Genetic diversity of pigeon pea (*Cajanus cajan* (L.) Millsp.) based on molecular characterization using randomly amplified polymorphic DNA (RAPD) markers

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Abstract. Pigeon pea (*Cajanus cajan* (L.) Millsp.) is an annual leguminous crop (perennial) which has advantages over other local leguminous crops as drought resistant, hold collapsed and strong pods. The research on drought resistance plant is very important to adapt to climate change adverse impact to support food security. The potential of pigeon pie has not been supported by accurate data. To explore the potential of pigeon pea, it is necessary to record the important properties by characterization, one of which is molecular. Increasing genetic diversity can be done through mutation which widely used gamma ray for the induction. The purpose of this study was to identify the genetic diversity of pigeon pea of black, white and brown seeds type resulted by gamma-ray irradiation with a wavelength of 100, 200 and 300 grays using RAPD method. The experiment resulted 14 bands, 12 of them are polymorphic bands and 2 of them are monomorphic with size varied from 300bp to 1.3kbp. The dendrogram showed from 30 accessions are divided into two main clusters, B shows clear genetical divergence from other clusters and some others split randomly. The range of similarity coefficient is from 0.43 to 1.00

1. Introduction

Climate change becomes one of the problems which quite influence on agriculture activities. It is clearly visible to notice an Indonesian climate change cycle. It switches off rainy and dry which influence on plant metabolism growth process and development especially in photosynthesis, transpiration, and respiration speed that is determined by plant production level.

To anticipate the climate change, a plant that tolerant toward this condition become necessary to develop. Pigeon pea has several advantages compared other leguminous, such as tolerant toward dryness source, resistant to collapse, pod not easy to crack, and suitable for any kinds of soil. *C. cajan* can live in dry area condition because it can adapt to the long rooting system [1]. Therefore, Pigeon pea plant has the potential for being developed in dry areas and rather barren, that is an area that cannot grow well soy [2].

Pigeon pea (*Cajanus cajan* (L.) Millsp.) is plant that contains good nutrition sources for family (every 100 gram seed contain 57 – 59% of carbohydrate, 14 – 30% of protein, 1 -9% of fat, and high vitamin A, B complex, and C); can be created for veggie friends eat, such made for soybean cake or cooked with milk squeezed from coconut; all parts of plant (root, steam, leaf, flower, and seed) can be established as natural medicine for treating many kinds of disease; it also can use as wood fire; its
ability for binding nitrogen from air from root nodule that contain rhizobium that can help to repair the soil fertility: and often used for reforestation plant especially in dry climate area [3].

Pigeon pea potential commodities are not supported yet by an accurate data such as peanuts, soy, and etc, unlike Pigeon pea genotype. Therefore, digging abilities, it needs an urgent characteristic data collection by operating characterization. Its function was for qualifying the plant diversity that has in the field as morphology characteristic diversity, agronomy, physiology, as well as molecular markers.

Utilizing expected molecular markers can contribute a high accuracy character description and enough on individual genetic diversity appearance, both at the species level or as well as distant relatives. RAPD is one of the molecular techniques for employing certain markers to study genetic diversity. Its basis analysis is to utilize a PCR machine which capable of amplifying DNA sequences in vitro. This technique involves single primary attachment with random nucleotide sequences [4].

The RAPD method is based on a homologous DNA sequence with the oligonucleotide primer sequence [5]. It has been used for numerous applications in plant molecular genetics research despite having disadvantages of poor reproducibility and not generally being associated with gene regions [6]. With the simplest and fastest detection technology, have been successfully employed for the determination of intraspecies genetic diversity in several grain legumes [7]. These include Cajanus cajan [2,8], Vigna radiata [9], Phaseolus sp. [10], Glycine sp. [11], and Pisum sp. [12]. Genetic variability of pigeon pea has been studied using several genetic markers such as RAPD [13,14,15], Amplified Fragment Length Polymorphism (AFLP) [16], and Restriction Fragment Length Polymorphism (RFLP) [17,18].

The application of gamma-ray plant recovery has huge advantages in developing new mutant varieties. 64% of 1585 varieties which were released since 1985 have been developed by practicing gamma-ray [19]. The x-ray and gamma-ray induction mutation were the most utilization for developing mutant varieties [20]. The irradiation gamma ray manipulating was one of increasing alternation to escalate genetic varieties of C.cajan. Mutation enlarges the genetic diversity opportunity of plant population. The relative relation among accession can provide information about the special character of each accession formed group. The information of among accession relatives can be used in plant breeding for determining its potentials which can be developed more.

The purpose of this study was to identify the genetic diversity of pigeon pea of black, white, and brown seeds type which was resulted by gamma-ray irradiation with a wavelength of 100, 200 and 300 grays by using RAPD markers.

2. Materials and Methods
Research materials were C. cajan leaf sample. It consists of brown guided seed type, white, and black which was radiated by gamma light with long of wave 100, 200, and 300 grays (table 1). The sampling method was carried out in accordance with the DNA sampling guide in the field.

| Population | Accession Name | Population | Accession Name | Population | Accession Name |
|------------|----------------|------------|----------------|------------|----------------|
| Black      | H0             | White      | P0             | Brown      | C0             |
| Black      | H1.1           | White      | P1.1           | Brown      | C1.1           |
| Black      | H1.2           | White      | P1.2           | Brown      | C1.2           |
| Black      | H1.3           | White      | P1.3           | Brown      | C1.3           |
| Black      | H2.1           | White      | P2.1           | Brown      | C2.1           |
| Black      | H2.2           | White      | P2.2           | Brown      | C2.2           |
| Black      | H2.3           | White      | P2.3           | Brown      | C2.3           |
| Black      | H3.1           | White      | P3.1           | Brown      | C3.1           |
| Black      | H3.2           | White      | P3.2           | Brown      | C3.2           |
| Black      | H3.3           | White      | P3.3           | Brown      | C3.3           |
| Sum        | 10             |            | 10             |            | 10             |

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2.1 Extraction and DNA Isolation

Extraction and DNA Isolation genome *C. Cajan* were based CTAB method that modified. The quantity of every DNA isolation result was measured by Fluorometer (Shimadzu UV1201), while its quality looked on electrophoresis 1.0%. DNA extraction result which producing a good enough quantity and quality DNA, and was continued by Polymerase Chain Reaction (PCR).

2.2 PCR Optimization and DNA Amplification

DNA genome *C. Cajan* was amplified by using a random primer that consists of 6 bases (decamer) (Operon Tech). PCR Optimization was done for getting PCR optimal condition. Several variables such as primer concentration, concentration DNA mold, and primer adhesive were exploited for PCR learning and trial for getting an optimal PCR product. To choose the primer will be used on RAPD analysis, every population *C. Cajan* represented by one individual that took at random and amplified using 6 primers from Operon Tech. (OPA 4, OPA5, OPB 17, OPC1, OPD 3, and OPD 5). Primer gave ribbons accretive amplification and clearly also producing polymorphic DNA ribbons of which was taken to amplify all of *C. cajan* DNA sample. DNA amplify was done based on Williams method [4] that modified. Furthermore, PCR was accomplished on total volume 10 μl every PCR reaction which consists of 5 μl PCR mix Go Taq polymerase, 0.25 μl 100 μM primer (Sigma-prologue), 2.5 μl DNA sample (template), and 2.25 nuclease-free water.

The amplification DNA was operated using PCR equipment BIO-RAD system t100-Thermal Cycler. pre-denaturation did 94°C during 1 minute, then followed 45 cycles with temperature and time every cycle was denaturation on temperature 94°C during 30 seconds, annealing on temperature 37°C during 30 seconds, and elongation on 72°C during 1 minute 30 second, which followed by post elongation on 72°C during 7 minutes.

PCR DNA result then electrophoresed using 1.0 % (b/v) agarose that was added *florosafe* DNA stain as coloring, on TBE buffer (that consist from 0.45 M Tris-HCl pH 8, 0.45 M Boric acid, 20 mM EDTA) by voltage 100 volt during 45 minutes. The result was visualized by applying UV light.

2.3 Data Analysis

Base on data analysis which results by having or nothing DNA band. DNA profile ribbons were translated into Byner data utilizing provisions value 0 for nothing ribbons and 1 for having DNA ribbons on 1 position equal to a variety of *C. cajan* that compared. Analysis of colony (cluster analysis) done using program NTSYSpc (Numerical Taxonomy and Multivariate Analysis System) version 2.02i by method Unweight Pair Group Method Arithmetic (UPGMA) function SIMQUAL (Similarity Qualitative) [21]. Genetic similarity matric was counted based Dice coefficient.

3. Results and Discussion

3.1 Profile RAPD

The total DNA genome amplification results using two random primers (OPA 5 and OPB 17) at 30 accessions of *C. cajan* produced PCR outputs that could be read and scored, so the results could be analyzed (Fig. 1, 2, 3, 4, 5, 6). The 2 primers produced 87.5% of polymorphism with OPA 5 and 83.3% of polymorphism with OPB 17 (Table 3). The experiment resulted 14 bands, 12 of them were polymorphic bands and 2 of them were monomorphic. The band's size was varied from 300bp to 1.3kbp. This result was RAPD markers which used to have a high level of polymorphism (> 50% polymorphic bands). Present study obtained 85.7% polymorphic band with an average 7 band per primer which was comparable to earlier studies on pigeon pea using RAPD marker [8,22]. The band's number which was generated after DNA amplification with PCR was highly dependent on how the primer recognizes the complementary DNA sequence in the DNA template (DNA template) used [23].
3.2 Genetic Diversity
Genetic similarities was measured through data analysis on the 14 RAPD markers revealed varying degrees of genetic relatedness among black, white, and brown seeds type and the genetic diversity which resulted by gamma-ray irradiation with a wavelength of 100, 200, and 300 grays. Dice similarity coefficients were between the C. cajan accession from 0.89 to 0.99. The value of this inequality shows that changes were not large but can be distinguished [24].

![Amplification patterns of different pigeon pea produced brown seeds type accession by primer OPA 5](image1.png)

**Figure 1.** Amplification patterns of different pigeon pea produced brown seeds type accession by primer OPA 5

![Amplification patterns of different pigeon pea produced white seeds type accession by primer OPA 5](image2.png)

**Figure 2.** Amplification patterns of different pigeon pea produced white seeds type accession by primer OPA 5

![Amplification patterns of different pigeon pea produced black seeds type accession by primer OPA 5](image3.png)

**Figure 3.** Amplification patterns of different pigeon pea produced black seeds type accession by primer OPA 5
3.3 Analysis cluster between accession

The phylogenetic dendrogram results obtained using NTsys software pc 2.2 from 27 accessions of *C. cajan* was divided into two main groups. Distance similarity coefficients were achieve 0.43 to 1.00 by splitting into at random.

Cluster analysis of genetic similarity in 30 accessions of *C. cajan* shows accessions separation into two main clusters, namely (A) similarity coefficient 0.69 and (B) similarity coefficients of 0.43. A cluster consists of two sub-cluster, namely sub-cluster which consists of C (which consists of E and F). Cluster F split randomly. While the cluster D consists of C31 (fig 7).
It shows the genetic diversity exists between individuals and populations. The first prediction was polymorphic loci which used in the high polymorphism analysis that have been selected. Due to genetic mutations in gamma-ray irradiance with different wavelengths was resulting in considerable genetic diversity. The same was also reported by Fox [25] by using isozyme. The high rates effect of genetic inequality because of several things. The first prediction was that the polymorphic loci which were used in this analysis have been selected which has a high polymorphism.

![Figure 7. Dendrogram of 30 accession pigeon pea showing genetic similarity](image)

### Table 2. Random primer selected for RAPD analysis result of amplification

| Primers | Average size of the fragments amplified | Total number of bands amplified | Total number of monomorphic bands | Total number of polymorphic bands | Percentage of polymorphic bands |
|---------|--------------------------------------|--------------------------------|----------------------------------|----------------------------------|--------------------------------|
| OPA 5   | 300-1100                             | 8                              | 1                                | 7                                | 87.5                            |
| OPB17   | 325-1300                             | 6                              | 1                                | 5                                | 83.3                            |

4. Conclusion

Genetic observations *C. cajan* by using two RAPD primer shows that 30 accessions that were exploited in the DNA fragment research have 14 bands with the size of 300bp until 1.3 kb. From 14 bands, 12 of them were polymorphic bands and 2 of them were monomorphic. The two primers produced 87.5% of polymorphism with OPA 5 and 83.3% of polymorphism with OPB 17. Cluster analysis of genetic similarity in 30 accessions of *c. cajan* displays separation of accession into the two main cluster, one of them shows clear genetical divergence from other clusters and some others split randomly. Based on the results of these studies can be concluded that to anticipate the climate changes which required plant varieties that are tolerant to these conditions. Pigeon pea has advantages such as drought resistant. Induction mutations with x-rays and gamma rays were used for the mutant varieties development in escalating the genetic diversity which employed as plant breeding agents and to
produce more DNA markers that can be used to generate varieties that are tolerant to the climate change.

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