Relationship of some upland rice genotype after gamma irradiation

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Abstract. The objective of the research was to group local upland rice genotypes after being treated with gamma irradiation. The research materials were upland rice genotypes resulted from mutation of the second generation and two parents: Pae Loilo (K3D0) and Pae Pongasi (K2D0) Cultivars. The research was conducted at the Indonesian Sweetener and Fiber Crops Research Institute, Malang Regency, and used the augmented design method. Research data were analyzed with R Program. Eight hundred and seventy one genotypes were selected with the selection criteria were based on yields on the average parents added 1.5 standard deviation. Based on the selection, eighty genotypes were analyzed with cluster analyses. Nine observation variables were used to develop cluster dendrogram using average linked method. Genetic distance was measured by euclidean distance. The results of cluster dendrogram showed that tested genotypes were divided into eight groups. Group 1, 2, 7, and 8 each had one genotype, group 3 and 6 each had two genotypes, group 4 had 25 genotypes, and group 5 had 51 genotypes. Check genotype formed a separate group. Group 6 had the highest yield per plant of 126.11 gram, followed by groups 5 and 4 of 97.63 and 94.08 gram, respectively.

1. Introduction
Indonesia has 50 million hectares of dry land but only 35 million hectares that can be utilized for cultivation [1]. The problem of dry land is low soil fertility. Working with cassava plants showed that there was a yield loss of 6.2 tons ha⁻¹ due to low soil fertility [2]. One alternative solution to solve the problem of dry land is by cultivation of plants tolerant with the condition of the land, such as upland rice [3,4]. Upland rice (Oryza sativa L.) is rice cultivated on dry land. Upland rice has become an alternative in increasing rice production and has been widely consumed by the community. Upland rice has nutrient content [5] that is able to meet 63% of total energy sufficiency and 37% protein. Policies of Government of Indonesia through the increasing rice production [4] and also promote local food [6] as an alternative food.

The production of upland rice is still far below the production of wetland rice. Productivity of wetland rice was 4.79 tons ha⁻¹ [7], while upland rice was only 2.69 tons ha⁻¹. The low production of upland rice was due to the decrease of harvested area caused by land conversion, varied soil and climate conditions, high pest and disease rate, and not optimal application of cultivation technology, especially in the use of superior varieties. The most crucial problem faced by farmers in the cultivation of upland rice was the lack of availability of varieties and superior seeds [8]. In general, farmers cultivate local varieties which have good taste, tolerance to marginal land, resistant to several types of...
pests and diseases, require low input of fertilizer and easy and simple maintenance, although the production is still considered low.

Improvement of rice varieties continues to be done by researchers through plant breeding [4,5], both conventional and unconventional methods. Conventional assembly of varieties takes a long time and more than 5 years, more over by using a variety of varieties or parents that have desirable traits or the available population has low diversity for traits will be fixed. The method of mutation is one method to accelerate the assembly of new superior varieties. Induction of mutations is one way to increase plant diversity. Mutations are sudden and random changes in genetic material. The purpose of induction of mutation in plants is to create a population with a high level of genetic diversity [9], including the diversity of production supporting factors such as number of productive tillers, number of grains per panicle and 100 grain weight. Genetic diversity is very important in a plant breeding program to assemble a new superior variety [10]. New genotypes from mutation breeding can then be analyzed for their kinship relationship to facilitate further breeding programs [11].

Cluster analysis is useful to determine kinship and similarity between mutant genotypes. Genotypes in one group have a high degree of similarity, so if researchers want to select genotypes, they can choose one genotype per group depending on superiority of each group. Analyzing kinship relationships among local crops has been done, among others, on local rice in Central Java [12], local red rice from Central Kalimantan [13], taro [14]. However, similarity information between the parents and their derived mutants is still very limited. The cluster results will allow researchers to select which genotypes are re-selected, where the genotypes have almost the same characters, or close genetic distance, so one of them is selected for plant breeding further. This will improve research efficiency as it saves time, land and energy.

2. Materials and methods
The research materials were the second generation of upland rice mutants, *Pae Loilo* cultivar and *Pae Pongasi* cultivar as parent and check test, *Inpago 7* and *Inpago Unram* as check tests. The research design used was Augmented Design which was divided into 6 blocks. The number of mutant genotypes was 18 genotypes. Genotype that already existed on one block was not repeated in the next blocks except check tests. Each genotype was planted as many as 50 plants, numbered according to the genotype code of the parent and the sequence of plants in the row. Observations were done to several parameters such as plant high, the number of productive tillers, the length of panicle, harvest age, the number of empty grains, the number of full grain, the weight of 1000 grains, and grain number per clump.

Research data were analyzed with R Program. There were 871 genotypes analyzed for selection. Selection was done based on production above 1.5 standard deviation plus the average of both parents. Based on the results of the selection, 84 genotypes were analyzed by cluster analysis to determine the relationship between genotypes. Genetic distance between genotypes was tested by the euclidean distance method.

3. Results and discussion
The kinship relationship or similarity relationship can be used as a foundation for cross breeding to obtain new superior varieties. The crosses will be better if it done between cultivars of distant kinship [14]. Kinship relationships can also be used to streamline the selection process. Similar genotypes or close kinship relationships are assumed to have similar genotypes and phenotypes that can be selected as a further breeding material.

A total of 871 genotypes were analyzed for selection. Selection was based on production above 1.5 standard deviation plus the mean of both parents (*yield = ≥ 60.694 + (1.5 * 18.009)*). The selection resulted in 84 genotypes being analyzed to know the kinship relationship between upland rice genotypes.
Figure 1. Dendrogram of upland rice genotypes.

Dendrogram as a result of cluster analysis is shown in Figure 1. Similarity analysis of 84 mutant upland rice genotypes on quantitative characters resulted in the division of 8 genotype groups reflecting similarities within groups or subgroups and dissimilarities between groups or subgroups. *Inpago Unram* (check variety) has the highest genetic distance (> 7) compared to other genotypes. The high genetic distance indicates the greatest dissimilarity. Another check genotype, *Inpago 7*, has a higher similarity with SSJ 23.190-27 compared to other mutant genotypes. The genetic distance between the two genotypes namely *Inpago 7* is the only high-yielding varieties of upland red rice released by the Indonesian government in 2011. As with *Inpago 7*, all mutant genotypes and parent are an upland red rice, contain anthocyanins from the group sianidin [15].

There were nine genotypes of upland rice in Southeast Sulawesi (*Paebiu Tamalaki, Paebiu Kolopua, Pae Tanta Mohalo, Paebiu Tamalaki Mataiwoi, Paebiu Sitoro, Paebiu Tamalaki Pewutaa, Paebiu Kolopua Kosebu, Ranggo Hitam, and Hitam Lapedidi*) which had high anthocyanin content [16].

Group 1, 2, 7 and 8 consisted of one genotype (Figure 1; Table 1). This was due to the considerable dissimilarities between genotypes in each group with other group genotypes. *Inpago 7* (group 1) and *Inpago Unram* (group 2) genotypes were superior varieties of upland rice that served as comparative plants. The separation of *Inpago Unram* into a separate subgroup with the highest genetic distance was because the genotype had a distinct difference compared to other genotypes. *Inpago Unram* was the shortest plant (125.2474 cm) and the shortest harvest age (111.59 day) compared to the other 7 groups. Group 7 (SSJ23.190-27) had the highest total number of grains but with the highest grain of hollow (90,000 grains) compared to the other groups. Group 8 (SSJ31.6-27) was the highest plant (193.00 cm) with the longest harvest age (138.00 day), the least productive tiller (16.00 tillers).

The advantage of this genotype was the highest 100 grain weight (4.25 g) (Table 2). The two parents (K2D0 and K3D0) formed a separate group from mutant genotypes. The genetic distance was 3. This suggested that gamma irradiation successfully altered the production and production support characteristics of the mutant genotypes. This also indicated a fairly high incidence between the
genotypes of the parents and the mutants. In group 4, the highest dissimilarity was between genotype SSJ21.239-41 and genotype SSJ21.86-27. The group was subdivided into 2 subgroups i.e. 4 subgroup 1 with the highest dissimilarity between genotype SSJ21.239-41 and genotype SSJ21.86-8 and 4 subgroup 2 between genotype SSJ21.72-24 with genotype SSJ21.86-27. Subgroup 2 consisted of 2 sub-sub groups with a sufficiently large number of members (17 genotypes). The highest dissimilarity in sub-subgroup 1 occurred between the genotype SSJ21.239-41 and genotype SSJ21.86-8 and 4 subgroup 2 between genotype SSJ21.72-24 with genotype SSJ21.86-27. The group was subdivided into 2 subgroups i.e. 4 subgroup 1 consisting of one mutant genotype and 4 subgroup 2 consisting of 50 mutant genotypes. The highest dissimilarity occurred between genotype SSJ21.167-21 and genotype SSJ21.27-38. The highest dissimilarity in sub-subgroup 2 occurred between genotypes SSJ21.78-38 and genotype SSJ21.86-27.

| Group | Genotypes | Number of group members |
|-------|------------|-------------------------|
| 1     | Inpago 7   | 1                       |
| 2     | Inpago Unram | 1                     |
| 3     | K2D0; K3D0 | 2                       |
| 4     | SSJ21.239-41 SSJ21.81-9 SSJ21.81-10 SSJ21.86-10 SSJ21.86-8 SSJ21.72-24 SSJ23.190-11 SSJ23.190-7 SSJ21.167-21 SSJ21.81-18 SSJ21.167-42 SSJ21.185-17 SSJ21.239-40 SSJ21.27-14 SSJ21.239-4 SSJ21.27-38 SSJ21.27-38 SSJ21.239-3 SSJ21.78-45 SSJ21.27-60 SSJ21.185-45 SSJ21.167-46 SSJ21.185-32 SSJ21.86-27 | 25 |
| 5     | SSJ31.6-12 SSJ31.37-22 SSJ21.185-15 SSJ31.104-36 SSJ21.185-1 SSJ21.72-6 SSJ21.72-31 SSJ21.72-19 SSJ21.72-22 SSJ21.185-33 SSJ21.72-5 SSJ31.24-3 SSJ31.24-5 SSJ31.37-3 SSJ33.203-25 SSJ31.6-2 SSJ33.203-33 SSJ33.203-10 SSJ33.203-5 SSJ31.6-21 SSJ31.6-8 SSJ31.104-4 SSJ31.104-8 SSJ31.104-40 SSJ31.104-6 SSJ31.6-20 SSJ31.170-9 SSJ31.6-10 SSJ31.6-25 SSJ31.37-34 SSJ33.203-20 SSJ33.203-23 SSJ31.6-28 SSJ31.6-7 SSJ31.104-11 SSJ31.104-39 SSJ31.102-45 SSJ31.104-33 SSJ31.102-4 SSJ31.104-35 SSJ31.104-35 SSJ31.104-9 SSJ31.104-10 SSJ31.6-16 SSJ31.6-23 SSJ31.6-5 SSJ33.203-35 SSJ31.102-2 SSJ33.203-3 SSJ31.162-29 SSJ33.203-48 | 51 |
| 6     | SSJ21.185-35; SSJ21.72-11 | 2 |
| 7     | SSJ23.190-27 | 1 |
| 8     | SSJ31.6-27 | 1 |

Group 5 had the largest number of member namely 51 genotypes (Figure 1; Table 1). This group was divided into two subgroups namely 5 subgroup 1 consisting of one mutant genotype and 5 subgroup 2 consisting of 50 mutant genotypes. The highest dissimilarity occurred between genotype SSJ31.6-12 and genotype SSJ33.203-48. The highest similarity existed between the genotype SSJ31.104-10 and SSJ31.8-6. Group 6 consisted of 2 genotypes i.e. genotype SSJ21.185-35 and SSJ21.72-11, had the lowest number of empty grain (7.00 grains) and the highest number of filled out grains (248.00 grains). This caused the groups to have the highest yield (126.115 g clump\(^{-1}\)) (Table 2). This result was higher than 8 genotypes from crosses of upland rice with wetland rice obtained by that was 35.92 -61.61 g clump\(^{-1}\) [17].
Table 2. Characters of production and production support in each group

| No. Group | Harvest age (day) | Plant height (cm) | Productive tillers (tiller) | Number of filled out grains (grain) | Number of empty grains (grain) | Number of total grains (grain) | Long panicle (cm) | Yield (g clump^{-1}) | 100 grain weight (g) |
|-----------|-------------------|-------------------|----------------------------|-----------------------------------|-------------------------------|-------------------------------|-----------------|---------------------|---------------------|
| 1         | 131.2727          | 151.80            | 22.34667                   | 145.0865                          | 55.95870                     | 201.0452                     | 27.27           | 66.91               | 2.58                |
| 2         | 111.5918          | 125.25            | 24.85537                   | 130.2697                          | 17.38421                     | 147.6539                     | 22.90           | 66.72               | 2.49                |
| 3         | 137.7500          | 178.32            | 19.08606                   | 162.7965                          | 16.36257                     | 179.1590                     | 24.37           | 61.24               | 3.08                |
| 4         | 132.6000          | 184.29            | 21.32000                   | 208.7200                          | 11.56000                     | 220.2800                     | 27.45           | 94.08               | 2.99                |
| 5         | 136.0196          | 176.53            | 25.66667                   | 147.6275                          | 11.41176                     | 159.0392                     | 26.83           | 97.63               | 3.67                |
| 6         | 129.0000          | 188.50            | 27.00000                   | 248.0000                          | 7.50000                      | 255.5000                     | 29.78           | 126.11              | 2.83                |
| 7         | 132.0000          | 181.30            | 22.00000                   | 186.0000                          | 90.00000                     | 276.0000                     | 24.70           | 91.59               | 2.91                |
| 8         | 138.0000          | 193.00            | 16.00000                   | 197.0000                          | 13.00000                     | 210.0000                     | 31.90           | 88.42               | 4.25                |

4. Conclusions
The results of cluster dendrogram showed that tested genotypes were divided into eight groups. Group 1, 2, 7, and 8 each had 1 genotype, group 3 and 6 each had 2 genotypes, group 4 had 25 genotypes, and group 5 had 51 genotypes. Check genotypes formed a separate group. Group 6 had the highest yield per plant of 126.11 gram, followed by groups 5 and 4 of 97.63 and 94.08 gram, respectively.

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