Individual selection of roselle plant m₄ generation (*Hibiscus sabdariffa* L.) based on anthocyanin, vitamin C and water content

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Abstract. Mutation of a plant is included to be one of the most effective methods to create a high genetic diversity in plants. This study aimed to get the selected individuals of M₄ generation of Roselle (*Hibiscus sabdariffa* L.) in rows through gamma-ray irradiation based on the flower calyx production, the content of anthocyanin and vitamin C. This research used Roselle seed of Roselindo 2 variety (control) as many as 16 numbers selected selection. The study was conducted at the Agrotechnology field Universitas Sumatera Utara (± 25 m asl) starting from December 2018 until April 2019 with the germination date of December 7, 2018. The data obtained were tested using a t-test analysis and presented descriptively. Results showed that the observation of all the average dry weights of plants-1 did not show any significant effect between one and other plants (control and number of selected plants). The average wet weight of plant-1 showed a significant effect in plant numbers of R141 and R148 compared to the other sample numbers. Levels of plants-1 parameters of anthocyanin and vitamin C showed a significant effect between one plant to another in all plant numbers (control and number of selected plants).

1. Introduction

Indonesia is a tropical country that has a lot of plant diversity. Various kinds of plants can be used as food or medicine. One of the plants that can be used as medicinal ingredients is the red roselle plant (*Hibiscus sabdariffa* L.) [1]. This plant was first spread in India and then spread to Malaysia and Indonesia [2].

Red roselle is roselle which has red flower petals. This plant has a very high anti-oxidant content in its flower petals [3]. Anthocyanin is a natural pigment that gives red roselle petals steeping. Anthocyanins are also classified as flavonoid pigments which are generally soluble in water. Anthocyanin pigments are red, blue, violet and are usually found in flowers, fruits and vegetables [4].

Roselle flower farming has good prospects for development in Indonesia. This is evident from the existence of foreign market demand for dried roselle in 2007, especially Malaysia, which is around 15 tons per year. Actually, to meet this demand, Indonesia is only able to meet around 5 tons to 8 tons per year. Limitations of the superior character of roselle plants can be overcome by the use of breeding techniques [5].

The selection is expected to improve one or more of the desired characters. These characters are spread across several genotypes. To collect these characters or to bring out the desired character, high genetic diversity is needed, so that the selection is more effective. The expansion of genetic diversity is
needed to make the selection more effective. The most common expansion of genetic diversity is hybridization (crossing) and mutation [2].

Roselindo 2 is a variety derived from Jamaican/Roselle purple with genotype no.1596. The advantage of this Roselindo 2 variety is the anthocyanin content in flower petals which is 146.97 ppm and 203.3524 mg.100g-1 vitamin C [6]. Mutations are included in the most effective method to create a high genetic diversity in plants [7]. Change in mutations that occur in a plant is one of the breeding techniques used to improve the properties of plants [8].

The morphology of mutant plant populations is affected by genetic and environmental factors. The M₃ generation population is considered having a wider variety of characteristics. Variations obtained from each character in the M₃ generation affect plant growth and development. Variation of character differences between the two populations is thought to be a mutant, causing a significant effect compared to the average value of the control plant population character. The heritability of observed characters ranged from low to high. Characteristic selection for the next plant generation can use the diameter size of the canopy and the total number of fruits per plant [9].

The 600 Gy application showed the best effect in increasing the total number of fruits per plant compared to the control sample [10]. Moreover, mutation induction with LD50 value at 477.803 Gy and LD20 value at 408.422 Gy on gamma-ray radiation dose range 150-600 Gy influenced the morphological character performance of each population irradiation in M₁ generation [11]. Furthermore, research of [12] showed that 150-600 Gy gamma-ray irradiation affected the morphological character performance of each population in the M₂ generation. Then, in the study of [9] showed that the average value of plant height, plant calyx weight and canopy diameter in the mutant population at 150 Gy differed significantly when compared with the average value of the control population character.

Based on the description above, the writer was interested in conducting further research in order to get selected individuals based on the content of anthocyanin, vitamin C and water content in the M₄ generation of Roselle (Hibiscus sabdariffa L.).

2. Materials and methods

This research was conducted in Faculty of Agriculture, Universitas Sumatera Utara, Medan (± 32 meters above sea level), which started in December 2018 until April 2019 with a germination date on December 7, 2018.

The material used in this study was M₄ roselle seed from population of roselle seed M₃ [13], which was obtained from M₂ roselle seed [14], which was obtained from M₁ roselle seed [15], then analysed using t-test analysis, which was comparing the planting material Roselle seed varieties of Roselindo 2 (control) as many as 40 pcs with Roselle seed varieties of Roselindo 2 were given irradiation treatment with a dose of 450 Gy using Co60 radiation sources in the M₃ generation as many as 16 selected selection numbers, each of which consisted of 10 pcs. The next ingredient was compost, top soil, NPK 16:16:16, as much as 20 g. plants⁻¹, insecticides to control pests, fungicides to control fungi and other materials that support this research. The tools used in this study were hoes, machetes, ratters, gauges, rulers, plastic ropes, random samples, buckets, hand sprayers, analytical scales, slide callipers, cameras, envelopes and stationery.

3. Result and discussion

Average of plant⁻¹ calyx dry weight and wet weight along with the t count at 450 Gy irradiation dose of selected plants of M₄ generation at 21 MST can be seen in Table 1. Table 1 showed that the observations of all dry weight of petals plant⁻¹ did not show any difference between plants with each other in all plant numbers (control and number of selected plants). Table 1 showed that the observations of the wet weight average of plants⁻¹ showed a significant effect in plant numbers R141 and R148 compared to the fresh weight average of the calyx. plants⁻¹ in other numbers.
The result showed that the induction of mutations with LD50 and LD20 values at 477.803 Gy and 408.422 Gy, respectively, on gamma-ray radiation doses range from 150 to 600 Gy affected the morphological character performance of each population irradiation which is in accordance with statement of [11]. The difference could be seen from the average value of morphological characters compared to the population’s character without irradiation. The dose of 300 Gy gamma-ray radiation significantly increased the average value of total branches. While the radiation dose of 600 Gy did not significantly influence the average value of plant height, total branches, canopy diameter and did not differ significantly from the non-irradiated populations.

Variations derived from total branches and canopy diameter size affected the plant growth. Modification of branches, leaves and flower’s colours of roselle plants was occurred, which indicated a morphological modification. This condition developed due to irradiation [9]. The M₃ generation population was considered having a wider character diversity. Variations obtained from each character in the M₃ generation affected the growth and development of the plant. The difference in character diversity of two populations was thought to be a mutant, causing a significant effect compared to the control plant. The heritability of all characters observed ranged from low to high. Based on observations of the plant² calyx wet weight parameters, this was in accordance with the statement of [11] which stated that the induction of mutation with LD50 and LD20 value at 477.803 Gy and 408.422 Gy, respectively on gamma-ray radiation dose range of 150-600 Gy affected the morphological character performance of each irradiated population. The difference could be compared to the average value of morphological characters without irradiation.

Table 2 showed the composition of anthocyanin and water in plant² at 21 MST. From the result of the wet weight average of plant² calyx, it can be seen that there was a significant difference in plant

| No. | Plant Number | Calyx Dry Weight | Calyx Wet Weight |
|-----|--------------|------------------|------------------|
|     |              | Average | t-count | Average | t-count |
| 1   | R0 (Control) | 22.42 ± 6.68 | -     | 56.94 ± 9.87 | -     |
| 2   | R4           | 20.63 ± 4.32 | 1.04  | 61.45 ± 7.45 | -1.60 |
| 3   | R7           | 22.65 ± 2.85 | -0.16 | 56.90 ± 10.0 | 0.02  |
| 4   | R9           | 23.15 ± 2.73 | -0.54 | 54.28 ± 7.47 | 0.94  |
| 5   | R10          | 21.77 ± 4.77 | 0.34  | 60.20 ± 10.6 | -0.85 |
| 6   | R11          | 21.22 ± 2.99 | 0.71  | 56.11 ± 8.48 | 0.20  |
| 7   | R28          | 22.30 ± 1.97 | 0.1   | 59.77 ± 9.35 | -0.85 |
| 8   | R43          | 24.09 ± 4.73 | -0.91 | 56.10 ± 10.5 | 0.23  |
| 9   | R44          | 24.25 ± 2.68 | -1.29 | 52.87 ± 7.65a | 1.30 |
| 10  | R63          | 20.80 ± 6.93 | 0.61  | 58.09 ± 9.86 | -0.30 |
| 11  | R74          | 16.98 ± 8.69 | 1.58  | 52.21 ± 8.14 | 1.37  |
| 12  | R86          | 22.38 ± 3.89 | 0.03  | 53.42 ± 6.19 | 1.41  |
| 13  | R97          | 24.00 ± 4.05 | -0.92 | 58.51 ± 9.80 | -0.43 |
| 14  | R118         | 20.38 ± 7.79 | 0.65  | 55.00 ± 12.80 | 0.38 |
| 15  | R121         | 21.57 ± 4.24 | 0.48  | 57.20 ± 10.80 | -0.08 |
| 16  | R141         | 19.72 ± 6.30 | 1.20  | 49.03 ± 7.90* | 2.68 |
| 17  | R148         | 23.09 ± 3.80 | -0.42 | 75.58 ± 3.80** | -9.46 |

Note: * = significantly different with control population (0 Gy) at the level of 5% based on t-test ; ** = significantly different with control population (0 Gy) at the level of 1% based on t-test
numbers of R141 and R148 compared to the wet weight of plant\(^1\) calyx in other samples. Moreover, Table 2 also presented the results of anthocyanin and vitamin C content in plants\(^1\) at irradiation doses of 450 Gy from selected plants in the M\(_4\) generation at 21 MST descriptively. Based on Table 2, it can be seen that the anthocyanin and vitamin C content in plants\(^1\) were different significantly between one plant to another in all plant numbers (control and number of selected plants).

| No. | Plant Number | Anthocyanin Content Plant\(^1\) (ppm) | Vit. C Content Plant\(^1\) (mg.100g\(^{-1}\)) |
|-----|--------------|--------------------------------------|---------------------------------------------|
| 1   | Control (13) | 386.37                               | 142.66                                      |
| 2   | 4 (10)       | 124.60                                | 85.94                                       |
| 3   | 7 (5)        | 201.04                                | 85.09                                       |
| 4   | 9 (10)       | 194.76                                | 137.97                                      |
| 5   | 10 (4)       | 207.32                                | 114.76                                      |
| 6   | 11 (6)       | 271.20                                | 84.44                                       |
| 7   | 28 (4)       | 102.61                                | 57.09                                       |
| 8   | 43 (8)       | 172.77                                | 138.81                                      |
| 9   | 44 (10)      | 185.33                                | 110.83                                      |
| 10  | 63 (5)       | 241.88                                | 86.15                                       |
| 11  | 74 (7)       | 224.08                                | 86.12                                       |
| 12  | 86 (9)       | 346.59                                | 85.19                                       |
| 13  | 97 (8)       | 48.17                                 | 86.11                                       |
| 14  | 118 (6)      | 152.87                                | 143.22                                      |
| 15  | 121 (10)     | 208.37                                | 201.05                                      |
| 16  | 141 (4)      | 271.20                                | 82.64                                       |
| 17  | 148 (5)      | 283.76                                | 113.67                                      |

Results of anthocyanin and vitamin C content in plants\(^1\) from control plants and selected plant numbers were in accordance with the statement of [7] and also supported by [9]. Thus, there were variations in morphological and genetic character diversity in populations suspected of mutants in populations of 150 Gy and 450 Gy. The difference in character diversity was thought to be significant compared to the control plant population [population of Rosellindo variety 2]. Then, the diameter size of the canopy and the total number of fruits per plant can be used as a selection character for the next generation.

4. Conclusions
The average dry weights of plants\(^1\) did not show any significant effect between one plant to another in all plant numbers (control and number of selected plants). The average wet weight of plant\(^1\) showed a significant effect in plant numbers of R141 and R148 compared to the other sample numbers. Anthocyanin and vitamin C levels parameters of plants\(^1\) showed significant effect between one plant to another in all plant numbers (control and number of selected plants).
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