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Yield Performance Evaluation of Arabica Coffee Progenies Resulted from Three Way Cross Method

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Abstract

Breeding for high yielding, pest and disease resistance, and good quality Arabica coffee is the direction of the coffee breeding program in Indonesia. Three-way cross-breeding method becomes the alternative method to improve plant genetics to achieve those goals. This research was aimed to evaluate the agronomic performance and grouping of Arabica coffee genotypes from three-way cross progeny. This study was conducted in Andungsari Experimental Station, Bondowoso, East Java from July until September 2021. A randomized complete block design with three replications was used as an experimental design. It consisted of eight Arabica genotypes resulting from three-way cross method and one check variety (Andungsari 1) as treatments. The tested genotypes were resulted from crossing parents i.e. S 1934, AB 3, RC, YC, S 795, and Catimor that was planted in 1997. The results of the yield performance evaluation showed that TWC (Tree Way Cross) 2 ((S 1934/AB 3 x AB 3) x AB 3) and TWC 3 (RC/S 795 x Catimor) had good yield characteristics i.e. weight of 100 cherries and cherry weight per tree. While grouping using heatmap clustering method showed that there were three groups of genotypes. One of the groups which consisted of TWC 1 ((S 1934/AB 3 x AB 3) x S 1934), TWC 2 ((S 1934/AB 3 x AB 3) x AB 3), and TWC 5 (S 1934/YC x S 1934) were categorized as moderate yield group.

Keywords: correlation, heatmap clustering analysis, path analysis

INTRODUCTION

Coffee is one of the important plantation crops with high economic value. The composition of coffee plantations in Indonesia is dominated by smallholder farmers with 98% of the total area and the remains are private and government corporations (BPS, 2019). The total coffee production is 752.5 thousand tons with total area of around 1.25 million ha (BPS, 2019). Indonesia is ranked fourth among the world's coffee producers with an export value is US$883.12 million (BPS, 2019). Arabica coffee has an advantage of being more expensive than Robusta coffee, has low caffeine content, and has special sensory characteristics (Dias & Benassi, 2015). Increasing Arabica coffee production can improve the economy of Indonesia. In general, Arabica coffee is mostly planted at the highland around 900-2000 m asl. (Nugroho *et al.*, 2016). Coffee tastes mostly is influenced by genetics and environmental factors (Bicho *et al.*, 2013; Ramadiana *et al.*, 2018). This information becomes important to improve coffee plant genetics for a breeding program (Benti, 2017).

Future strategies of Arabica coffee breeding are directed to obtain high productivity, resistant to leaf rust disease, and good quality (Melese & Kolech, 2021). The availability of genetic variations provides immense possibilities for the improvement of the crop for any desirable traits of interest (Tounekti *et al.*, 2017). Genetic variations can be obtained through conventional breeding and biotechnology breeding (Babiye *et al.*, 2020). Breeding through hybridization has the opportunity to improve the genetic potential of coffee plants (Akpertey *et al.*, 2019; Hulupi *et al.*, 2012). This method will be effective if crosses are carried out on advanced genotypes using the three-way cross. The three-way cross had the advantage in terms of genetic diversity compared with the single cross because it uses three different types of inbred lines.

The Indonesian Coffee and Cocoa Research Institute (ICCRI) has carried out a three-way cross-based breeding program and already released a variety namely Andungsari Tiga Composite Varieties (Komasti) based on the Minister of Agriculture Decree No. 200/Kpts/SR.120/1/2013. The Komasti variety has high yield potential (2.1 tons ha^{-1}) and resistant to leaf rust (Hulupi, 2013). Komasti was composed of several genotypes namely Red Caturra (RC), S 795, Catimor, and S 1934. Red Caturra has high production characteristics, S 795 has good taste, S 1934 and Catimor have leaf rust resistance in the moderate to resistant category. Based on these advantages, the development of three-way cross varieties has the opportunity to obtain better results in terms of productivity and resistance to leaf rust. This research was aimed to evaluate the yield performance and grouping of Arabica coffee genotypes from three-way cross progenies.

MATERIALS AND METHODS

Plant genetic materials consisted of eight three-way cross progenies and one check variety namely Andungsari 1 (Table 1). Andungsari 1 has characteristics of high productivity and good taste. This study was conducted in Andungsari Experimental Station, Bondowoso, East Java from July until September 2021. The experiment location had an altitude of 1,400 m asl. with a range of temperature 21.1-23.3°C and annual precipitation was 1750-3000 mm with three dry months. A randomized complete block design with three replications was used as an experimental design. It consisted of eight genotypes from three-way cross progeny and one check variety (Andungsari 1) as treatments, so there was 27 experimental units. Plant genetic materials were planted with spacing 2.5 m x 2.5 m. Plant maintenance followed the standard of Arabica coffee cultivation. The observation variables were internode length (cm), leaf length (cm), leaf width (cm), cherry length (cm), cherry width (cm), cherry thickness (cm), bean length (cm), bean width (cm), length/width ratio of bean, weight of 100 cherries (g), weight of 100 beans (g), volume of 100 beans (mL), cherry weight per tree (g). Cherry length was measure as average of five normal mature green fruits, measured at the widest part. Fruit/cherry width was measured on average of five normal mature green cherries measured at widest part. Cherry thickness was measured on the average of five normal mature green cherries, measured at the thickest part.

Data were subjected to analysis of variance following randomized complete block design format. If there was a significant difference, an LSD test was performed at the level of 5% significance. Then, the data of different traits were subjected to analysis of Pearson correlation followed by path analysis. The

Table 1. Eight genotypes of 3-way cross progenies used in the experiment and Andungsari 1 as control

Code	Genotypes	Explanation
TWC 1	(S 1934/AB 3 x AB 3) x S 1934	Three way cross progeny
TWC 2	(S 1934/AB 3 x AB 3) x AB 3	Three way cross progeny
TWC 3	RC/S 795 x Catimor	Three way cross progeny
TWC 4	YC/S 1934 x S 1934	Three way cross progeny
TWC 5	S 1934/ YC x S 1934	Three way cross progeny
TWC 6	RC/ S 795 x CTM x Catimor	Three way cross progeny
TWC 7	S 795/RC x Catimor	Three way cross progeny
TWC 8	YC/S 795 x Catimor	Three way cross progeny
AS 1	Andungsari 1	Released (check) variety

genotypes were grouped using a heatmap to visualize hierarchical clustering analysis. The software used in the analyses were STAR 2.0.1 from IRRI for analysis of variance, Microsoft Excel 2019, and R version 4.1.1 for correlation, path, and heatmap clustering analysis.

RESULTS AND DISCUSSION

Agronomic and Yield Characters

The results of variance analysis showed that genotype had a significant effect on all observed characters except on leaf length, bean width, and cherry weight per tree (Table 2, Table 3). The coefficient of variation on all observed characters ranged from 2.52-12.21%. The coefficient of variation showed heterogeneity in the population (Mattjik & Sumertajaya, 2013). Agronomic traits performance is presented in Table 2 and Table 3. The internode length of the Arabica genotype ranged from 3.35 cm (AS 1) to 7.04 cm (TWC 1) (Table 2). Two genotypes (TWC 3 and TWC 4) had internode length not significantly different from AS 1 (Andungsari 1). According to these characteristics, these genotypes were grouped as a dwarf. The advantage of dwarf coffee is that it has a dense population per hectare, which increases productivity but it requires intensive care and fertilization.

In general, Arabica coffee has a smaller leaf area compared to Robusta coffee. Arabica coffee is more susceptible to leaf rust, especially when it is planted at low to medium altitude. TWC 3 had the shortest leaf length while TWC 2 had the longest leaf length compared to other tested genotypes. There was no difference in leaf length for all genotypes. Meanwhile, the leaf width of tested genotypes ranged from 5.90 cm (TWC 7) to 8.00 cm (TWC 2). TWC 2 and TWC 5 had leaf length significantly longer compared to AS 1. TWC 2 had leaf characteristics derived from S 1934 and AB 3, while TWC 5 had leaf characteristics derived from Yellow Caturra, S795, and Catimor which had leaf rust resistance from S 795.

TWC 8 had the shortest cherry length compared with tested genotypes, while AS 1 had the longest cherry length. All tested genotypes had cherry length significantly shorter compared to AS 1. Cherry width of tested genotypes ranged from 1.30 cm (TWC 5) to 1.53 cm (AS 1). Moreover, the cherry thickness of tested genotypes ranged from 1.12 cm (TWC 3) to 1.36 cm (AS 1). All tested genotypes had red mature cherries except on TWC 4 which had yellow mature cherries. This genotype got that character from Yellow Caturra or Caturra Amarelo. Setoyama *et al.* (2013) sugested that tryptophan metabolism may be tightly linked to the development of coffee cherries and or beans.

Tabel 2. Internode, leaf and cherry characteristics of 8 coffee genotypes of 3-way cross progenies used in this study and Andungsari 1 as control

Average	Internode length (cm)	Leaf length (cm)	Leaf width (cm)	Cherry length (cm)	Cherry width (cm)	Cherry thickness (cm)
TWC 1	7.04 a	15.7	6.70 bc	1.62 bc	1.36 b	1.20 b
TWC 2	6.13 ab	17.6	8.00 a	1.62 bc	1.35 b	1.17 bc
TWC 3	4.24 cde	12.4	6.93 b	1.61 bc	1.32 b	1.12 c
TWC 4	4.02 de	14.3	6.43 bc	1.58 bc	1.35 b	1.19 bc
TWC 5	6.30 a	17.0	7.13 a	1.65 b	1.30 b	1.12 c
TWC 6	4.49 cd	16.3	7.23 ab	1.64 b	1.33 b	1.18 bc
TWC 7	4.56 cd	14.3	5.90 c	1.64 b	1.31 b	1.14 bc
TWC 8	5.23 bc	13.5	6.07 c	1.56 c	1.35 b	1.19 bc
AS 1	3.35 e	15.2	6.60 bc	1.75 a	1.53 a	1.36 a
Average	5.04	15.1	6.78	1.62	1.36	1.20
CV (%)	12.04	12.21	7.32	2.54	2.87	3.46
MS Genotype	4.44 **	8.58 ns	1.23 **	0.01 **	0.01 **	0.01 **

Notes: Values followed by the same letter in the same column were not significantly different according to the LSD test at 5%. CV: coefficient of variation, MS: mean square, **: significantly different at α 0.01, *: significantly different at α 0.05, ns: not significantly different at α 0.05.

Tabel 3. Bean and cherry characteristics of 8 coffee genotype of 3-way cross progenies used in this study and Andungsari 1 as control

Code	Bean length (cm)	Bean width (cm)	Length/width ratio of bean	Weight of 100 beans (g)	Volume of 100 beans (mL)	Weight of 100 cherries (g)	Cherry weight per tree (g)[a]
TWC 1	1.29 a	0.87	1.50 d	27.7 a	34.0 ab	199 abc	567
TWC 2	1.29 a	0.85	1.51 cd	26.6 abc	35.3 a	191 abc	646
TWC 3	1.30 a	0.83	1.57 abc	24.0 bcd	28.0 c	203 ab	689
TWC 4	1.27 a	0.84	1.50 cd	23.7 cd	30.0 bc	172 bc	215
TWC 5	1.35 a	0.85	1.59 ab	26.8 ab	31.0 abc	172 bc	450
TWC 6	1.30 a	0.81	1.60 a	24.0 bcd	30.0 bc	181 bc	531
TWC 7	1.25 a	0.82	1.53 bcd	23.2 d	30.0 bc	169 c	227
TWC 8	1.25 a	0.83	1.51 cd	22.9 d	28.7 c	172 bc	150
AS 1	1.33 a	0.86	1.55 abcd	27.2 a	36.0 a	219 a	775
Average	1.29	0.84	1.50	25.1	31.4	199	472
CV (%)	2.52	2.77	2.57	6.73	9.69	9.94	11.08
MS genotype	0.00 *	0.00 ns	0.01 *	10.89 *	25.66 *	922.08 *	0.15 ns

Notes : Values followed by the same letter in the same column were not significantly different according to the LSD test at 5%. CV: coefficient of variation, MS: mean square, **: significantly different at 0.01, *: significantly different at á 0.05, ns: not significantly different at α 0.05, [a] data transformed with formula log (x).

TWC 4 had the shortest bean, while AS 1 had the longest bean (Table 3). All tested genotypes had bean lengths not significantly different compared to AS 1. TWC 6 had the narrowest bean width, while TWC 7 had the widest bean. All the tested genotypes had seed length : width ratio which is not significantly different to AS 1. These results showed that in terms of bean size, tested genotypes were already the same with check variety. Bean size is important to create composite variety.

TWC 1, TWC 2, and TWC 3 had weight of 100 cherries not significantly different compared to AS 1. This result showed that the cherry weight of these genotypes had the same potential as AS 1. Meanwhile, in terms of weight of 100 beans, TWC 1, TWC 2, and TWC 5 had weight of 100 beans not significantly different compared to AS 1. Cherry weight per tree of tested genotypes ranged from 215 to 775 g. Five genotypes including check variety had a cherry weight per tree higher than average weight.

Secondary Character

Coffee breeders are unable to study the interaction of genetic and environmental variation using only one location trial. Thus, selection might be ineffective if it only focuses on the yielding character, especially when the yield character heritability is low. To increase the effectiveness, selection must include some secondary characters supporting the yielding character so that the selected genotypes can be relatively stable across environments (Fellahi *et al.*, 2018). Besides, according to Fellahi *et al.* (2018), secondary characters or the yield supporting characters including cherry weight per tree have to possess a significant impact. Therefore, the determination of secondary characters has significant impact and can be conducted by employing the multivariate analysis approach, which can improve the accuracy of determination (Dallastra *et al.*, 2014; Kumar & Paul, 2016).

Pearson's correlation analysis revealed that bean length (r = 0.40), bean width (r = 0.41), weight of 100 cherries (r = 0.42), weight of 100 beans (r = 0.57), volume of 100 beans (r = 0.62) were significantly correlated to cherry weight per tree (Figure 1). The highest correlation value with cherry weight per tree was observed in volume of 100 beans (r = 0.62). Meanwhile, the other characters had low-value correlation, indicating that more specific multivariate analyses such as path analysis could be used.

Path analysis is identical to direct effects as the critical point to identify specifically related characters to the main character (Akbar *et al.*, 2021). High direct effects indicated the magnitude of the character's influence to the total standard deviation of the main characters (Singh & Chaudhary, 2013). Path analysis indicated that volume of 100 beans had the highest direct effect on the cherry weight per tree with a value of 0.51 (Table 4), followed by weight of 100 cherries and weight of 100 beans with values of 0.28 and 0.37 respectively. These characters could be used as a secondary characters for selection.

Heatmap Clustering Analysis

Characterization is done to obtain information on the initial performance of coffee genotypes (Akbar *et al.*, 2019). This information can be used for grouping purposes. Whereas, grouping based on agronomical characters can reveal the relationship between plant genotypes so that they can support the selection process (Anshori *et al.*, 2020). Selection through grouping will be effective if the characteristics of the group are known. One approach that can be taken is the heatmap clustering analysis method. The use of this analysis can provide a simple understanding related to the grouping and the determinants of the group, to increase the effectiveness of selection (Yuan *et al.*, 2016). This method had been used in studies of rice (Darmadi *et al.*, 2019; Wening *et al.*, 2019), cocoa (Zasari *et al.*, 2020), and coffee (Zakidou *et al.*, 2021; Montero-Vargas *et al.*, 2013).

Dendrogram analysis was one of the grouping methods based on similarity or dissimilarity among genotypes in a population according to its characters (Anshori *et al.*, 2020). The grouping could be useful if the specific characters in the group have been

Table 4. Path analysis coefficient of several characters of Arabica coffee to cherry weight per tree

	Bean length	Bean width	Weight of 100 cherries	Weight of 100 beans	Volume of 100 beans
PBI	0.07	-0.04	0.09	0.07	0.20
LBI	0.03	-0.10	0.12	0.08	0.28
BSB	0.02	-0.04	0.28	0.05	0.11
BSI	0.05	-0.08	0.13	0.10	0.36
VB	0.03	-0.05	0.06	0.07	0.51

Notes: PBI: bean length; LBI: bean width; BSB: weight of 100 cherries; BSI: weight of 100 beans; VB: volume of 100 beans.

known. This knowledge will help a researcher to determine the important character for selection. Heatmap clustering could cover the disadvantage of the dendrogram. This analysis combined two dendrograms in the same dimension attached to heatmap analysis (Verbanck & Pagès, 2013; Metsalu & Vilo, 2015). The combination produced a relationship pattern among genotypes and characters in color intensity which was easy to understand (Bowers, 2010). The higher the color intensity indicated that the genotype has higher responses than the others in a current character (Verbanck & Pagès, 2013). The approach had been conducted by Montero-Vargas *et al*. (2013) for identifying metabolic phenotyping for the classification of coffee trees. Therefore, the analysis also could be applied for grouping three-way cross progeny of Arabica coffee.

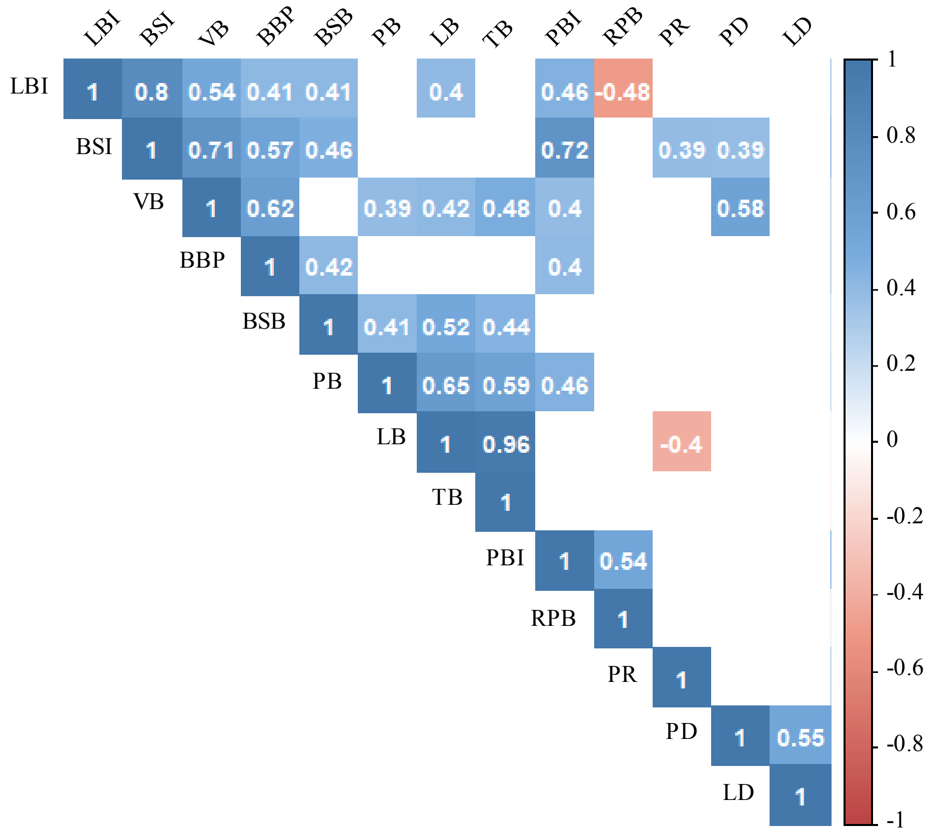

Figure 1. Pearson correlations among all observed characters of Arabica coffee

Notes: PR: internode length, PD: leaf length, LD: leaf width, PB: cherry length, LB: cherry width, TB: cherry thickness, PBI: bean length, LBI: bean width, RPB: length/width ratio of beans, BSB: weight of 100 cherries, BSI: weight of 100 beans, VB: volume of 100 beans, BBP: cherry weight per tree. The significance was focused on the character BBP, color box: r >0.42 was significantly correlated at p < 0.01, blank box: not significant.

Heatmap clustering analysis showed that three genotype groups had a reverse orientation to character responses (Figure 2). The first group (1) which consisted of three genotypes (TWC 1, TWC 2, TWC 5) were categorized as a moderate yield group. The group had a moderate weight of 100 cherries, weight of 100 beans, volume of 100 beans, and cherry weight per tree. The second group (2) which consisted of five genotypes were categorized as a low yield group. The group had low yield characters and its supporting characters. The third group (3) which consisted of one genotype (AS 1) were categorized as a high yield group. The group had high yield characters and its supporting characters. Eventhough there were no genotypes gathered in the same group with AS 1, there were three potential genotypes in group one (1) which had a moderate yield to be developed further.

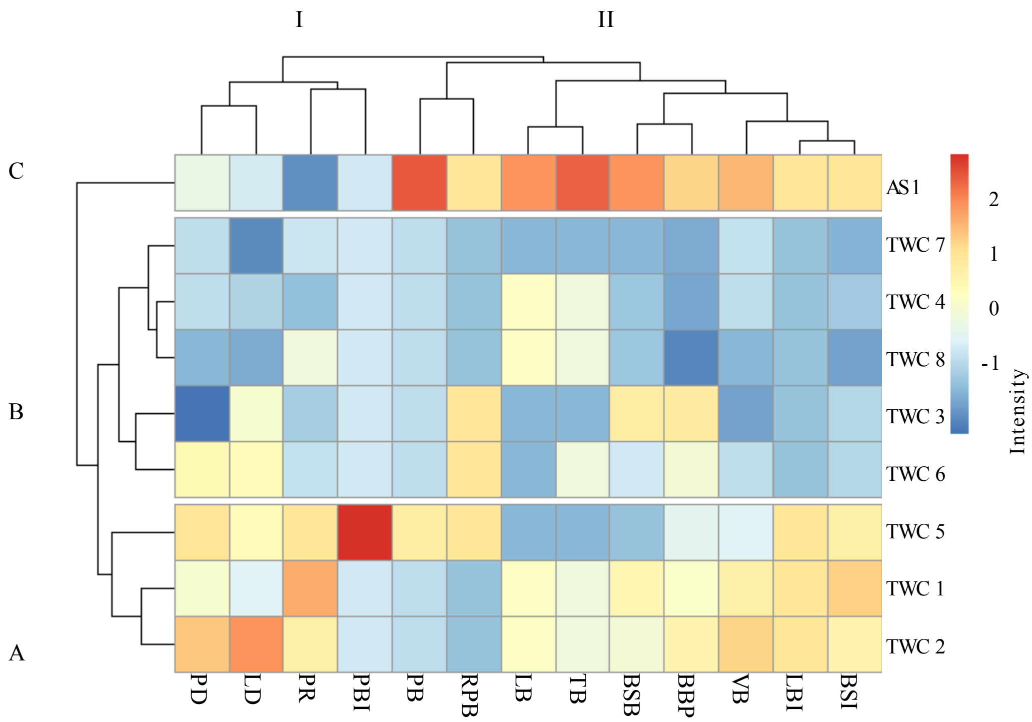

Figure 2. Heatmap clustering analysis of agronomic and yield characters of nine genotypes of Arabica coffee

Notes: PR: internode length, PD: leaf length, LD: leaf width, PB: cherry length, LB: cherry width, TB: cherry thickness, PBI: bean length, LBI: bean width, RPB: length/width ratio of beans, BSB: weight of 100 cherries, BSI: weight of 100 beans, VB: volume of 100 beans, BBP: cherry weight per tree.

TWC 1 [(S 1934/AB 3 x AB 3) x S 1934)] was a genotype from three-way cross progeny consisting of S 1934 as a female parent for crossing and male parent for backcrossing and AB 3 as a male parent for crossing and backcrossing. S 1934 was an introduction genotype from India with a yield potential of 0.8 kg per tree and resistant to leaf rust (Hulupi, 2016). AB 3 was an introduction genotype from the exploration in Ethiopia with a yield potential of 0.3 kg per tree (Hulupi, 2016). TWC 2 [(S 1934/AB 3 x AB 3) x AB 3] was genotypes from three-way cross progeny consisting of S 1934 as a female parent for crossing and AB 3 as a male parent for crossing and backcrossing. TWC 1 and TWC 2 had the same genetic composition because both of them had the same female parents. The difference was in the backcrossing process, TWC 1 is the combination between S 1934 and AB 3 as a source of pollen, while TWC 2 used only AB 3 as a source of pollen. TWC 5 (S 1934/ YC x S 1934) was a genotype from three-way cross progeny consisting of S 1934 as a female parent for crossing and male parent for backcrossing and Yellow Caturra (YC) as a male parent for crossing. YC was the first generation of Arabica coffee dwarf variety with good yield potential. This crossing combination was directed to obtain a genotype with dominant characteristics of S 1934 and also have the dwarf characteristic of YC. This information can become initial information for further evaluation.

CONCLUSIONS

The results of the yield performance evaluation showed that TWC 2 ((S 1934/AB 3 x AB 3) x AB 3) and TWC 3 (RC/S 795 x Catimor) had good yield characteristics i.e. weight of 100 cherries (191-203 g) and cherry weight per tree (646-689 g). Grouping with heatmap clustering showed that there were three groups of genotypes. The results of the correlation and path analysis showed that the supporting selection characters for the yield characters were the volume of 100 beans and the weight of 100 beans.

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