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Research article

Genetic and phenotypic variabilities of 24 *Impatiens platypetala* accessions from South Sulawesi, Indonesia

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Abstract

Impatiens platypetala (IP) is an important ornamental plant. The genetic parameters were estimated and the genotype clustering determined of 24 IP accessions collected in 2008 from South Sulawesi, Indonesia. A randomized complete block design was used with the 24 IP accessions as treatments. The agronomic characters measured were plant height, canopy width, leaf length, leaf width, petiole length, and flower diameter. The morphological characters recorded were the colors of the leaves and flowers and the presence of a flower eye zone. Genotypic and phenotypic variations were calculated using analysis of variance. The results were expressed in SD values, genotypic and phenotypic variance components, broad-sense heritability estimates, genetic advance, degree of similarity for pairs of species, cluster analysis of the morphological data and matrix coefficient analysis. The averages of the taxonomic distance and the matrix of genetic similarity were compared through the correlation test function of MXCOMP with the NTSYS v2.1 software. Plant height, canopy width, leaf length, flower diameter, flowering time and fruit ripening time had higher heritability estimates ($h^2_{bs} = 0.57 – 0.98$), more extensive genetic variability and higher genetic advance, thus they can be used as criteria for future breeding programs. Clustering of the genotypes, based on combinations of the agronomic and morphological data suggested differences in the genotypes. The combination data revealed that the 2008_17 genotype is possible a new *Impatiens* species.

Introduction

The *Impatiens* genus (impatiens) accounts for a large portion of the world's ornamental plant trade. This genus is among the top-four world's selling ornamental herbaceous varieties, with one of the major producers and breeder companies is Sakata Seed Corporation (SSC) (Kate and Laird, 2019). Globally, about 850 species of *Impatiens* have been recognized (Grey-Wilson, 1980; Utami, 2006). In Java, Indonesia, approximately 50 species with 10 types of impatiens have been identified (Baker and Bakhuizen, 1963; Utami, 2006), while the remaining species are in Sumatra, Papua, and Sulawesi. This situation has prompted the exploration, collection and characterization of *Impatiens* genetic resources in Indonesia.

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One of the unique *Impatiens* species originating in Indonesia is *Impatiens platypetala* (Ip). The Indonesian Ornamental Crops Research Institute (IOCRI) explored and collected the genetic resources of Ip in Sumatra, Java, Sulawesi, Bali, Flores and the Moluccas from 2008 to 2016, and the materials obtained have been assembled in an experimental garden at the IOCRI. These plants, which are commonly found throughout Indonesia, including Java, are known as the mother plants for the breeding program of a commercial seed company. The varieties of Ip found in South Sulawesi have been variously identified as *Impatiens platypetala* var. *aurantiaca*, *I. platypetala* Lindl. subsp. *aurantiaca* (Teijsm. ex Koord., 1898) and *I. aurantiaca* (Steenis). This *aurantiaca* IP variety produces the anthocyanin aurantinidin (Morgan, 2007). It can reach a height of 1 m bearing flowers with the colors of orange, white and pink-purple with a white 'eye zone'. The leaf is 5–12 cm long, ovate to lanceolate-ovate (Morgan, 2007).

This variety show variations in botanical, agronomic and habitat characteristics. Information on the botanical and agronomical characterization of *Impatiens* genetic resources are useful for genotypic cluster analysis, and these data can be used for analyzing genetic parameters. The genetic parameter components (selection methods, started time of selection, and genetic variability) are also important for ensuring effective and efficient breeding programs, where determining genetic variability among genotypes is essential for selection (Lee, 1995; Poehlman and Sleeper, 1995). Genetic variability information also provides guidance for developing an effective breeding scheme (Mumm et al., 1994), and knowledge of the heritability characters is also important for targeted selection (Pinaria et al., 1995).

Estimation of the genetic parameters, including the genetic variability, genotypic, phenotypic and environmental variance, heritability, genetic advance, phenotypic and genotypic correlation, provides basic information for the crop improvement achieved through the selection of a character for breeding activities (Hill, 2010; Akhtar et al. 2011). The estimation of genetic parameters in relation to the characterization of a plant's properties is also a major component in improving those properties for breeding (Wardiana and Pranowo, 2011). Therefore, the aim of the present study was to estimate the genetic parameters (the coefficient of genetic variability, heritability and genetic advance) and to determine the genotype clustering for 24 IP accessions from South Sulawesi.

Materials and Methods

Study area

The study was conducted at the IOCRI, at 1,100 m above sea level, from January to December 2015. The experiment used a randomized complete block design with the 24 Ip accessions as genotype treatments. The treatment was replicated three times, with each replication having 10 plants. The Ip accessions were collected during exploration activity in 2008 in South Sulawesi, Indonesia (Table 1).

Seeds produced in 2009 were germinated on wet tissue paper placed on plastic petri dishes. The seeds germinated after 1–2 weeks; after 4 weeks, they were ready for transfer to trays containing a rice husk medium. The seedlings were transferred to polybags (5 cm) after 4 weeks in the trays. For the field experiment, the soil was fumigated and then mixed with organic fertilizer. After 4–6 weeks in the polybags, the plants were transferred to the soil. Data were collected after the plants had been in the field for 16 weeks.

The vegetative and generative characters were recorded using quantitative and qualitative variables. The quantitative variables were plant height, canopy width, leaf length, leaf width, petiole length, flower diameter, flowering time and fruit ripening time. The qualitative characters were the form of the upper leaves, presence of anthocyanin in the leaves, color of the leaf underside, color of the underside leaf veins, types of flower, number of flower colors, distribution of secondary color, presence of a flower eye zone, size of the eye zone, main color of the flower and secondary color of the flower.

Data analysis

The data were assessed using analysis of variance in the SAS software version 9.1 (SAS® 9.1, SAS Institute Inc., NC, USA) as shown in Table 2.

Table 1 *Impatiens platypetala* accessions collected during South Sulawesi exploration activity in 2008.

No.	Accession number	Collection location	No.	Accession number	Collection location
1	2008_003	Takapala, Gowa	13	2008_8A	Mengkendek, Toraja
2	2008_007	Pattapang, Gowa	14	2008_28B	Mengkendek, Toraja
3	2008_008	Pattapang, Gowa	15	2008_29A	Mengkendek, Toraja
4	2008_009	Takapala, Gowa	16	2008_29B	Mengkendek, Toraja
5	2008_10	Pattapang, Gowa	17	2008_31	Mengkendek, Toraja
6	2008_11	Pattapang, Gowa	18	2008_32	Mengkendek, Toraja
7	2008_12	Pattapang, Gowa	19	2008_37	Bori, North Toraja
8	2008_15	Tombolo pao, Gowa	20	2008_39	Pangli, North Toraja
9	2008_17	Tombolo pao, Gowa	21	2008_41	Tanimbayong, North Toraja
10	2008_18	Tombolo pao, Gowa	22	2008_42	Tanimbayong, North Toraja
11	2008_19	Tombolo pao, Gowa	23	2008_47	Buntao, North Toraja
12	2008_24	Gunung Nona, Enrekang	24	2008_50	Madandan, North Toraja

Table 2 Analysis of variance

Sources of variation	Df	SS	MS	Expected MS
Replication	r−1	SS replication	MSr	
Treatment	t−1	SS genotypes	MSt	$\sigma^2_e + r\,\sigma^2_g$
Error	t (r−1)	SS error	MSe	σ^2_e

Df = the degrees of freedom; SS = the sums of squares; MS = the mean squares; r = the number of replications; t = the number of treatments

The results were then used to calculate the genotypic and phenotypic variance component (σ^2_g and σ^2_p) based on Equations 1 and 2 (Allard, 1960):

Estimate of phenotypic variance $(\sigma^2_p) = \sigma^2_g + \sigma^2_e$ (1)

Estimate of genotypic variance $(\sigma^2_g) = (MSt - MS_e)/r$ (2)

where, r is the number of replications, MSt is the mean square of treatments, and MSe is the mean square of error.

Estimation of variance components:

Determination of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation GCV) was made based on Equation 3 and 4, as described by Singh and Chaudhury (1987):

$PCV = (\sqrt{\sigma^2_p}/\mu) \times 100\%$ (3)

$GCV = (\sqrt{\sigma^2_g}/\mu) \times 100\%$ (4)

where σ^2_p = phenotypic variance; σ^2_g = genotypic variance and μ = sample mean.

Estimation of heritability in a broad sense

The estimation of heritability in the broad sense (h^2) can be determined using Equation 5 (Falconer and Mackay, 1996).

Broad sense heritability $(h^2_{bs}) = (\sigma^2_g) / (\sigma^2_g + \sigma^2_e/r)$ (5)

The criteria for classification of heritability values were based on McWhirter (1979). Low heritability was indicated by a value of $h^2 < 0.2$, medium heritability by $0.2 \leq h^2 \leq 0.5$, and high heritability by $h^2 > 0.5$.

Estimation of genetic advance

The genetic advance (GA) was estimated by the formula on Equation 6 and 7.

$GA = h^2\,\sigma^2 p.k$ with $k = 2.06$ (6)

The value of genetic advance of mean $(GAM) = (GA/\mu) \times 100\%$ (7)

where μ is the average mean value of the character (Comstock and Robinson, 1952). The genetic advance of mean is considered high if it is greater than 20%, moderate if it was between 10–20%, and low if it is less than 10% (Johnson et al., 1955).

Cluster analysis

The average taxonomic distance matrix was assessed using Equation 8:

$E_{ij} = [\Sigma_k (n^{-1}) (X_{ki} - X_{kj})^2]^{1/2}$ (8)

where, E_{ij} is the average taxonomic distance, i and j are two compared genotypes, k is the phenotype, X is the observed values, and n is the number of genotypes. The matrix of average taxonomic distance for genotype and morphological traits was computed with the similarity interval (SIMMINT) function and the Dist coefficient. This dissimilarity coefficient is based on interval measured data collected for the morphological traits (Tatineni et al., 1996). Cluster analyses were performed using the unweighted pair group method with arithmetic mean (UPGMA) in the NTSYS program (Rohlf, 1993).

The morphological data were clustered using the unweighted pair group method with arithmetic mean (UPGMA). The similarity for qualitative (SIMQUAL) data function in the NTSYS program (Rohlf, 1993) was also applied. The data matrix coefficients were calculated using the Dice similarity coefficient. The degree of similarity among the pairs of species found on different lines was based on Equation 9 (Nei and Li, 1979):

$S = 2n_{ab} / (n_a + n_b)$ (9)

Where S is the similarity coefficient, n_{ab} is the number of morphological traits shared between sample/genotype a and b, $(n_a + n_b)$ are the total number of morphological traits for genotypes of a and b, respectively.

The average distance matrix taxonomic and genetic similarity matrices were compared using the correlation test function MXCOMP with the NTSYS program to see the agreement between the matrix of the morphological data and the matrix of the combined agronomic and physiological data (Rohlf, 1998).

Results

Analysis of variance

All the observed characters showed significant difference among accessions at the 0.01 and 0.05 probability levels. Significant effects at $p < 0.01$ were found for plant height, canopy width, leaf length, flower diameter, flowering time and fruit ripening time. Petiole length and leaf width were significantly different at $p < 0.05$ (Table 3). This result shows that there is a large variability among the genotypes observed and thus can be used for further breeding programs.

Estimation of variance components, heritability and genetic advance

The estimation of phenotypic variances ($\sigma^2 p$), genotypic variances ($\sigma^2 g$), phenotypic coefficients of variation (PCV) and genotypic coefficients of variation (GCV) are given in Table 4. The PCV was higher than the GCV for all characters (Table 4) indicated that there were environmental effects on genetic expression. The PCV ranged from 5.79% in flowering time to 56.09% in plant height, and GCV ranged from 4.37% in flowering time and 55.47% in plant height. Plant height, canopy width, petiole length and flower diameter showed broad genotypic and phenotypic coefficient of variation among all the genotypes of *Impatiens platypetala.* This indicated that these characters were more variable among the genotypes studied as compared to the other characters. Flowering time and fruits ripening time had narrow genotypic and phenotypic coefficient of variation. Genetic variability is a basic prerequisite for plant breeding program, so that the selection process is directed at developing superior genotypes. Thus, the higher the genetic variation for the characters in the selected materials, the greater the genetic improvement and selection would be effective.

Plant height, canopy width, leaf length, flower diameter, flowering time and fruit ripening time had high heritability estimates (Table 5). Heritabilities ranged from the lowest of 0.48 for leaf width to the highest of 0.98 for plant height.

The broad-sense heritability estimates for plant height, canopy width, leaf length, petiole length and flower diameter indicated that these characters were influenced more by genetics than the environment, and thus the selection would be effective even based on phenotypic appearance.

The genetic advance of mean for all characters were high except for petiole length and fruit ripening time. The association of broad-sense heritability with high genetic advance and GCV was reported in the case of plant height, canopy width, and flower diameter (Table 5), indicating the presence of additive gene action and consequently a high genetic gain from phenotypic selection.

Table 3 Mean square values and average mean values of each character from the 24 *Impatiens platypetala* accessions from South Sulawesi

Characters	Mean square of treatment		Mean value ± SD
Plant height (cm)	1,855.761	**	44.34 ± 25.43
Canopy width (cm)	1,721.576	**	56.31 ± 25.39
Leaf length (cm)	5.588	**	8.02 ± 1.62
Petiole length (cm)	0.500	*	1.38 ± 0.59
Leaf width (cm)	2.675	*	4.07 ± 1.34
Flower diameter (cm)	1.577	**	3.27 ± 0.78
Flowering time (days after planting)	87.541	**	93.36 ± 7.41
Fruit ripening time (days after planting)	5.308	**	19.66 ± 1.94

* and ** denote significant difference at $p < 0.05$ and $p < 0.01$, respectively.

Table 4 Estimation values of genotypic and phenotypic variance components of 24 *Impatiens platypetala* accessions from South Sulawesi

Characters	σ^2g	σ^2p	μ	GCV (%)	Criterion	PCV (%)	Criterion
Plant height (cm)	605.00	618.59	44.34	55.47	broad	56.09	broad
Canopy width (cm)	546.47	573.86	56.31	41.51	broad	42.54	broad
Leaf length (cm)	1.44	1.86	8.02	14.97	medium	17.01	medium
Petiole length (cm)	0.08	0.17	1.38	20.98	broad	29.52	broad
Leaf width (cm)	0.42	0.89	4.07	16.02	medium	23.19	broad
Flower diameter (cm)	0.48	0.53	3.27	21.26	broad	22.16	broad
Flowering time (days after planting)	16.68	29.18	93.36	4.37	narrow	5.79	narrow
Fruits ripening time (days after planting)	1.02	1.77	19.66	5.14	narrow	6.77	narrow

σ^2g = genotypic variance; σ^2p = phenotypic variance; μ = sample mean; GCV = genotypic coefficient of variation; PCV = phenotypic coefficient of variation

Table 5 Estimation of heritability of 24 *Impatiens platypetala* accessions from South Sulawesi

Characters	Estimated value of genetic parameter			Criterion h^2_{bs}	Genetic advance of means (%)	Criterion of genetic advance
	σ^2g	σ^2p	h^2_{bs}			
Plant height (cm)	605.00	618.59	0.98	high	2,810.80	high
Canopy width (cm)	546.47	573.86	0.95	high	1,999.17	high
Leaf length (cm)	1.44	1.86	0.77	high	37.02	high
Petiole length (cm)	0.08	0.17	0.50	medium	12.52	medium
Leaf width (cm)	0.42	0.89	0.48	medium	21.51	high
Flower diameter (cm)	0.48	0.53	0.92	high	30.45	high
Flowering time (days after planting)	16.68	29.18	0.57	high	36.81	high
Fruit ripening time (days after planting)	1.02	1.77	0.58	high	10.69	medium

σ^2_g = genotypic variance; σ^2_p = phenotypic variance; h^2_{bs} = broad-sense heritability

Genotype clustering

Cluster analysis was performed based on the quantitative characters of the IP accessions. The 24 IP genotypes were classified into six groups based on the average taxonomic distance of 1.32. This grouping revealed that the genetic variability of the 24 Ip genotypes was based on agronomic characters. The presentation of this grouping as a dendrogram resulted in the genotypes with similar agronomic characters being classified into the same cluster. This could be attributed to the possibility that the spread of Ip genetic material occurred randomly by either human or natural influences (Fig. 1 and 2).

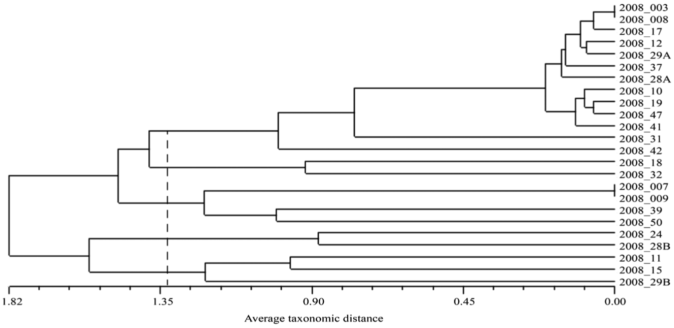

Fig. 1 Dendrogram showing cluster analysis of 24 *Impatiens platypetala* accessions collected in 2008 from South Sulawesi based on the mean taxonomic distance from eight agronomic characters clustered using unweighted pair group method with arithmetic mean.

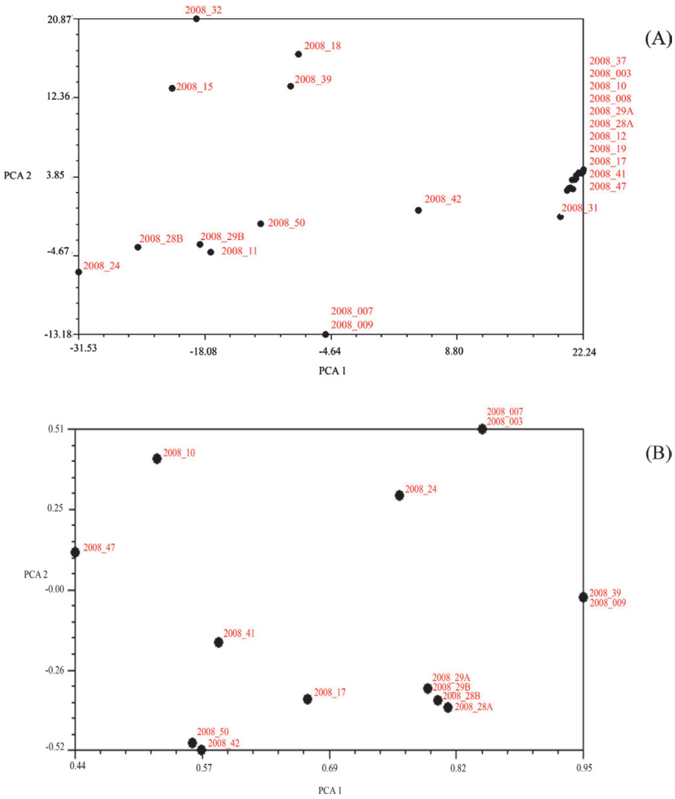

Fig. 2 Distribution of 24 *Impatiens platypetala* accessions collected in 2008 from South Sulawesi using two main components (PCA1 and PCA2) based on agronomic characters (A), and morphological characters (B)

Analysis of the main components, conducted to reduce all eight characters, revealed four main components that had root characteristics higher than 1 that explained 88.03% of the data variability. The first component (PC1) consisted of three characters (leaf length, flower diameter and flowering time) that were mutually correlated and could explain 34.34% of the data variability. The second component (PC2) consisted of petiole length and fruit ripening time, which were correlated and explained 21.95% of the data variability. The third and fourth components had one character each, i.e. canopy width and plant height, respectively (Table 6).

Table 6 Score of each main component

Observation variable	PC1	PC2	PC3	PC4
Plant height (cm)	1.30	1.63	6.47	**6.70**
Canopy width (cm)	4.09	3.72	**−7.62**	−1.18
Leaf length (cm)	**−5.84**	−4.78	−2.07	4.09
Petiole length (cm)	−5.70	**7.29**	1.41	−2.55
Leaf width (cm)	−5.45	−5.56	−4.30	0.67
Flower diameter (cm)	**−8.47**	3.98	6.27	−1.19
Flowering time (days after planting)	**9.33**	6.67	−4.95	0.14
Fruit ripening time (days after planting)	1.10	**−5.99**	4.74	−6.01
Proportion	34.34%	21.95%	18.48%	13.26%
Cumulative	34.34%	56.29%	74.77%	88.03%
Root characteristic	2.75	1.76	1.48	1.06

Note: PC = principal component

Genotype clustering based on morphological characters

Observations were made for 11 morphological characters, however, only six characters were further analyzed as the remaining five could not be analyzed because they were grouped into monomorphic characters. Cluster analysis based on morphological appearance resulted in the successful classification of the 24 genotypes into five groups at 0.595 similarity coefficient. Group I consisted of 10 genotypes with 2 sub-groups (A and B), which had similar characters. The linkage of these data with flower diameter resulted in the IA group containing six genotypes with small yellow flowers collected from the town Malino in Gowa district of South Sulawesi. The IB group consisted of three genotypes, one with orange flowers (accession 2008_32) and two with yellow flowers. One genotype (accession 2008_24) formed a separate subgroup in Group I showing similar characteristics to those of the other groups, but with a larger eye zone. Group II had similar characters to those of Group I, but with eye zone. Group III had nine genotypes which were divided into two sub-groups (C and D). Group IV had three genotypes, while Group V consisted of only one genotype (Figs. 2 and 3).

The grouping of genotypic similarity was based on the agronomic and morphological characters. The data were further examined using correlation analysis of the two matrices constructed based on the agronomic and morphological data. Both groups showed very low correlation (r = 0.15142, p = 0.9156). The results suggested that the agronomic and morphological characters may be controlled by different set of genes. Therefore, grouping analysis had to be developed on the basis of a combination of agronomic and morphological characters. This resulted in two groups, group I consisted of 12 genotypes and group II consisted of two genotypes (Fig. 4). However, 10 accessions did not belong to any group. The genetic distance coefficient used in this classification was 1.265.

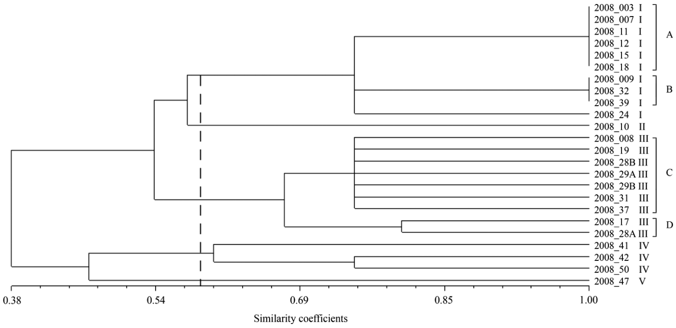

Fig. 3 A dendrogram of cluster analysis of 24 *Impatiens platypetala* accessions collected in 2008 from South Sulawesi using the Dice similarity coefficient from six morphological characters and clustered by unweighted pair group method with arithmetic mean

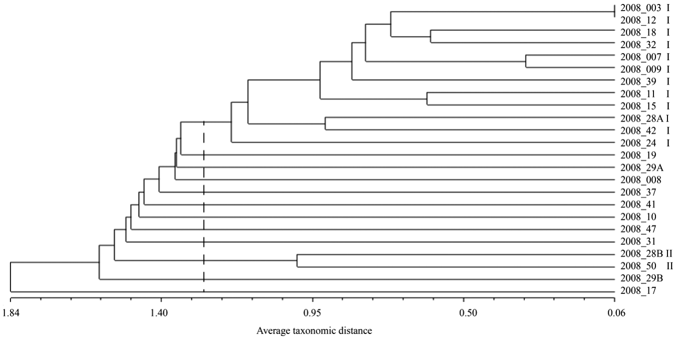

Fig. 4 Dendrogram of cluster analysis of 24 *Impatiens platypetala* accessions collected in 2008 from South Sulawesi was based on average taxonomic distance using agronomic and morphological characters and clustered by unweighted pair group method with arithmetic mean.

Correlation analyses of the agronomic data, morphological data and their combination were performed to determine whether the agronomic or morphological data could possibly be combined for analysis. The correlation was low between the matrices of the agronomic data and the combined agronomic and morphological data ($r = 0.16436$, $p = 0.8865$). In contrast, the correlation was high between the matrices of the morphological data and the combined agronomic and morphological data ($r = −0.66643$, $p = 0.0000$).

Discussion

The study of genetic variation is important for determining the possible variation among genotypes. The analysis of variance of the 24 Ip indicated significant genotypic variation in all characters. Other researchers reported similar results for row crops such as *Plectranthus eduli* (Garedew et al., 2009), *Solanum anguivi* (Denton and Nwangburuka, 2011), beans (Malek et al., 2014) and eggplant (Solaimana et al., 2015).

The parameters of genetic variability for the 24 Ip is important for the next selection process. Allard (1960) stated that wide genetic variability is the prerequisite for the effectiveness of a selection process because it will provide more flexibility in genotype selection. The results of the present study indicated that wide variability in the genotype and phenotype of plant characters provides narrow genetic variability but wide phenotypic variability. This result was consistent with the findings on genetic variability in chili (*Capsicum annuum* f.) for yield and quality characters (Jogi et al., 2015). The difference in the appearance of a phenotypic character is influenced by genetic and environmental factors (Falconer and Mackay, 1996). The main source of variability was separated by calculating the values for genetic variability, environment, heritability and genetic progress because these parameters are important in relation to selection of the characters. The combination of the predictive value of genetic variability and the heritability estimate can provide a broad picture of the characters that can be inherited (Burton, 1952). In addition, both values are good predictors of the size of the response expected from a selection (Akhtar et al., 2011). In the present study, the environment showed pronounce effect on petiole length, leaf width, flowering time and fruit ripening time. The significant genetic variability indicated the difference in individual genotypic values in the population (Murdaningsih et al., 1990), thereby showing the potential and opportunity for the success of a selection.

The heritability estimates of characters were used to predict whether the characters were influenced mainly by environmental or genetic factors. High broad-sense heritability values indicate that the genetic factors have a greater influence than the environmental factors on phenotypic appearance. High heritability values also play a role in improving the effectiveness of a selection (Syukur et al., 2009). The results of the present study demonstrated that plant height, canopy width, leaf length, petiole length and flower diameter had high heritabilities, however, heritability alone is not sufficient to produce high genetic progress (Ibrahim and Hussein 2006). If the predictive value of a genetic parameter is based on the value of heritability without the support of the predicted genetic progress, substantial progress in the selection will not be assured (Shukla et al., 2005). Plant height, canopy width, and flower diameter had broad variability, high heritability and high genetic advance. Broad variability with high heritability and genetic advance implied the presence of the additive effects of genes (Ibrahim and Hussein 2006), thus selection of the character will be effective even if it is done in early generations (Yousaf et al., 2008). Conversely, if the broad-sense heritability is high but the genetic progress is low due to the presence of non-additive genetic effect (Subramayan et al., 1995), the opportunity for genetic improvement is relatively limited for the character of that crop (Sharma and Garg, 2002).

Cluster analysis based on the appearance of the agronomic and morphological characters of the 24 Ip accessions resulted in classification of the genotypes with similar agronomic characters into the same cluster, with each group not being identical to the original genotype. This group separation was possible because the spread of Ip genetic material occurred randomly by either human or natural selection. These results were consistent with Gohil and Pandya (2008), Popluechai et al. (2009) and Wardiana and Pranowo (2011) who observed a lack of correspondence between genotype grouping and geographic origin.

The magnitude of the suitability of genotype groupings on the basis of agronomic and morphological characters was assessed by further correlation analysis between the two matrices and their combination. The agronomic and morphological data had a low correlation, indicating that agronomic and morphological characters each revealed different genomic regions. Thus, grouping cannot be viewed as a single group of characters, i.e. either agronomic or morphological, but as a combination of both groups. To determine whether the agronomic or morphological data were closer to the data combination, we assessed the correlation between their matrix data. The correlation between the matrix of the morphological data and the matrix of the combined agronomic and physiological data showed a higher correlation. These results indicated that the morphological data were more dominant in the cluster analysis. In addition, the combined data revealed that the 2008_17 genotypes are different from the other genotypes, with the possibility of their being a new species. An analysis of either agronomic or morphological data did not reveal this difference, however.

It should be noted that this experiment was conducted in only a single year and single environment. More trials are required for a more conclusive result.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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