Preliminary study on genetic diversity and relationship of 12 White Turmeric (Curcuma zedoaria (Christm.) Roscoe) accessions based on morphological traits

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Abstract. White turmeric (Curcuma zedoaria (Christm.) Roscoe) is one of the important plants in the genus Curcuma (Zingiberaceae). This plant grows in Asia and has wide variety of morphological characteristics. White turmeric can be used as a spice for food additive and the raw material for traditional medicine. Indonesian Spice and Medicine Crop Research Institute (ISMCRI) has collected 12 white turmeric accessions from several localities in Indonesia. This study aims to determine the relationship between 12 white turmeric accessions based on their morphological characteristics. The rhizome used weighs 30-50 grams with 2-3 shoots. The plots size was 2.5 x 3.5 m² with a spacing of 50 x 50 cm, contained 20 plants per plot. The experiment was arranged in a randomized block design with three replications. Morphological traits parameters observed were 11 morphological characters, namely plant height, leaf length, leaf width, number of leaves, leaf thickness, stem diameter, number of tillers, rhizome weight, rhizome length, rhizome width, and rhizome thickness. A significant morphological variation level was recorded for rhizome weight and plant height, while genetic diversity is showed by rhizome weight and a number of tillers. The morphological characteristics observed were correlated with each other. Cluster analysis created four major groups.

1. Introduction

White turmeric (Curcuma zedoaria (Christm.) Roscoe) is one of a rhizomatous herbaceous perennial plant of the ginger family, Zingiberaceae. Nowadays, this species is widely cultivated in Asia, especially in South and Southeast Asia. Curcuma zedoaria has different vernacular names in other countries such as Er-chu (Chinese), Gajutsu (Japan), Gandamatsi (Hindi), Zitwer (German), and Rhizome de Zédoaire (French). White turmeric is a large plant having a height of 70-100cm, pseudostems of 30-35cm, green sheath. Propagation of plants is using rhizomes. Rhizomes are large, 5-6 x 9-10cm, 5-6 x 9-10cm, blue in the center, verging towards grey. The blue colour is highly variable, depending upon the nature of the soil and the rhizome's age. Rhizomes are strongly aromatic; sessile tubers branched, condensed. Roots are fleshy; root tubers many, ovate-oblong, pale, watery pearl colour [1].

White turmeric is useful as a spice and medicine. In Thailand, the young rhizomes can be used in the ingredient to make curry pastes. In India, it is sometimes used for a curry dye and flavor additive in seafood dishes or Indian cuisine. In Indonesia, C. zedoaria leaves are used as an additional seasoning to
enhance the taste of fish dishes and other foods and in Malaysia this species is widely consumed as a spice and postpartum food [2].

As a medicine, white turmeric is widely used by several countries such as India, China, Korea, and Japan. In India, it has been used in Ayurveda for the treatment of menstrual disorders, indigestion, nausea, and carcinomas. Meanwhile, in China, its rhizome has been used in traditional Chinese medicine for various cancers treatment [3]. In Asian countries such as China, Korea, and Japan it is used as traditional medicine for the improvement of blood circulation, menstrual flow, abdominal cramps, and rheumatic pain [4]. White turmeric is also useful for stomach diseases, toothache, blood stagnation, leucoderma, tuberculosis, spleen swelling, dyspepsia, cold, cough, fever, and used as anti-venom against the bite of Indian cobra [5].

The useful part of white turmeric is a rhizome. It is containing essential oil 1-1.5%, curcumin, gum, resin, starch, and tannins [6]. The rhizome of white turmeric has 10 sesquiterpenes and was able to structurally characterize 15 such compounds, namely furanodiene, furanodienone, zedorone, curzerenone, curzeone, germacrone, 13-hydroxy germacrone, dihydrocurdione, curcumenone, and zedoaronediol [7]. Generally, the main active compound of the curcuma rhizome is the non-volatile curcuminoid and the volatile oil [8]. The curcumin in the white turmeric rhizome has many functions such as anticancer, anti-inflammatory, antihypertensive, antidiabetic, antimicrobial, immunomodulator, antifungal, antiproliferation, and nephroprotective [9]. In addition, curcumin, a major component of turmeric, has been proven to have clinical therapeutic and its antioxidant properties have an important role in the management of chronic inflammation diseases [10].

Germplasm is an essential component of plant breeding programs where a large genetic diversity is required to assemble a superior variety. Germplasm selection is a collection of plants obtained from different regions with their respective characteristics of superiority. In order to determine genetic variation and superior morphological characteristics among collected plants, selection is carried out in germplasm. Genetic diversity can be calculated in many ways, but morphological characteristics (i.e., leaves, stems, branches and flowers, etc.), especially their form, size and variability, are the main source of genetic diversity [11]. In addition, genetic diversity information can also be very useful in conservation methods, as conservation should be carried out after recognizing genetic diversity [12]. This study aims to determine the genetic diversity and relationship of 12 white turmeric accession based on morphology characters.

2. Materials and methods

2.1. Time and place experimental

This experiment was conducted from 2014—2015 during the planting seasons (the beginning of the rainy season) at Cicurug Research Station of Indonesian Spice and Medicinal Crops Research Institute, in Cicurug, Sukabumi, West Java, Indonesia (550m above sea level). In 2014 climatic conditions were more dry months than wet months.

2.2. Plant material and experimental design

The twelve accessions of white turmeric used were collected from seven different Indonesia localities (Table 1). The rhizome used 10 months after cultivation, weighs 30-50 grams with 2-3 shoots. The scale of the plots was 2.5 x 3.5 m², spaced 50 x 50 cm², as 20 plants/plot. The experiment was arranged in a randomized block design with three replications. The plant maintenance follows the Standard Operating Procedures for the Zingiberaceae family [13].
Table 1. Passport data of white turmeric [5]

| Accessions | District        | Province     | Date of collection |
|------------|----------------|--------------|--------------------|
| Cuze 1     | Sukabumi       | West Java    | 2001               |
| Cuze 2     | Cianjur        | West Java    | 2008               |
| Cuze 3     | Cianjur        | West Java    | 2008               |
| Cuze 4     | Gunung Kidul   | Yogyakarta   | 2008               |
| Cuze 5     | Bunaken        | North Sulawesi| 2008              |
| Cuze 6     | N.I            | North Sulawesi| 2008              |
| Cuze 7     | Ciwidey        | West Java    | 2008               |
| Cuze 8     | N.I            | Bali         | 2088               |
| Cuze 9     | N.I            | East Nusa    | 2009               |
| Cuze 10    | Gunung Kidul   | Yogyakarta   | 2009               |
| Cuze 11    | Puwakarta      | West Java    | 2009               |
| Cuze 12    | N.I            | East Java    | 2011               |

2.3. Observation on morphological traits
A total 11 phenotypic traits (growth and yields) were used for morphological characters. Plant growth parameters are plant height (the measurement from ground level to the apex of the main stem), leaf length (measured by digital vernier caliper), leaf width (measured by digital vernier calliper), number of leaves (number of leaves was counted manually), leaf thickness (measured by digital vernier calliper), stem diameter (measured by digital vernier caliper), and number of tillers (number of tillers was counted manually). Yield parameters are rhizome weight (measured by digital scales), rhizome length (measured by digital vernier calliper), rhizome width (measured by digital vernier calliper), and rhizome thickness (measured by digital vernier calliper). Observations of the morphological characters referred to Tjitosoepomo [14].

2.4. Statistical analysis
The measurement of genetic diversity and correlation coefficient was based on mean values of accessions detected in each replication using R software. The correlation coefficient for evaluates the relationships among the different variables in the experiment using R software. The broad-sense heritability was calculated by using Ahsan formula [15].

\[
\text{Genetic Variance} \left( \sigma_g^2 \right) = \frac{\text{Genotype Mean Square (GMS)} - \text{Error Mean Square (EMS)}}{\text{Number of replications (r)}} \tag{1}
\]

\[
\text{Environmental Variance} \left( \sigma_e^2 \right) = \text{Error Mean Square (EMS)} \tag{2}
\]

\[
\text{Phenotypic Variance} \left( \sigma_p^2 \right) = \sigma_g^2 + \frac{\sigma_e^2}{r} \tag{3}
\]
Genotypic, Phenotypic, and Environmental coefficient of Variation was calculated as

\[ \text{Genotypic Coefficient of Variation (GCV)} = \frac{\sigma_g^2}{x} \times 100 \]  

\[ \text{Phenotypic Coefficient of Variation (GCV)} = \frac{\sigma_p^2}{x} \times 100 \]  

\[ \text{Environmental Coefficient of Variation (GCV)} = \frac{\sigma_e^2}{x} \times 100 \]

Where, GCV% = Genotypic Coefficient of variation; \( \sigma_g^2 \) = Genotypic Variance; PCV % = Phenotypic Coefficient of variation; \( \sigma_p^2 \) = Genotypic Variance; ECV % = Environmental Coefficient of variation; \( \sigma_e^2 \) = Environmental Variance.

Broad sense heritability \( (H^2) \) was calculated as

\[ H^2 = \frac{\sigma_g^2}{\sigma_p^2} \]  

Cluster analysis is carried out to assess the level of dissimilarity using PBSTAT software. Principal component analysis for 11 morphological traits was calculated using Software R to identify traits that contributed to the observed variation among accessions.

3. Results and discussion

3.1. Genotypic and phenotypic diversity

The results of the analysis showed a significant level of variation morphological between 12 accessions of white turmeric using morphological characters (Table 2). The largest variance is the rhizome’s weight with a variance value of 33792.73 and the plant height with a variance value of 73.58. Meanwhile, the low levels of variability differ in leaf length, leaf width, number of leaves, leaf thickness, stem diameter, number of tillers, rhizome length, rhizome width, and rhizome thickness. The mean value of the white turmeric genotype for rhizome weight characters has a value of 609.79 with a range of 344.44—923.89 and plant height characters have a value of 52.48 with a range of 37.76—62.10. The highest coefficient variety of genetics is the characteristics of rhizome weight and a number of tillers. The value of the coefficient variety of each character is 30.15% and 25.16%.

The diversity value is based on the value of the genotypic coefficient of variation (GCV) and the phenotypic coefficient of variation (PCV). Characters with low GCV value and low PCV value are classified as narrow diversity, however, characters with relatively high GCV value and high PCV value are classified as broad diversity [16]. Heritability is a visualization of the phenotype traits from genetic traits influences. The diversity of genotypic, phenotypic, environmental, and heritability values are shown in Table 3. Genotypic and phenotypic diversity of twelve white turmeric accession is low that is shown by the value of the genotypic coefficient of variation and phenotypic coefficient of variation for all observations under 25%. Low heritability with a value of <0.25 is indicated by leaf thickness (0.16), rhizome width (0.11), and rhizome thickness (0.05). Meanwhile, other observations indicate the medium heritability.
Table 2. Variation in morphological traits of 12 white turmeric accessions

| Traits          | Mean   | Minimum | Maximum | SD      | CV (%) | Variance |
|-----------------|--------|---------|---------|---------|--------|----------|
| Plant height (PH) | 52.48  | 37.76   | 62.10   | 8.58    | 16.35  | 73.5825  |
| Leaf length (LL) | 27.63  | 20.18   | 32.94   | 4.24    | 15.35  | 17.9918  |
| Leaf width (LWi) | 10.83  | 7.62    | 13.76   | 1.80    | 16.66  | 3.2530   |
| Leaf thickness (LT) | 0.27   | 0.22    | 0.29    | 0.02    | 7.49   | 0.0004   |
| Number of leaf (NoL) | 7.06   | 5.17    | 8.33    | 0.92    | 13.10  | 0.8550   |
| Stem diameter (SD) | 10.95  | 7.09    | 12.78   | 1.86    | 16.97  | 3.4555   |
| Number of tiller (NoT) | 2.38   | 1.33    | 3.06    | 0.60    | 25.16  | 0.3592   |
| Rhizome weight (RWe) | 609.79 | 344.44  | 923.89  | 183.83  | 30.15  | 33792.73 |
| Rhizome length (RL) | 22.04  | 17.69   | 25.83   | 2.64    | 11.97  | 6.9599   |
| Rhizome width (RWi) | 9.99   | 7.33    | 11.83   | 1.36    | 13.61  | 1.8492   |
| Rhizome thickness (RT) | 14.01  | 12.36   | 15.20   | 0.93    | 6.66   | 0.8720   |

*CV : Coefficient of Variation

Table 3. Genotypic, phenotypic, and environmental variance and coefficient of variation, broadsense heritability of 12 white turmeric accessions

| Traits          | $\sigma^2_a$ (100%) | $\sigma^2_e$ (100%) | $\sigma^2_n$ (100%) | PCV (100%) | $H^*$ |
|-----------------|---------------------|---------------------|---------------------|------------|-------|
| Plant height (PH) | 45.10               | 12.80               | 85.45               | 130.55     | 7.26  | 0.35  |
| Leaf length (LL) | 10.85               | 11.92               | 21.43               | 5.58       | 32.28 | 6.85  | 0.34  |
| Leaf width (LWi) | 2.03                | 13.16               | 3.66                | 5.89       | 5.70  | 7.35  | 0.36  |
| Leaf thickness (LT) | 0.0001              | 4.52                | 0.0008              | 3.45       | 0.009 | 3.76  | 0.16  |
| Number of leaf (NoL) | 0.57                | 10.66               | 0.87                | 4.40       | 1.43  | 5.66  | 0.39  |
| Stem diameter (SD) | 1.76                | 12.12               | 5.08                | 6.86       | 6.84  | 7.96  | 0.26  |
| Number of tiller (NoT) | 0.23                | 20.26               | 0.38                | 8.62       | 0.61  | 10.95 | 0.38  |
| Rhizome weight (RWe) | 18702.16           | 22.43               | 45271.73            | 11.63      | 63973.88 | 13.83  | 0.29  |
| Rhizome length (RL) | 3.83                | 8.88                | 9.38                | 4.63       | 13.21 | 5.50  | 0.29  |
| Rhizome width (RWi) | 0.51                | 7.18                | 4.00                | 6.68       | 4.52  | 7.09  | 0.11  |
| Rhizome thickness (RT) | 0.11                | 2.38                | 2.28                | 3.59       | 2.39  | 3.68  | 0.05  |

A plant's genetic diversity is an essential advantage in a breeding program as this will make it easier to pick superior plants that are used in crossing as the parent. The low value of genetic variation indicates that the genetic variation is low. The heritability value of white turmeric is medium which shows that environmental and genetic influences are balanced in the appearance of these characters (see Table 3). This result is in contrast to Bahadur et al [17] which states that high heritability, accompanied by high genetic gain, was recorded for plant height, mother rhizome diameter, length of primary rhizome, length of primary rhizome, secondary rhizome, recovery from dry matter, curcumin content, and yield of rhizome.

3.2 Traits correlations
Correlation is an analysis of plant traits that do not pay attention to cause and effect factors. Correlation analysis aims to study the closeness of relationships between variables [18]. The correlation coefficient of rhizome weight is given in Table 4. Rhizome weight trait has a significant correlation with stem diameter, rhizome length, rhizome width, and rhizome thickness. Other components correlate with the rhizome weight but not significant. All morphological characteristics show a positive correlation, it is shown that the morphological traits of white turmeric interact with each other. Components of growth and yields from 12 white turmeric accessions were correlated with each other, although not all
components provide a significant correlation. The morphological characteristics of stem diameter, rhizome length, rhizome width, and rhizome thickness have a significant correlation with rhizome weight. The production of fresh rhizome per hectare is influenced by plant height, leaf area index, number of tillers per plant, number of leaves per plant, stem circumference, length of primary rhizomes, number of secondary rhizomes, rhizome length secondary, oleoresin content, percentage of curing, and essential oil content [19]. Correlation between traits is so important because it would enable the breeder to easily select important characters from the studied traits [20].

Table 4. Correlation coefficients among morphological traits.

|     | PH  | LL  | LWi | LT  | NoL | SD  | NoT | RWe | RL  | RWi | RT  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| PH  | 1   |     |     |     |     |     |     |     |     |     |     |
| LL  | 0.981** | 1  |     |     |     |     |     |     |     |     |     |
| LWi | 0.884** | 0.937** | 1  |     |     |     |     |     |     |     |     |
| LT  | 0.798** | 0.777** | 0.552 | 1  |     |     |     |     |     |     |     |
| NoL | 0.496 | 0.426 | 0.124 | 0.808** | 1  |     |     |     |     |     |     |
| SD  | 0.955** | 0.927** | 0.769** | 0.882** | 0.689* | 1  |     |     |     |     |     |
| NoT | 0.721** | 0.738** | 0.695* | 0.53 | 0.374 | 0.675* | 1  |     |     |     |     |
| RWe | 0.556 | 0.465 | 0.387 | 0.538 | 0.497 | 0.594* | 0.176 | 1  |     |     |     |
| RL  | 0.610¹ | 0.558 | 0.578¹ | 0.456 | 0.305 | 0.57 | 0.293 | 0.934** | 1  |     |     |
| RWi | 0.416 | 0.347 | 0.347 | 0.411 | 0.261 | 0.426 | 0.105 | 0.891** | 0.850** | 1  |     |
| RT  | 0.634* | 0.630¹ | 0.642¹ | 0.630¹ | 0.371 | 0.620¹ | 0.482 | 0.773** | 0.862** | 0.737** | 1  |

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

3.3 Cluster analysis

Cluster analysis was done to understand the relationship among the accessions of the white turmeric. Relationships between accessions can provide information about the characteristic traits of each group of accessions that are formed [21]. Twelve white turmeric accessions were clustered into four major groups, namely cluster I (1, 7, 8, 11, and 9), cluster II (2, 3, 10, and 12), cluster III (6), and cluster IV (4 and 5) (Figure 1). The biggest group is cluster I, it consists of 5 accessions, and the smallest one is cluster III that has only one accession. Clusters I was divided into two sub-groups, viz. subgroups I (1 and 7) and subgroups II (8, 9 and 11). These clusters established were not based on the locality origin of the white turmeric accession, but they were based on the morphological characteristics of the growth and the yield components. The yield component is a basis in the cluster analysis because it is based on the results of morphological variations, genetic diversity, and the correlation between morphological traits. This is consistent with the research findings of Dabalo et al. [22], according to which the clustering of 36 genotypes of linseed is not of genotype geographical origin but reflects morphological similarities between genotypes.

Principal Component Analysis (PCA) is performed to simplify the observational variables or parameters that influence plant morphology. The PCA results show that all variables can be described into 2 dimensions (Figure 2). The first dimension with a value of 64.2% and the second dimension with a value of 17.4%. The variable rhizome width, rhizome weight, rhizome length, and rhizome thickness were the first principal components influence plant morphology. Meanwhile, the variable plant height, leaf length, leaf width, number of leaves, leaf thickness, stem diameter, and number of tillers were the second principal components. The principal components of yield traits affected the white turmeric accession number 1 and 7. The second principal component of the growth traits affected the accessions of the white turmeric numbers 8 and 11.
The results of the PCA were in accordance with the results of the correlation among morphological traits analysis where positive and significant correlation occurred in the yield components. Therefore, it can be concluded that cluster 1 (1, 7, 8, 11, and 9) is superior better than another cluster because of morphological traits near in five accessions of white turmeric. Five accessions of white turmeric have the potential to be the parents to create superior variety. However, the selection of parents should avoid the level of relationship that is too close because it could increase the chance of gathering recessive genes and results in "deep cross depression" in the offspring [23]. Genetic diversity and superior in morphological characteristics provide breeders with opportunities or challenges to assemble new superior varieties.

4. Conclusion
Based on the results of this study, there were significant morphological variations among the twelve white turmeric accessions. The highest value of coefficient of variation is the rhizome weight trait with a value of 30.15% and the number of tillers traits with a value of 25.16%. The genetic diversity of the 12 accessions of white turmeric is low. This can be shown from the value of the genotypic coefficient of variation and phenotypic coefficient of variation for all observations under 25%. The correlation coefficients among morphological traits are shown by the rhizome weight traits which are significantly related to stem diameter, rhizome length, rhizome width, and rhizome thickness. The results of the cluster analysis showed that 12 white turmeric accessions were divided into 4 major groups is cluster I (1, 7, 8, 11, and 9), cluster II (2, 3, 10, and 12), cluster III (6), and cluster IV (4 and 5). Group 1 is formed based on similarities in yield traits, group 2 is formed based on growth traits, group 3 is based on character approaching yield character, and group 4 does not approach yield and growth character. This study has an important role for future breeding programs.

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