	0.69	0.69	0.67	0.68	0.66	0.73	0.69	0.65	0.67	0.71	0.66	0.77	0.73	0.78	0.77	1.00

Various responses of the shallot genotypes against *A. porri* as one of the most devastative disease in shallot cultivation were identified from susceptible to resistance. Based on phylogenetic tree, some cultivars were clustered according to their resistance and others were not. For instance, in sub-cluster IA, a total of 4 shallot genotypes were moderately resistance to *A. porri* including 8NC, 8NA, 11 1TA, and 11 2TA genotypes. While in sub-cluster IB, a total of 4 genotypes such as Kramat1, Kramat2, Tiron, and Manjung were all susceptible to *A. porri*. However, in sub cluster IA (ii), two susceptible shallot genotypes (22N and Kuning) were grouped together with two moderately resistance genotypes (Maja and Batu Putih) and one resistance cultivar (Sumenep). Similarly, clustering of the shallot genotypes in sub-clustered IIA and IIB were not related to *A. porri* resistances. It implied that the clustering of shallot genotypes adapted in peatland which genotyped using SSR markers was mostly related to their genetic relationships rather than their correlation with character of resistance to *A. porri*. Of the total 21 shallot genotypes evaluated in this study, several genotypes in a cluster showed a closely relationship to each other such as between 8NC and 8NA in sub-cluster IA (i), 11 1TA and 11 2TA in sub-cluster IA (ii), Kramat 1 and Kramat 2 in sub-cluster IB (i). In addition, 12NA and 12NC accessions were the closest one that was grouped in sub-cluster IIA (ii).

The closest genetic relatedness in sub-cluster IA was observed between two shallot genotypes, 8NC and 8NA, with genetic similarity coefficient of 0.85 (Table 7) which implies that the genetic distance was only 15 %. The close relatedness between 8NC and 8NA genotypes might be due to the same genome sources in developing these genotypes which derived from Sembrani cultivar as female parent (Table 1). On the other hand, molecular analysis of the 21 shallot genotypes using SSR markers showed that the highest genetic distance (0.41) was observed between 11NA and Kramat2 implying their potential parental lines for breeding programs. Crosses involving parents belonging to more divergent clusters would be expected to manifest maximum heterosis and wide variability in genetic architecture in rice cultivars (Singh, Singh, Maurya, & Verma, 1987). Furthermore, Singh et al. (2013) reported that selection of genetically diverse parents and that too without testing all possible hybrid combinations is of immense importance for successful recombination breeding program. Vajire, Thakare, Solunke, Panche, & Tiwari (2017) predicted the future of hybrid performance of elite lines of onion based on a positive correlation between genetic distance and heterosis using RAPD markers. They suggested that highest genetic distance observed between two onion lines (356-2 and 378M) would express higher performance if crossed. From 12 shallot clones adapted in the peatland growing area, six were grouped into the same sub-cluster with one or two of their parental lines (clones 8N, 8 NA, 11 1TA, 11 2TA, 22 N, and 11 NA,), while the remaining six clones seemed to be in different sub-cluster from their both parents (Fig. 2). Despite number of SSR markers used in present study is limited (18 polymorphic primers), the phylogenetic tree demonstrated that those markers could be able to trace the genetic relationship of certain clone to their parent according to the pedigree with different genetic background.

Overall, based on agronomical and molecular characterization, 12 shallot clones adaptive to peatland could be valuable for further improved varieties. Furthermore, these shallot clones will be beneficial for future evaluation in the wide areas of peat-lands in Indonesia. In order to identify the importance traits conferred by these peatland adapted shallot clones, gene (s) specific related markers are needed to apply.

CONCLUSION

Based on phenotypic evaluation, yield potential of shallot genotypes tested in peatland area ranged from 6.66 to 14.21 t/ha. Seven out of 12 shallot clones including 11NA, 11I1TA TA, 12NA, 12NC, 20NA and 22N had good yield potential and comparable with those of five released shallot varieties. Nine out of 12 shallot clones were moderately resistant to *Alternaria porii*. Based on molecular analysis, eighteen SSR primers used in present study could detect 184 alleles. Shallot genotypes used in the study were clustered into two main groups, namely Cluster I (13 shallot genotypes) and Clustered II (8 genotypes). The closest genetic relatedness was observed between 8NC and 8NA with genetic similarity coefficient of 0.85. While, the highest genetic distance was observed between 11NA and Kramat2 (0.41).

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