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Morphological Characterization of Tea Hybrid Progeny

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Abstract
Hybridization is the main method for obtaining genetic diversity and breeding new varieties of tea. The purpose of this research is to determine the morphological potential of each progeny from crossing between male and female parents which can then be used as a source of information in developing high quality superior tea clones through tea breeding activities. Male and female parents with the desired traits were crossed to form genetic diversity. 34 progenies resulted from crosses formed from crossing 4 male parents and 3 female parents. All parents and progeny were characterized morphologically and yield components. Data on morphological characters and yield components were analyzed descriptively. Correlation analysis and Principles Component Analysis Biplot (PCA Biplot) were also performed using R software. Male tea parents and female tea parents are in different groups based on morphological characteristics. Progeny-17, Progeny-18, Progeny-4, Progeny-5, and Progeny-27 had a higher total number of shoot and potential yield per bush compared to the other progenies.

1. Introduction

Tea is the most widely consumed non-alcoholic beverage in the world that provides important economic and health benefits as it is a source of polyphenols, catechin, amino acids, flavonoids, carotenoids, vitamins, and polysaccharides (Wang et al. 2022). The quality of tea is mainly determined by the content of catechins and several elements that affect its biological properties as well as its quality and safety (Koch et al. 2018). Tea contains polyphenols (including flavanols, flavonols, flavones, proanthocyanins, and phenolic acids, which account for 10-36% of the dry weight of tea, of which 60-80% are catechins), alkaloids, and free amino acids which determine taste, aroma, and its health benefits (Chen 2002; Too et al. 2015).

The development of superior varieties of tea is an important thing that must be done to support a sustainable tea plantation industry. Hibridization is one of the efforts to increase the new genetic diversity that is directed through the recombination of genes that come from the two parents. Two parents with the desired character are selected to be crossed so as to bring out genetic diversity. In tea plants, genotypic selection was carried out in stages starting from progeny resulting from crosses grown from seeds to several selections made after vegetative propagation. Selection was made both on morphological characters and yield components.

Catechins are the main reactive materials involved in tea fermentation which determine the final quality and taste of tea (Dong et al. 2021). Catechins are the main astringent substances in tea which are synthesized via phenylpropanoid pathway (Liu et al. 2015). In general, the main catechins in tea leaves include (+)-catechin (C), (-)-epicatechin (EC), (+)-gallocatechin (GC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin gallate (EGCG), and (+)-gallocatechin gallate (GCG) (Lee et al. 2014). EGCG, EGC, ECG, and EC are the main polyphenols present in green tea. EGCG with 50-70%of total catechins has a chemopreventive effect on cancer and other diseases such as diabetes, neurological, cardiovascular, and obesity (Khan & Mukhtar 2018). EGCG is an antioxidant compound in which these flavonoids are able to suppress swelling, multiplication, and initiation of carcinogenesis (Thawonsuwan et al. 2010).

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Like catechins, anthocyanins are also products of the phenylpropanoid pathway (Kerio et al. 2012). Some F1 crosses have a combination of characters from Male and Female parents, the presence of anthocyanin pigments in the leaf shoot adds to the specialization of tea. Tea breeders in various tea-producing countries have developed purple tea varieties which are rich in anthocyanins (Joshi et al. 2017; Kerio et al. 2013). The results of the study revealed that the antioxidant value of purple leaf tea was higher than that of green leaf tea due to the presence of catechins and anthocyanins (Joshi et al. 2017; Kottawa-Arachchi et al. 2019). According to Pang et al. (2021), anthocyanin-rich tea cultivars with purple shoot have high amino acid content, where the amino acids in tea leaves are highly correlated with taste and aroma.

The crosses between clone cultivars of the male parents which have green shoot and the female parent which have red-brown shootis expected to produce new hybrid progenies with a combination of morphological characteristics for better productivity and a combination of metabolite characteristics for better quality. Characterization of hybrid progenies is the first step in the proper utilization of genetic resources (Thuvaraki et al. 2017). Information regarding the morphological characters of male and female parents and their progeny is needed to determine the morphological potential of each progeny which can then be used as a source of information in developing high quality superior tea clones through tea breeding activities.

2. Method

2.1. Materials and Methods

The genetic material used included accessions of parents there were Parent-1, Parent-2, Parent-3, Parent-4, Parent-5, Parent-6, Parent-7, and 34 hybrid progenies from crosses between male and female parents (Table 1). Morphological characterization of parents and hybrid progeny was carried out using a descriptive method by observed the morphological characters of shoot, leaves, and yield components. All parents and progeny were characterized morphologically such as anthocyanin pigmentation in shoot and petiole, presence of trichomes on pekoe and first leaves, leaf length, and leaf width. Yield components included the number of shoots (p+3), the number of dormant shoots, the total number of shoot, the dry weight per shoot, and the potential yield per bush on the progeny.

Each tea shoot is harvested by picking at the ¾ position of the internode between the 3rd and 4th leaves, with shoot characteristics including:

1. Density of trichomes at pekoe, coded as 1-glabrous, 2-light, 3-dense.
2. Density of trichomes at bottom of first leaves, coded as 1-glabrous, 2-scattered, 3-half to edge, 4-dense.
3. Young leaf color, coded as 1-light green, 2-green, 3-purplish green, and 4-purplish.
4. Petiole color, coded as 1-light green, 2-green, 3-purplish green, and 4-purplish.

Characteristics of maintainer leaves, including:

1. Leaf length (cm), measured from the base of the leaf to the tip of the leaf.
2. Leaf width (cm), measured of the widest position on the leaf.
3. Leaf length/width ratio.
4. Leaf angle (degrees), the angle formed between the branch and the leaf.
5. Petiole length (cm), measured from the brach to the base of the leaf.

Stem characteristics in progeny plants, include:

1. Stem diameter (cm), measured 5 cm above the ground.
2. The number of main branches, calculated on the last observation.
3. The length of the plucking surface (cm), observed in the last observation.
4. The width of the plucking surface (cm), observed in the last observation.

Characteristics of yield components, include:

1. Number of pekoe shoot (p+3), the average value of mature shoots that can be harvested (3 first leaves and 1 peko).
2. Number of dormant shoot (b+1), the average value of dormant shoots that can be harvested (1 first leaves and 1 dormant shoot).

3. The fresh weight of shoots, obtained by dividing the weight of pekoe shoots (p+3) that can be harvested by the number of pekoe shoots (p+3) that can be harvested.
4. The dry weight of shoots with a moisture content of 3.5-4%, was obtained by dividing the weight of the pekoe shoots (p+3) that can be harvested after drying in the oven to a moisture content of 3.5-4% by the number of pekoe shoots that can be harvested (p+3).
5. The ratio of fresh weight/dry weight of shoots.
6. Yield potential per shrub, obtained by multiplying the dry weight of shoots by the total number of shoots.

Table 1. List of genetic material

No.	Genotipe
1	Progeny-1
2	Progeny-2
3	Progeny-3
4	Progeny-4
5	Progeny-5
6	Progeny-6
7	Progeny-7
8	Progeny-8
9	Progeny-9
10	Progeny-10
11	Progeny-11
12	Progeny-12
13	Progeny-13
14	Progeny-14
15	Progeny-15
16	Progeny-16
17	Progeny-17
18	Progeny-18
19	Progeny-19
20	Progeny-20
21	Progeny-21
22	Progeny-22
23	Progeny-23
24	Progeny-24
25	Progeny-25
26	Progeny-26
27	Progeny-27
28	Progeny-28
29	Progeny-29
30	Progeny-30
31	Progeny-31
32	Progeny-32
33	Progeny-33
34	Progeny-34
35	Parent-1
36	Parent-2
37	Parent-3
38	Parent-4
39	Parent-5
40	Parent-6
41	Parent-7

Data analysis

Data on morphological characters and yield components were analyzed descriptively. Quantitative morphological data and yield components were calculated for the mean value, minimum and maximum value, standard deviation calue, and the coefficient of variation (CV). The value of the CV is used to estimate the level of observed character diversity, CV of 0%-25% indicated low diversity (Nilasari et al. 2013; Hadi et al. 2014), while high diversity if the value of CV > 25% (Purwati et al. 2015). Correlation analysis and Principle Component Analysis Biplot (PCA Biplot) were also performed using R software. Secondary metabolite analysis data were compared using analysis of variance (ANOVA). Characters with a significant F value (p <0.05) were further tested using Duncan's Multiple Range Test.

3. Result and Discussion

In tea plants, shoot characterization is very important because it determines the yield and quality of tea. In this study, clones of male parents were used which had the character of green shoot, and female parents which were pigmented with anthocyanin, where male parents had higher productivity than female parents, so it was expected that the progeny would have a combined character of the two parents. There were differences in the morphological characters of the progenies in the field.

In this study the cultivars Parent-5, Parent-6, and Parent-7 had the character of shoot with anthocyanin pigment. Crosses between male and female parents produced progeny with shoot color distribution from light green to dark green with and without the addition of anthocyanin pigment on the shoot or petioles. According to Kottawa-Arachchi et al. (2013), variation of pigment content in tea are influenced by cultivar and environmental differences such as shade and fertilizer levels. The female parents, which consisted of Parent-5, Parent-6, and Parent-7 with several progeny, had morphological characteristics of anthocyanin pigmentation in shoot and petiole and the presence of trichomes on the pekoe and the bottom of first leaf which was denser than that of male parents. Male parents, which consisted of Parent-1, Parent-2, Parent-3, and Parent-4 with several progeny, have shoot and petioles that have a color from light green to green without anthocyanin pigment with medium presence of trichomes. According to Thuvaraki et al. (2017), the presence of anthocyanin pigmentation in shoot, petiole, and the presence of trichomes were correlates with antioxidant activity and tea quality. Leaf trichomes play an important role in plant resistance and tea quality (Liu et al. 2021).

Based on the descriptors developed by IPGRI, qualitative and quantitative characters were observed to estimate morphologicaldeversity. The results of the analysis showed that the characters with low variability were leaf length : leaf width, leaf angle, fresh weight : dry weight per shoot, leaf length, and leaf width as indicated by the CV value of 7.12%; 9.64%; 16.95%; 20.46% and 21.43%. High diversity was indicated by the long petiole, length of 1-3 internode, fresh weight per shoot, dry weight per shoot, number of pekoe shoot (p+3), number of dormant shoot (b+1), and yield potential per shrub which was refrected by the value of CV > 25%, which were 28.02%; 29.83%; 36.76%; 70.92%; 55.26% and 83.45% (Table 2). The high diversity of these characters provides an opportunity to obtain superior progeny with low number of dormant shoots through negative selection. In addition, positive selection can also be carried out to obtain progeny with long petiole, number of pekoe shoots (p+3), fresh weight per shoot, dry weight per shoot, and high yield potential per shrub.

Table 2. Descriptive statistics of morphological and yield component characteristics.

No.	Character	Mean	Standard deviation	Minimum	Maximum	CV (%)
1.	Leaf length (cm)	9,87	2,02	6,00	14,7	20,46
2.	Leaf width (cm)	4,13	0,88	2,59	7,24	21,43
3.	Leaf angle (°)	36,55	3,52	30,00	45,00	9,64

4.	Petiole length (cm)	0,38	0,09	0,20	0,70	25,48
5.	Length of 1-3 internode (cm)	3,68	1,35	1,90	9,50	36,76
6.	Number of pekoe shoot (p+3)	10,22	6,11	1,00	39,00	70,92
7.	Number of dormant shoot (b+1)	15,63	8,63	1,00	49,00	55,26
8	Fresh weight per shoot (gram)	1,15	0,32	0,60	1,87	28,02
9	Dry weight per shoot (gram)	0,30	0,08	0,12	0,56	29,83
10.	Yield potential per shrub (gram)	8,37	6,41	0,37	36,97	83,45
11.	Leaf length : leaf width	2,41	0,17	2,00	2,79	7,12
12.	Fresh weight : dry weight per shoot	4,02	0,68	2,23	5,61	16,95

The desired varieties have high yield potential and shoot numbers (Das et al. 2012). The highest yield potential of superior tea clones in Indonesia, the GMB series, reached 5,800 kg/ha/year (Astika et al. 1999). The results showed that Progeny-17, Progeny-18, and Progeny-4 had potential shoot yield >20 g per bush was higher than the other progeny. Progeny-18, Progeny-17, Progeny-5, Progeny-4, and Progeny-27 with a number of pekoe shoots (p+3) >15, but these progenies also had a number of dormant shoots >20, except for the Progeny-27 with an average number of dormant shoots 17.20.

Progeny-17, Progeny-27, Progeny-18, and Parent-1 had shoot dry weight >0.4 gram per shoot. Shoot dry weight is directly related to shoot fresh weight. The main yield components for tea are the number of shoots harvested per unit area and the average weight of each shoot. Tea productivity is calculated in terms of the weight of made tea per unit area of land per year, where made tea is a form of tea that is obtained after harvested and through factory processes such as withering, fermentation, and drying (De Costa et al. 2007).

Leaf size characteristics consisting of leaf length and width can be used to identify the type of tea accession and also to characterize the yield. Male parent leaf length with an average leaf length of 12.58 ± 0.78 cm and a leaf width of 5.53 ± 0.61 cm. Female parent leaf length with an average leaf length of 10.19 ± 0.57 cm and a leaf width of 3.91 ± 0.12 cm. Meanwhile, in progenies resulting from crosses, the average leaf length was 9.52 ± 1.76 cm and leaf width was 3.98 ± 0.65 cm. This indicated that the progeny leaf length and leaf width were between the sizes of the male and female parents.

Table 3 showed that there was a significant relationship between several morphological characteristics and yield components. Leaf length, leaf width, node length 1-3, and petiole length were positively correlated and significantly related to fresh weight and dry weight per shoot. Fresh weight and dry weight per shoot were positively correlated and significantly related to yield potential per shrub which was supported by the number of pekoe shoots (p+3) and the number of dormant shoots (b+1) which were also positively correlated and significantly related to yield potential per shrub. Leaf angle was not significantly related to leaf length, leaf width, and dry weight, and negatively correlated with 1-3 internode length, petiole length, fresh weight, number of pekoe shoots (p+3), number of dormant shoots (b+1), total number of shoots, and yield potential per bush, this is in accordance with the statement of Tanton et al. (1992), that the angle of the leaf affects the effectiveness of photosynthesis, a large leaf angle was less effective in receiving sunlight for photosynthesis. Observations on the leaf angles showed that the average of leaf angle of progeny was 36.36°, between the average leaf angles of male parents (39.38°) and female parents (35°).

Table 3. Correlation coefficient of 13 morphological characters and yield components of parent and progeny.

	LL	LW	LI	LA	PL	FW	DW	NS	ND	NTS	YP	LL:LW	FW:DW
LL	1	0.90*	0.68*	0.01	0.51*	0.79*	0.68*	0.63*	0.67*	0.63*	0.68*	0.19	-0.12
LW	0.90*	1	0.69*	0.07	0.55*	0.80*	0.68*	0.57*	0.65*	0.59*	0.68*	-0.19	-0.02

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LI	0.68*	0.69*	1	-0.07	0.62*	0.78*	0.62*	0.68*	0.74*	0.63*	0.72*	0.04	0.14
LA	0.01	0.07	-0.07	1	-0.14	-0.05	0.04	-0.12	-0.15	-0.21	-0.09	-0.13	-0.17
PL	0.51*	0.55*	0.62*	-0.14	1	0.48*	0.40*	0.49*	0.60*	0.52*	0.53*	-0.09	0.12
FW	0.79*	0.80*	0.78*	-0.05	0.48*	1	0.74*	0.78*	0.78*	0.75*	0.86*	-0.03	0.11
DW	0.68*	0.68*	0.62*	0.04	0.40*	0.74*	1	0.56*	0.67*	0.60*	0.82*	-0.07	-0.34
NS	0.63*	0.57*	0.68*	-0.12	0.49*	0.78*	0.56*	1	0.89*	0.93*	0.88*	0.14	0.07
ND	0.67*	0.65*	0.74*	-0.15	0.60*	0.78*	0.67*	0.89*	1	0.94*	0.94*	0.08	0.03
NTS	0.63*	0.59*	0.63*	-0.21	0.52*	0.75*	0.60*	0.93*	0.94*	1	0.90*	0.10	0.01
YP	0.68*	0.68*	0.72*	-0.09	0.53*	0.86*	0.82*	0.81*	0.94*	0.90*	1	-0.04	-0.01
LL:LW	0.19	-0.19	0.04	-0.13	-0.09	-0.03	-0.07	0.14	0.08	0.10	-0.04	1	-0.21
FW:DW	-0.12	-0.02	0.14	-0.17	0.12	0.11	-0.34	0.07	0.03	0.01	-0.01	-0.21	1

Notes: *) significant different 5%

LL	= Leaf length	NS	= Number of shoot (p+3)
LW	= Leaf width	ND	= Number of dormant shoot (b+1)
LI	= Length of internode 1-3	TSN	= Total shoot number
LA	= Leaf angle	YP	= Yield potential per shrub
PL	= Petiole length	LL : LW	= Leaf length : leaf width
FW	= Fresh weight per shoot	FW : DW	= Fresh weight : dry weight
DW	= Dry weight per shoot		

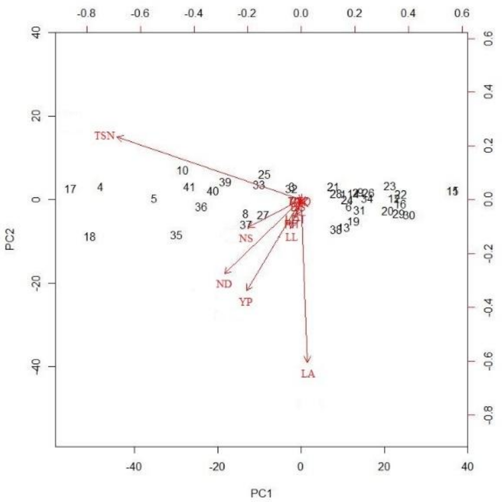

Figure 1. PCA Biplot of 13 morphological traits in parents and progeny.

The results of the PCA Biplot (Figure 1), progeny and parents were grouped based on the correlation between morphological traits that can be used to identification. Some of the morphological characteristics as the main factor component were yield potential per shrub (YP) which was significantly correlated with the total shoot number (TSN), the number of dormant shoot (ND), and the number of shoots (NS), which is supported by the shoot fresh weight (FW), shoot dry weight (DW), length of internode 1-3, length of leaf, width of leaf, and length of petiole. The progeny and parents that were in front of the main factor had a large number of shoots with high shoot fresh weight, shoot dry weight, lengths of internode 1-3, leaf length, leaf width, and petiole length. Production characteristics and number of shoot were used to measure the superiority of a tea variety or as selection criteria (Rahadi et al. 2016). Progeny that were in front of the main factor show progeny with high yield potential supported by yield component factors such as number of shoots, shoot fresh weight, shoot dry weigh (Figure 1). The potential progenies include Progeny-17, Progeny-18, Progeny-4, and Progeny-5 which have a higher total number of pekoe shoot and potential yield per bush compared to the other progenies. Progeny-27 had a higher number of pekoe shoot than the number of dormant shoot, while Progeny-17, Progeny-18, Progeny-4, and Progeny-5 had more dormant shoots than pekoe shoots.

Stem characteristics in progeny include stem diameter, the number of main branches, and the length and width of the picking surface. Progeny-17 had a larger stem diameter compared to the other progenies (4.17 cm), followed by the progeny Progeny-5 (3.85 cm), and Progeny-25 (3.70 cm). According to De Costa et al. (2007), tea stems play an important role in the formation of shoots and new shoots after pruning, because the stems contribute to the assimilation of CO_2, and also assimilation o starch which will give rise to new shoots on the stems after pruning. The progeny from crosses is 2 years so it still has a few main branches (1-3 main branches). In the early stages of observing the stem morphology, it can be seen that the difference in the number of main branches between progenies. The number of main branches on the stem determines the surface area of the picking area. Plants with different genetics have different numbers of primary branches (Pamungkas & Supijatno 2017).

4. Conclusion

Male tea parents and female tea parents are in different groups based on morphological characteristics. Progeny-17, Progeny-18, Progeny-4, Progeny-5, and Progeny-27 had a higher total number of shoot and potential yield per bush compared to the other progenies.

Author Contributions

Hani Widhianata: Conceptualization, Methodology, Software and Data curation, Writing- Original draft preparation. **Novan Noviansyah**: Visualization. **Eka Sudibya** and **Prayitno Ribut Suwasono**: Validation and Editing.

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Declaration of Conflicting Interests

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