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FRUIT TRAIT-BASED EVALUATION OF DIALLEL CROSSING COMPATIBILITY OF SIX ELITE CLONES OF COCOA

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SUMMARY

Cocoa cultivar development through diallel crosses is one of the national efforts in increasing cocoa productivity in Indonesia. However, these crosses require compatibility evaluation to increase the effectiveness of the crossing. Therefore, the aim of this study was to determine the best compatibility level of common cocoa clones and to identify the compatibility selection characters based on the characteristics of the fruit produced from the crossings. This study was designed using a full diallel cross design of six Indonesian superior cocoa clones, namely MCC-01, Sulawesi-1, THR, MCC-02, AFQ/MHP-01, and BB-01. Apart from diallel crosses, each clone was also subjected to self-pollination and natural crosses, so that there were 42 types of crosses. Each type of cross was carried out by 50 crosses per plant which was repeated for three plants, resulting in 150 crossing experiments for each type of cross. Observations were made on the 12 characters of the fruit resulted from crosses. The results show that the BB-01 was the clone with the best compatibility, followed by the MCC-01 and Sulawesi-1 clones. Meanwhile, the best selection character in measuring the cross-compatibility characteristic is the seed wet weight per fruit.

Keywords: Cauliflory, diallel crossing, inter-compatibility, self-incompatibility, *Theobroma cacao* L.

Key findings: The study showed that clone BB-01 had the best compatibility, followed MCC-01 and Sulawesi-1 clones. Meanwhile, the best selection character in measuring the cross-compatibility characteristics is the seed wet weight of the fruit. This character can serve as the compatibility success indicator in cacao crossing.

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INTRODUCTION

Cocoa (*Theobroma cacao* L.) is one of the plantation crops that play a role in increasing Indonesia's foreign exchange. The product of this plant, in the form of cocoa beans, is widely used as raw material for chocolate and cosmetics industry, hence there is an increasing demand for cocoa beans following the development of the lifestyle (Randriani and Dani, 2017; Lanaud *et al*., 2017). According to statistical data, cocoa plantations in Indonesia are dominated by smallholder plantations with a percentage of 97.29% and the rest are state and private companies. Production of cocoa beans in 2018 reached 561,400 tons and 41% of which was exported abroad in the form of cocoa butter (Statistics Indonesia, 2019). However, the export development is considered to be fluctuating. This is due to a decrease in the area of land for cocoa and availability of superior cocoa clones with optimum yield (Randriani and Dani, 2017). These problems can threaten the sustainability of cocoa plantations in Indonesia. Therefore, the Indonesian government launched a national cocoa movement to improve cocoa productivity and quality (Rubiyo, 2013). One of the steps to improve cocoa productivity is the assembly of new clones through a plant breeding program.

The effectiveness of plant breeding programs is dependent on the characteristics of the plants to be improved and the objectives of the program. In general, cocoa is a tropical woody plant originating from the Latin American continent (Lopes *et al*., 2011). This plant has flowers that are cauliflory, where the flowers appear on the bark of the plant (Groeneveld *et al*., 2010). This makes pruning management crucial in maintaining cocoa plant productivity. Cocoa plants are able to produce a large numbers of flowers, around 125,000 per year (Lopes *et al*., 2011). Each of these flowers has 14,000 pollen grains and 74 ovules (N'Zi *et al*., 2017). However, the anther and the stigma of the cocoa flower are located in separate parts of the flower hence pollination requires a pollinator, namely *Forcipomyia* (Zakariya *et al*., 2019). Pollination carried out by *Forcipomyia* cannot be carried out completely on all flowers causing about 50% to 75% of the cocoa flowers are not pollinated by pollen and experience abortion within 2-3 days (Reffye *et al*., 1978; Bos *et al*., 2007). This means that the cocoa pods are produced from only about 2% of the total flowers formed per year (Alvim, 1984; Aneja *et al*., 1999; Lopes *et al*., 2011). Therefore, breeding of this plant poses a major challenge.

The development of cocoa cultivars is more focused on increasing abiotic stress tolerance, biotic stress resistance, and increasing plant productivity (Randriani and Dani, 2017). Crosses between clones with these specific traits can result in good offspring improvement. In general, the cocoa clones that are widely planted in the community, especially in North Luwu Regency as one of the centers of cocoa cultivation in Indonesia, are cultivars MCC-01, Sulawesi-1, THR, MCC-02, AFQ/MHP-01 and BB-01. These six cultivars have specific characteristics, such as resistance to Cocoa Pod Borer (CPB) (AFQ/MHP-01), somewhat resistant to fruit rot (BB-01), has a lot of seeds (Sulawesi-1), large seed shape (MCC-01 and MCC-02), and has many flowers (THR). The advantages of each cultivar are valuable assets in the crossings. According to Syukur *et al*. (2015), crossing between parents with specific traits can produce traits showing hybrid vigor. Therefore, crossing between the six cultivars is thought to produce offspring with better combination characteristics than their parents.

The merging of the characteristics between the six clones can be done by means of diallel crossing. The advantage of diallel crossing is that it will allow to select best crossing combinations from all possible crosses between the parental clones (Mumtaz *et al*., 2015). This has resulted in using the diallel crossing both in self-pollinated plants (Souza *et al*., 2012; Ljubičić *et al*., 2017; Hijam *et al*., 2019) and cross-pollinated plants (Nyadanu *et al*., 2012; Nduwumuremyi *et al*., 2013; dos Santos *et al*., 2016). However, in the process of the diallel crossings, evaluation of its compatibility is very important. Compatibility evaluation is a stage of conformity assessment in the formation of fruit and seeds, both for inter-compatibility and self-pollination (Acquaah, 2007; Branco *et al*., 2018). The results of this assessment can provide an overview and basis for cocoa cultivar development.

Compatibility research is focused on the success of crossing (Susilo, 2006; N'Zi *et al*., 2017). Evaluation on the fruit traits resulted from successful crosses is rarely carried out. This is because the fruit traits are influenced by the maternal effects. Nevertheless, the compatibility between female and male parents is considered to be able to influence the hormonal conditions on fruit formation (Zhang *et al*., 2007; Singh *et al*., 2017). Therefore, the evaluation of the compatibility between the six clones used in this study through the diallel crossing design based on fruit characteristics of the crosses is interesting and important. The objective of this study was to determine the cocoa clones with the best compatibility level and to identify characters having cross-compatibility based on the fruit traits to be used in the cacao breeding programs.

MATERIALS AND METHODS

This research was conducted in Tarengge Village, Wotu Subdistrict, East Luwu Regency, South Sulawesi Province from November 2016 to June 2017. The research was designed using a full dialellic crossings design of six superior cocoa clones, namely MCC-01 (v1), Sulawesi-1 (v2), THR (v3), MCC-02 (v4), AFQ/MHP-01 (v5), and BB-01 (v6), resulted in 30 cross combinations. Apart from diallel crossing, each clone was also subjected to self-pollination and open pollination (OP), resulted in 42 types of crosses. Each type of crossing was carried out as many as 50 crossings per cross which was repeated three times per cross, hence there were 150 crossing experiments for each type of cross. As for the numbers of open-pollination observations 150 flowers were used. Meanwhile, the crosses naturally sampled using a completely randomized design (CRD) in evaluating the characteristics of the fruit produced from the crossing between clones.

Crossing procedure

Crossings were carried out in several stages according to Syukur *et al*. (2015) which consists of selecting male and female flowers, castration, emasculation, pollen collection, pollination, and isolation. Flowers that are used as females were flowers that have not yet bloomed. The flowers were covered with pipes to keep insects from pollinating the female flowers. Female flowers were selected on the productive stems, namely primary and secondary branches about 2 m above the ground. The male flower is a flower that has bloomed perfectly and has a fresh color. Castration was carried out by cleaning the dirt around the flowers using a brush. Castration was conducted when the flowers began to appear but have not yet broken. After the female flowers bloomed, emasculation or removal of the staminodes was conducted using tweezers. The anther of the female flower must be in an unbroken condition which is indicated by the white anther. After emasculation, pollination was done by smearing the pollen that were previously collected onto the stigma. The pollination process was carried out in the morning around 7 to 8 o'clock using a small brush. Subsequently, labeling and isolation were carried out as the final part of the cross. Especially for natural crosses, flowers were selected randomly on each plant. Meanwhile, plant maintenance was carried out by farmers using the standard cocoa cultivation practices.

Fruit characters

Observations of compatibility characters were carried out on several fruit characters, namely the percentage of successful crosses, fruit length, fruit diameter, number of seeds, furrow pod shell thickness, ridge pod shell thickness, pod shell thickness, furrow exocarp thickness, ridge exocarp thickness, seed length, seed width, seed thickness, and seed wet weight per fruit.

Data analysis

The data on cross compatibility were analyzed by MS Excel (Version 2013) program to determine the compatibility success percentage. The compatibility success percentage was calculated using the following formula:

$$Percentage\ of\ Successful\ Crossings\ (PS)\% = \frac{The\ number\ of\ success\ crossing}{Total\ of\ crossing\ treatment} \times 100\%$$

Data on natural crosses were analyzed to evaluate the diversity of parent clones based on all fruit characteristics. This analysis used the ANOVA test using STAR 2.01 software. The Student t-test was carried out by comparing the combination of crosses against their natural crosses. This analysis used the Minitab v 17 software as the analysis tool (Anshori *et al*., 2018). Each fruit character was correlated with Pearson correlation analysis in assessing the compatibility selection character. Meanwhile, identification of the diversity of female parents in the compatibility evaluation was carried out by biplot of principal component analysis (Fadhli *et al*., 2020). Pearson correlation analysis and principal component analysis were carried out with R Studio 3.6.1 software through the Agricolae package (Mendiburu, 2020) and Corrplot (Wei *et al*., 2017) for correlation analysis as well as Factoextra (Kassambra and Mundt, 2020) and GG-biplot (Vu, 2020) for biplot of principal component analysis.

RESULTS AND DISCUSSION

Compatibility of crosses

The results of crosses between six cocoa clones show that the MCC-01, Sulawesi-1, and BB-01 clones had good crossing compatibility compared to THR, MCC-2, and AFQ/MHP-01 (Table 1). Meanwhile, self-pollination showed fertilization failures in all clones. This is also consistent with the report of Susilo (2006), but it is different from the research of N'Zi *et al*. (2017) which shows a large percentage of success in self-pollinated crosses. In general, the cocoa plant is a cross pollinating crop. This is supported by the presence of stigma and anther located far apart in one flower hence pollination requires pollinators (Zakariya *et al*., 2019). The failure to form cocoa pods through self-pollination is known as self-incompatibility. Self-incompatibility is a condition of unsuccessful fertilization caused by the similarity of genes in pollen and ovules (Susilo, 2006; Syukur *et al*., 2015; Lanaud *et al*., 2017). This causes the pollen tube not to germinate or the germinated pollen tubes to not reaching the ovule, so that double fertilization does not occur and causes failure of fruit development (Acquaah, 2007). According to Groeneveld *et al*. (2010) and Schawe *et al*. (2013), in general, cocoa plants have a high level of self-incompatibility, but in several studies there were wild and cultivated cocoa clones with a high level of self-compatibility. This shows that the six clones used behaved as general cocoa clones showing high degree of self-incompatibility.

Based on the percentage of successful crosses, the THR, MCC-02, and AFQ / MHP-01 clones had a very high level of cross incompatibility (Table 1). This is evident by the failure of fruit formation among these crosses as well as by self-pollination. This situation may be due to the close genetic relatedness among the three clones, hence resembling a self-pollination situation. In cross-pollinated

Table 1. Diallel crossing of six cocoa clones and percentage success of crossing.

Female	Male						Total	PS (%)	OP		C/OP
	v1	v2	v3	v4	v5	v6			Total	PS (%)	
MCC-01 (v1)	-	5	3	7	8	4	27	3	9	6.00	0.50
Sulawesi-1 (v2)	1	-	4	4	4	3	16	1.78	7	4.67	0.38
THR (v3)	6	2	-	-	-	11	19	2.11	7	4.67	0.45
MCC-2 (v4)	1	3	-	-	-	3	7	0.77	7	4.67	0.17
AFQ/MHP-01 (v5)	2	11	-	-	-	2	15	1.67	6	4.00	0.42
BB-01 (v6)	2	6	4	7	1	-	20	2.22	6	4.00	0.56
Total	12	27	11	18	13	23	92		42		
PS (%)	1.33	3.00	1.22	2.00	1.44	2.56	1.70		4.67		

Note: C= Crossing, OP = Open pollination, PS = percentage of successful crossings

plants, the closer the genetic distance between the parents of the crossing, the offspring will experience very low hybrid vigor and can even induce inbreeding depression or failure in crosses (Acquaah, 2007; Syukur *et al*., 2015). Therefore, crossing between these three clones is not recommended.

The results of this study also showed that the percentage of successful crossings were lower than open pollination. Some combinations showed higher success than natural pollination, such as in THR × BB-01, AFQ/MHP-01 × Sulawesi-1, BB-01 × MCC-02 (Table 1). Based on the compatibility of male and female parents, the Sulawesi-1 and BB-01 clones were the two best males in diallel crosses with success rates of 3.00% and 2.56%, respectively. Meanwhile, the clones MCC-01 and BB-01 were the two best female parents with a success ratio of open pollination to crossings of 0.5 and 0.56, respectively. The lower level of successful crosses in the recent study is different from the research of Groeneveld *et al*. (2010) who reported higher success in hand pollinated crosses compared to their OP crosses.

This may be due to two factors, namely the compatibility of these crosses and the optimum capacity the plants have in forming fruits. In general, the failure to form fruit from the cross is caused by an imbalance between the energy capacity of fruit formation and the flowers that are induced to become fruit. This results in fruit abortion at the cherelle wilt stage of the crossed flower, especially if there is no good compatibility between male and female parents (Forbes *et al*., 2019). In addition, this is also supported by the low general ratio of the ratio of pod formation to total flower of the cocoa plant (Lopes *et al*., 2011), so that incompatible cherelle wilt will experience abortion. Although in other studies, increasing the chances of success can be done by adjusting the intensity of shade and fertilizer (Groeneveld *et al*., 2010). However, in general, if there was no synchronization regarding the intensity of the crosses, the capacity to form fruit, the compatibility of the parents and the success of the crosses would be lower than the OP fruit.

Based on the success of the crossings, the BB-01 clone demonstrated the best compatibility, both as a female parent and as a male parent. However, this compatibility needs to be evaluated further, such as examining the character performance of the fruit of the cross against the OP fruit. Therefore, it is necessary to evaluate the compatibility variability of the fruit characters resulting from crosses in order to increase the precision of parent compatibility assessment and selection of the best parents

Evaluation of cocoa parental clones based on open pollinated fruit characters

ANOVA results showed that the six clones had a significant difference in almost all

Table 2. Average values of fruit characters and ANOVA of open pollination crossings of six cocoa clones.

C	Clones						F-cal.	F-table		CV (%)
	MCC-01	Sulawesi-1	THR	MCC-02	AFQ/ MHP-01	BB-01		0.05	0.01	
NS	33.7	37.9	37.4	35.7	28.0	38.2	1.78	3.33	5.64	14.24
SWW	0.14a	0.072b	0.135a	0.075b	0.092ab	0.133a	3.55*	3.33	5.64	25.72
FL	16.08ab	17.22a	14.97bc	16.96a	14.18c	16.56ab	4.6**	2.39	3.38	10.62
FD	9.21a	8.49a	8.74a	6.81b	7.92ab	8.79a	2.85*	2.39	3.38	18.74
SL	3.12ab	2.32d	3.21a	2.33d	2.69c	2.88bc	17.98**	2.34	3.28	11.49
SW	1.69ab	1.41c	1.66ab	1.59bc	1.40c	1.79a	7.21**	2.34	3.28	14.16
ST	1.00b	1.25a	0.99b	1.09ab	0.85b	0.91b	3.91**	2.34	3.28	27.19
FPST	0.83bc	1.17a	0.92b	0.75c	0.82bc	0.9bc	8.91**	2.37	3.35	18.08
RPST	1.15b	1.41a	1.09bc	0.94c	0.97c	1.20b	9.60**	2.37	3.35	15.92
PST	0.113	0.144	0.150	0.086	0.116	0.133	2.18	2.39	3.38	18.53
FET	0.46	0.48	0.52	0.48	0.50	0.52	0.43	2.37	3.35	23.78
RET	0.83	0.75	0.74	0.69	0.70	0.82	2.25	2.37	3.35	17.31

Notes: Means with the same letter are not significantly different from each other. *significant at 5% , ** significant at 1% probability levels, C = Character,FD = fruit diameter, FL = fruit length, FET = furrow exocarp thickness, FPST = furrow pod shell thickness, NS = number of seeds, PST = pod shell thickness, RET = ridge exocarp thickness RPST= ridge pod shell thickness, SL = seed length, ST = seed thickness, SW = seed width, SWW = seed wet weight per fruit, F-cal =F calculation, CV = coefficient of variance.

characters of their open pollinated fruit, except for the number of seeds, pod shell thickness, furrow exocarp thickness, and ridge exocarp thickness (Table 2). This indicates that crossing between the six cocoa clones is expected to produce a wide diversity. According to Acquaah (2007), the more diverse a parent is, the higher the diversity of the offspring, so the crossing is considered to be ideal. The ANOVA results also show that the character of the fruit shell (pod shell thickness, furrow exocarp thickness, and ridge exocarp thickness) was not significantly different between clones. This indicates that the fruit skin of the crosses have a low diversity. According to Downie *et al*. (2003), the character of the fruit skin (testa and pericarp) shows maternal inheritance. The same observation was also reported by Indriyani *et al*. (2012) in durian crosses where the treatment of crossings did not have an effect on some of the characters of the fruit skin. Meanwhile, the number of seeds also did not show a significant difference between clones. However, focusing on the number of seeds is still important as this character is very useful in evaluating the compatibility of a cross. Based on the ANOVA results, the characters of pod shell thickness, furrow exocarp thickness, and ridge exocarp thickness were not considered in assessing the traits of crosses between cocoa clones.

Evaluation of compatibility of crosses based on fruit characters

Evaluation of the characters of fruit from open pollination shows that open pollinated fruit had a higher number of seeds and seed wet weight per fruit than fruits from crossings (Table 3). In general, the character of the number of beans and the wet weight of the fruit are the main characters of cocoa which correlate with its productivity (Groeneveld *et al*., 2010). This indicates that the crosses resulted in a higher incompatibility with regard to the traits of economic value of the fruit compared to the OP fruit. The lower number of seeds per fruit and seed wet weight per fruit of crosses may be due to the less optimal fertilization process and compatibility between parents compared to the open pollinated fruits. This is also in accordance with Singh *et al*. (2017) who reported that the development of seed and fruit size was highly dependent on the

Table 3. Average value and t-student test on fruits characters from crossing and open pollination of six cocoa clones.

Clone	Pollen	SWW (g)	FL (cm)	FD (cm)	NS	SL (cm)	SW (cm)	ST (cm)	FPST (cm)	RPST (cm)
v1	v2	0.12	17.55	8.60	32.6	3.18	1.74	1.14	0.93	1.23
v1	v3	0.13	17.35	8.95	30	3.12	1.58	0.96	0.76	1.20
v1	v4	0.13	16.21	8.68	31	3.22	1.62	1.06	0.86	1.35
v1	v5	0.13	16.20	8.53	36.5	3.18	1.72	1.12*	0.73	1.11
v1	v6	0.09	15.77	8.72	19.8	3.22	1.64	0.94	0.81	1.11
CPA		0.12	16.62	8.70	30	3.18	1.66	1.04	0.82	1.20
v1	OP	0.16	16.08	9.21	36.1	3.12	1.69	1.01	0.83	1.15
v2	v1	0.05	16.17	6.80	19	2.36	1.40	1.06	1.11	1.35
v2	v3	0.06	17.88	7.60	32.5	2.28	1.30	1.01	1.29	1.58*
v2	v4	0.06	18.17	7.60	35	2.36	1.21	0.99	1.29	1.43
v2	v5	0.05	18.07	7.48	31.8	2.48	1.40	1.03	1.29	1.53
v2	v6	0.06	16.96	7.28	44	2.30	1.36	1.23	1.27	1.50
CPA		0.06	17.45	7.35	32.5	2.36	1.33	1.06	1.25	1.48
v2	OP	0.07	17.22	8.49	38	2.32	1.41	1.25	1.17	1.41
v3	v1	0.12	15.10	8.14	46	3.38	1.72	0.99	0.89	1.07
v3	v2	0.08	14.53	8.35	39	3.48	1.83*	0.94	0.96	1.11
v3	v6	0.10	14.84	8.75	25.4	3.45	1.71	0.87	0.89	1.11
CPA		0.10	14.82	8.41	36.8	3.44	1.75	0.93	0.91	1.10
v3	OP	0.15	14.97	8.74	39.3	3.21	1.66	0.99	0.92	1.09
v4	v1	0.10	16.47	7.03	34	2.55	1.48	1.01	0.77	0.94
v4	v2	0.08	16.10	6.64	26.7	2.53	1.51	0.92	0.67	0.89
v4	v6	0.10	18.63(d)	7.40	30	2.71*	1.62	0.98	0.80	1.00
CPA		0.09	17.06	7.02	30.2	2.60	1.54	0.97	0.75	0.94
v4	OP	0.09	16.96	6.81	37.3	2.33	1.59	1.09	0.75	0.94
v5	v1	0.05	14.30	8.10	12.5	2.73	1.59	0.96	0.84	1.06
v5	v2	0.08	14.22	7.69	27.6	2.80	1.43	0.97*	0.85	1.03
v5	v6	0.10	15.28	7.94	38.5*	2.95	1.51	0.95	0.80	1.00
CPA		0.08	14.60	7.91	26.2	2.83	1.51	0.96	0.83	1.03
v5	OP	0.09	14.18	7.92	28	2.69	1.40	0.85	0.82	0.97
v6	v1	0.10	15.90	8.68	39.5	2.90	1.76	0.97	0.94	1.20
v6	v2	0.15	17.50	9.29*	43.7	3.08	1.84	0.97	0.98	1.34
v6	v3	0.13	16.29	8.83	45.5*	2.81	1.77	0.85	0.84	1.08
v6	v4	0.09	15.03	8.53	40.7	3.14*	1.84	0.82	0.86	1.30
v6	v5	0.05	16.30	8.93	29	3.07	1.94*	0.91	0.91	1.33
CPA		0.09	16.20	8.85	38.4	3.00	1.83	0.90	0.91	1.25
v6	OP	0.13	16.56	8.79	38.2	2.88	1.79	0.91	0.90	1.20

Notes: * significant difference at 5% probability level between the average values of crosses and OP based on t-student's test.,CPA= cross-pollination average, v1 = MCC-01, v2= Sulawesi-1, v3= THR, v4= MCC-02, v5= AFQ/MHP-01, v6= BB-01, OP = open polination, FD = fruit diameter, FL = fruit length, FPST = furrow pod shell thickness, NS = number of seeds, RPST= ridge pod shell thickness, SL = seed length, ST = seed thickness, SW = seed width, SWW = seed wet weight per fruit.

fertilization process, especially the suitability of the parent material characteristics. The results obtained in this study supported previous results, where OP fruit has better fruit formation compared to from crosses.

Based on the results of the compatibility evaluation given in Table 3 it also shows that there are several cross combinations that have a better phenotype than the OP fruit. The combinations are MCC-02 × BB-01 for fruit length, BB-01 × Sulawesi-1 for fruit diameter, AFQ/MHP-01 × BB-01, and BB-01 × THR for number of seeds, MCC-02 × BB-01 and BB-01 × MCC-02 for seed length, THR × Sulawesi-1, and BB-01 × AFQ/MHP-01 for seed width, MCC-01 × AFQ/MHP-01 and AFQ/MHP-01 × Sulawesi-1 for seed thickness, and Sulawesi-1 × THR for ridge pod shell thickness. These results indicate that crossings between clones can produce a wide diversity, so that there are several cross combinations that have a better phenotype than the OP fruits. In addition, based on these results, the BB-01 clone was the parent of the cross which had the best compatibility evaluation performance and it could be used to improve the characteristics of the seeds and fruit than from the OP fruit. Therefore, the BB-01 clone can be recommended as one of the best parents for crossing based on fruit character evaluation

Pearson correlation analysis of fruit characters from crosses

The results of the correlation analysis showed that the seed wet weight per fruit had a significant positive correlation with the percentage of successful crossings (0.43), number of seeds (0.44), fruit diameter (0.59), seed length (0.58), and seed width (0.50) (Figure 1). On the other hand, this character had a significant negative correlation with furrow pod shell thickness (-0.49). The character of fruit length had a significant positive correlation with seed thickness (0.44), furrow pod shell thickness (0.43), and ridge pod shell thickness (0.50), and had a significant negative correlation with seed length (-0.46). The character of fruit diameter showed a significant positive correlation with seed length (0.72), and seed width (0.66). The character of seed length had a significant positive correlation with seed width (0.76) and had a negative correlation with seed thickness (-0.38) and furrow pod shell thickness (-0.47). The character of seed width also had a significant negative correlation with seed thickness (-0.37) and furrow pod shell thickness (-0.50). The character of seed thickness had a significant positive correlation to furrow pod shell thickness (0.41) and ridge pod shell thickness (0.38). Meanwhile, a significant correlation was also found in the characters of furrow pod shell thickness and ridge pod shell thickness which had a significant positive correlation of 0.86.

Correlation analysis is used to identify the relationship between variables (Anshori *et al*., 2018). This analysis is an indicator in determining important characters in the initial evaluation of the results of crosses. Based on the results of the correlation analysis, the wet weight character of the fruit was a character that had a significant correlation in almost all characters, especially the percentage of success, and all seed parameters. In general, the wet weight of the fruit is an accumulation of various components of the cocoa pod yield (Groeneveld *et al*., 2010) and the heavier the wet pod weight of cocoa indicates that the fertilization has progressed successfully. Therefore, the wet weight character of the fruit can be the main character, in addition to the percentage of success, in evaluating the compatibility of crosses between cocoa clones.

Mapping of initial diversity of fruits from crossings and open pollination

The results of the principal component analysis (PCA) biplot show that the total diversity among the fruit characters was 61.7% (Figure 2). This diversity is

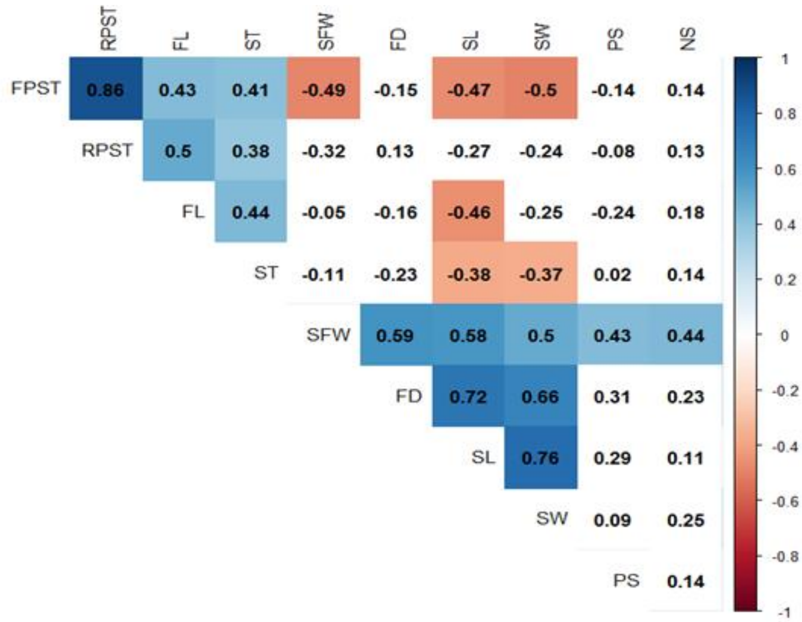

Figure 1. Pearson correlation analysis of cocoa fruit characters of crosses. The cell color indicates significant correlation at 5% probability level, FD = fruit diameter, FL = fruit length, FPST = furrow pod shell thickness, NS = number of seeds, RPST= ridge pod shell thickness, PS = percentage of successful crossings, SL = seed length, ST = seed thickness, SW = seed width, SWW = seed wet weight per fruit.

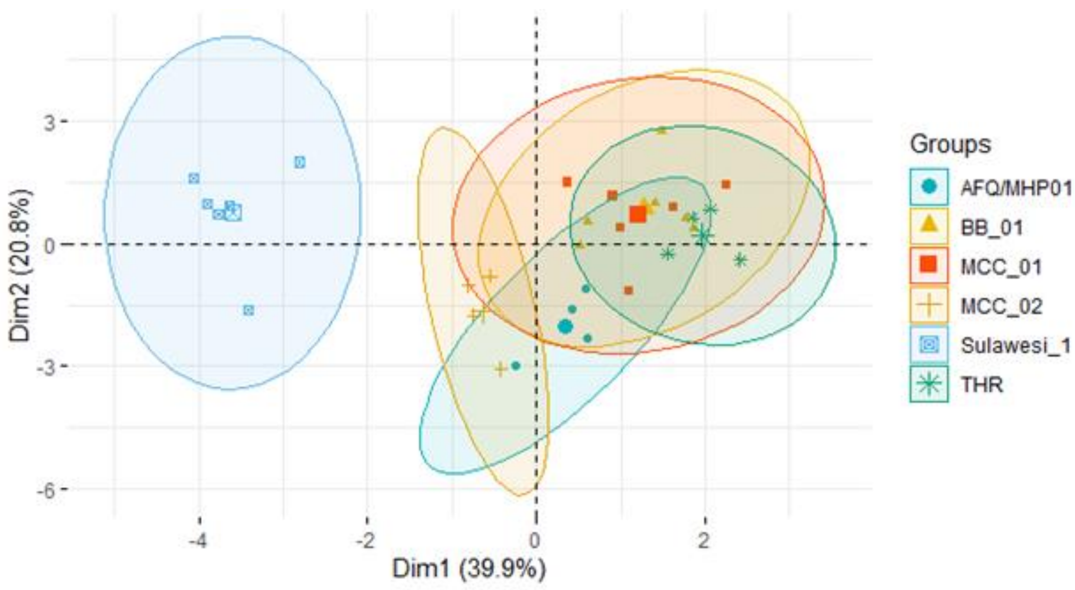

Figure 2. Principal component analysis (PCA) biplot analysis on the crossing combinations of cocoa clones and genetic clustering based on female parents.

considered good enough to map the pattern of cross diversity based on female parents. As female parents the clones of MCC-01, BB-01, and Sulawesi-1 have shown a wide cross diversity pattern on the success rate. Sulawesi-1 clone is the only clone that does not intersect with other clones. On the other hand, the MCC-01 and BB-02 clones had a diversity pattern that overlapped with almost all clones, except for Sulawesi-1. The combination distribution of Sulawesi-1 crosses was also relatively centered. On the other hand, the MCC-01 and BB-02 clones had a relatively wide distribution in several directions. Based on these differences in patterns, it can be indicated that the diversity of Sulawesi-1 clones is conservative in terms of fruit characters compared to other clones. This conservative trait is thought to be due to the high maternal effect of the Sulawesi-1 clone. An inverse relationship was shown by MCC-01 and BB 02 clones where cross combinations were relatively less affected by maternal effects. This shows the possibility of exploiting hybrid vigor in MCC-01 and BB 02 crosses for fruit characters. The use of PCA analysis enables to objectively assess and map the combination of crosses. In general, PCA analysis compresses a large data set into a simpler one while maintaining most of the diversity of the initial data (Mattjik and Sumertajaya, 2011; Anshori *et al*., 2019; Farid *et al*., 2020). In addition, each PC generated in the principal component analysis has no overlapping variance (Mattjik and Sumertajaya, 2011; Fadhli *et al*., 2020). This makes the PCA analysis useful in mapping the diversity of crosses between cocoa clones. Therefore, based on the overall consideration of the compatibility analysis in this study, the BB-01 clone was rated as the best parent in crossing the six cocoa clones.

CONCLUSION

In summary, the crosses of the six cocoa clones showed various compatibility properties. The clones with the best compatibility level were the BB-01 clone followed by the MCC-01 and Sulawesi-1 clones. On the other hand, THR, MCC-02 and AFQ/MHP-01 clones were not suitable to be crossed with each other and they serve as better female parents. Meanwhile, the best selection character in measuring the compatibility characteristics of a cross is the seed wet weight of the fruit. However, this character needs to be correlated with the phenotype of the other fruit traits. Based on the results of this study, it is recommended that BB-01 clone should be used for cross-breeding in the development of new cultivars, both as female and male parents.

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