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Developing Somaclonal Variation of Bogor Taro to Expand the Diversity of Character

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Abstract

Taro (*Colocasia esculenta* (L.) Schott) is a monocot which is included in the Araceae Family, and one of the staple foods as a source of carbohydrate. In the framework of breeding, the diversity of traits is required, and the induction of somatic mutation is one of the technical options utilized. The aim of this study was to develop somatic asexual variation of botanic mites to expand the diversity of traits, leading to the production of new genotypes that were then propagated to meet the needs of quality seeds. Top bud culture of the botanic taro was induced by gamma ray irradiation at 0, 5, 10, 15 and 20 Gy. The results showed that γ-rays had an effect on the number of leaves, and had the greatest effect at 10 Gy dose, but had no effect on shoots. Compared with the parental, the induction of somatic clonal variation produced up to 51% diversity.

Keywords

Bogor Taro; Somaclonal variation; Gamma ray; Dendogram.

Introduction

Taro (*Colocasia esculenta* (L.) Schott) is a monocot which is included in the Araceae Family, and one of the staple foods as a source of carbohydrate. First domesticated in Southeast Asia afterwards spread to the worldwide and now is one of the important crops in Asia-Pacific, Africa, and Caribbean. Taro's biological resources with its variegated types in Indonesia have not been optimally used in food diversification program as a choice carbohydrate sources. This commodity potential has yet to be supported with decent data compared to other root crops such as cassava, yam, and potato. On the contrary of its potential and usages, based on the data of Bogor District (Moreno-Vazquez et al., 2014; Shooshtari et al., 2011).

Department of Agriculture, the productivity of taro in Bogor District is still low and the production has decreased in the last 5 years. This matter is due to the unoptimized breeding program thus not fulfilling the

high quality and variant seeds provision. Therefore, a superior seed development is required through the breeding program.

In the context of breeding program, either through selection or the assembly of superior varieties, a diversity of character is needed. One of the technologies that could be utilized to improve the genetic diversity of crops is through in vitro culture technology. The genetic diversity resulted from in vitro culture is known as diversity (variation) somaclonal (Yasin et al., 2017).

On the in vitro multiplication, what happened is a somatic mutation. A mutated cell when undergoing a cell division process will form a group of cell different from the origin cell (Tripathy et al., 2016). Crops resulted from mutated cells might form clones that differs from the parent.

Induced mutation through in vitro multiplication technique on several crops were proved efficient and could evoke somaclonal variation. Somaclonal variation can be caused by genetic diversity that existed in the explant. With the diversity induced through in vitro culture, the opportunity of obtaining a new superior genotype is wide open. On Bogor Taro crop, research concerning genetic diversity through the induction of somaclonal variation to obtain a crop with new genotype is yet to be available up until now.

Gamma ray irradiation can be utilized to induct mutation thus resulting genetic diversity which afterwards can be selected to obtain the desired mutant. A factor that affects the formation of a mutant is the amount of irradiation dosage. Irradiation dosage is measured in Gray units (Gy), 1 Gy is equivalent with 0,10 Krad which is 1 J energy per kilogram irradiation produced (Francischini et al., 2017).

The purpose of this research is improving Bogor taro through the induction of somaclonal variation to expand character diversity which leads to a new genotype that will be mass-produced to fulfill the need for superior Bogor taro seeds later on.

Materials and Methods

Research was conducted at Tissue Culture Laboratory and Greenhouse of the Universitas Nusa Bangsa (UNB), Bogor, West Java, Indonesia and Molecular Laboratory of Research Center for Bioresources and Biotechnology (RCBIO), Institut Pertanian Bogor, West Java, Indonesia from January to October 2013. Plant materials were obtained from a farmer in Ciapus, Bogor, whereas irradiations were done at National Nuclear Energy Agency of Indonesia (BATAN) Pasar Jumat, Jakarta, Indonesia.

Bogor taro explant that was used in the research is obtained from 7-8 months old Bogor taro crops. Surface sterilization was done to the tip of the shoot which was obtained by cutting and peeling midrib to the deepest part along with the surrounding roots about 3 cm width. After that the explant was washed with soap and placed under flowing water for 1 hour. Then surface sterilization was done with 10% chlorox and 2 drops of Tween 20 for 45 minutes and put inside laminar airflow cabinet. It was then washed with sterile distilled water twice and the midrib was then peeled until an approximately 3 mm apical bud is seen, afterwards dipped in 70% ethanol for 30 seconds, washed with sterile distilled water, then soaked in 5% chlorox for 5 minutes, washed with sterile distilled water for three times and later on planted.

The base medium used was MS with 30 g L^{-1} sucrose, 1 mg L^{-1} BAP growth regulator condensed with 8 g L^{-1} gelatin with pH of 5.7. The medium was sterilized in an autoclave for 20 minutes with a temperature of 121℃ and a pressure of 15 psi.

Gamma irradiation treatment were administered at a dose of 0, 5, 10, 15, and 20 Gy using Gamma Chamber 4000A irradiator (^{60}Co). Each treatment was repeated three times. After the irradiation, the culture was immediately moved to a new medium. To multiply buds, the explant was sub-cultured into the MS medium which has been added with 4 mg L^{-1} of BAP and 1 mg L^{-1} of IAA. Afterwards sub-cultures were done every 5 weeks, and explant growth was observed each week with leaf and bud numbers as the parameter. All cultures were incubated inside a room with a temperature of 25℃ and illumination from a 40 Watt fluorescent lights for 12 hours. Mutant selections were carried out to explants that showed alterations or differences from the parent plant. Explant growth data were analyzed using ANOVA and if there are differences a multiple Duncan range test is to be carried out. Somaclonal variations

were detected on DNA level using Amplified Fragment Length Polymorphism (AFLP) marker. The DNA of the chosen mutant was then isolated from the leaf for AFLP marker analyzed.

AFLP analysis using Vos et al. (1995) which has been modified on primary labeling. AFLP analysis consists of cutting the genome of DNA and ligation with adaptor, pre-amplification, selective amplification, amplification result visualization and data analysis. The genome of the DNA was cut with *Pst*I and *Mse*I enzyme. Ligation process were carried out using *Pst*I adaptor (CTGCAG) and *Mse*I (TTAA). Said restriction reaction and ligation resulted in diluted *RL*. Pre-amplification was carried with mixing RL result with primary P00 (*Pst*I) and primary M02 (*Mse*I). Every said mixture were amplified using PCR *PTC-100™ MJ Research* tool. Selective amplification was carried out using two primary combination, which was P00+AA (P11) as the forward primary and M02+AC (M48) and M02+AG (M49) as the reverse primary. P11 primary was labeled IRD 700 as Ultra Violet marker. Electrophoresis was then done for approx. 3 hours with a power of 40 watt and a voltage of 1500 volt. Electrophoresis result was then immediately visualized through computer monitor that is connected to the LI-COR 4300 DNA Analyzer machine.

Data analysis from AFLP result was carried out using Numerical Taxonomy and Multivariate Analysis System (NTSYS) ver.2.02 program. Fragments resulted from the AFLP analysis that appear as DNA fragments were translated into binary data based on the existence of the shared fragments within the analyzed individual. Said binary data is then used to compile genetic similarity matrix and dendogram similarity using Unweighted Pair Group Method Arithmetic (UPGMA) method and simple matching coefficient, the clustering using Sequential Agglomerative Hierarchal Nested Cluster Analysis (SAHN) from SIMQUAL NTSY ver. 2.02 program.

Results and Discussion

During the First week after irradiation, all cultures appear to be in a healthy condition. All shoots appear green and leaves were seen on several cultures. During the second week, cultures with 15 Gy and 20 Gy treatments underwent browning process (Figure 1.A; Figure 1.C). Whilst cultures with 0, 5, and 10 Gy treatments were showing healthy signs of growth (Figure 1.B), one culture with a 10 Gy treatment even appeared to have its root growing (Figure 1.C) and culture with a 20 Gy treatment haven't survived. Through the 20th week, observational data showed that gamma ray irradiation are giving significant effects on the parameter number of leaves but not on the bud numbers (Figure 1.D).

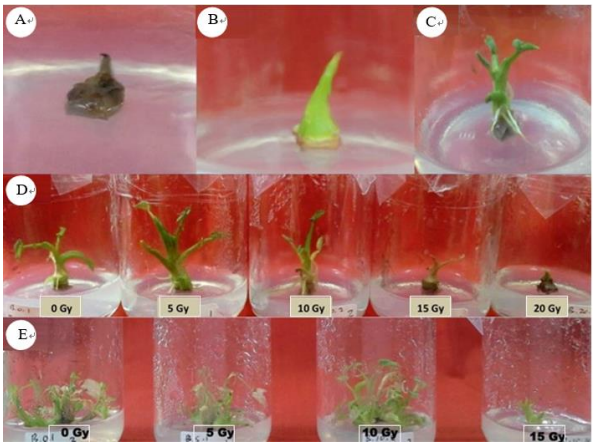

Figure 1: Bogor taro apical bud cultures irradiated with gamma ray dose of 0-20 Gy growth: (A) browning condition; (B) healthy condition; (C) cultured have its root growing; (D) cultures during 5th week; (D) cultures during 20th week.

Observations on numbers of leaf growth showed that during the 5th through 10th week most cultures with 5 Gy treatments have the highest number of leaves, whereas during the 15th week through 20th week cultures that have the highest number of leaves are cultures with 10 Gy treatments. Based on the observations, it can be concluded that the dose of 10 Gy is an effective mutation dose on numbers of leaf growth (Figure 2). Irradiation Treatments at 15 Gy and 20 Gy appear to have given strong browning effect to Bogor Taro explants. Several culture samples with the dose of 15 Gy could not grow well, several died even after undergoing several times of subculture. Almost all cultures with 20 Gy treatment underwent browning process and all samples eventually died on the third week. Therefore it seems that the dose of 20 Gy is a lethal dose. These results are parallel with a research

done by Seetohul et al. (2008) on *Colocasia esculenta var esculenta* that showed 20 Gy is a lethal dose and effective mutation at 7.65 Gy. This shows that mutation dosage effectiveness are dependent on genotype, explant type, and explant's orientation inside a medium of the parent source.

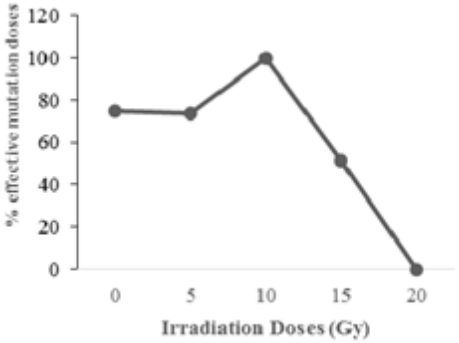

Figure 2: Effective mutation dose on the average number of leaves after irradiation for 20 weeks

Growth observations are also showing appearance of root growth. Cultures with 10 Gy treatments show the quickest root growth, which was on the second week after irradiation, parallel with the highest number of leaves at that dose. Whilst treatments on 0, 5 and 15 Gy average root growth occurred during sixth and seventh week after irradiation. These results seem to be related with several physiology reciprocal relationship processes in roots and leaves that affect absorption of mineral salts and water by root.

Gamma ray irradiation showed changes in phenotype on Bogor taro cultures at a dose of 10 Gy and 15 Gy in comparison to the non-irradiated ones (0 Gy). At a dose of 10 Gy there were 1 mutant with 2 suckers and 1 mutant at a dose of 15 Gy with curled leaves and midrib (Figure 3). Whilst Malamug et al. (1994) reported that gamma irradiation of shoot tips in vitro showed considerable variability within the first vegetative generation (V_1) including increased number of lateral shoots, flowering behavior, increase and decrease in plant height, presence and absence of corms. Gamma rays are ionizing rays that react with atoms and molecules present inside the cells to produce free radicals that causes damage or modification of components in plants, effecting morphology, physiology, anatomy, and biochemistry of plants.

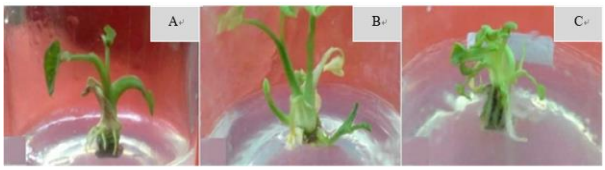

Figure 3: Phenotype changes of Bogor taro apical bud culture irradiated with gamma ray dose of 10 Gy (B) and 15 Gy (C) compared with no irradiation (A).

AFLP analysis were carried out to chosen mutant DNA that had been isolated using two primary selective combination (P11-700/M48 and P11-700/M49) and resulting in DNA fragment between <50 and >700 bp. Analysis were only carried to fragment of 50-255 bp (Figure 4) due to fragments below 50 bp being very tight and dense causing difficulty differentiating one fragment and another, whereas fragments above 255 bp can be vaguely seen. Total fragments resulted from utilizing two primary combination towards 13 Bogor taro samples were 235 fragments. DNA fragments that formed from the result of amplification are considered to be a character that represents one locus. All DNA fragments with the same migration rate are assumed as homolog locus. If one fragment is considered as one character, then a big amount of character could be analyzed through AFLP technique (Vos et al. 1995).

Analysis using quantitative similarity with simple matrix coefficient and clustering using Sequential Agglomerative Hierarchical Nested Analysis (SAHN) with UPGMA method resulting in dendogram with a similarity coefficient of 0.49-0.81 (Figure 5). At a similarity coefficient of 0.57 two clusters were formed, which was cluster I consisting of 11 samples including parent samples and cluster II consisting of 2 samples. Cluster I was divided into two sub-cluster at a similarity coefficient of 0.682, which was sub-cluster A consisting of 4 samples that have been irradiated with 0 Gy (B013), 5 Gy (B542 and B521), and 15 Gy (B1542a) gamma ray; and sub-cluster B consisting of parent samples and 6 samples that have been irradiated with 0 Gy (B012 and B021), 5 Gy (B511), 10 Gy (B1022 and B1023), and 15 Gy (B1542) gamma ray. Whereas sub-cluster II was only consisted of 2 samples that have been irradiated with 10 Gy (B1041) and 15 Gy (B1511) gamma ray.

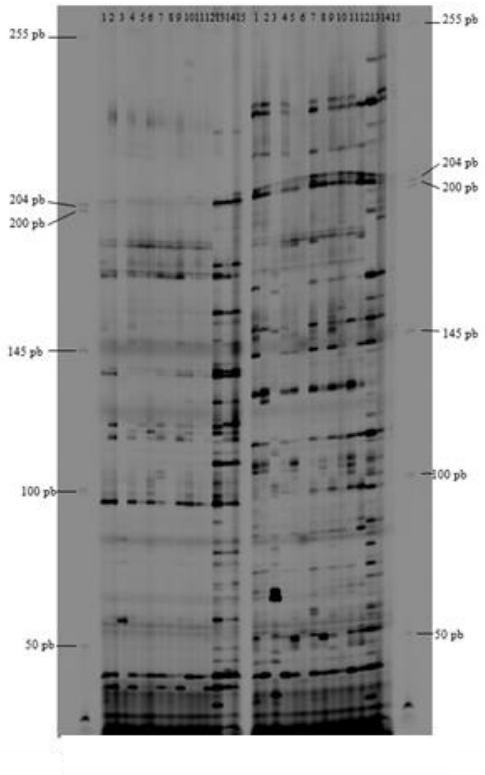

Figure 4: AFLP Analysis using two primary selective combination on parent (1,2,3) and gamma ray irradiation treatment 0 Gy (4,5,6); 5 Gy (7,8,9); 10 Gy (10,11,12); and 15 Gy (13,14,15). M = marker 50-255 bp.

The dendogram also shows that gamma ray irradiation could result in random changes for every given dose and the higher the dose is given, the higher the genetic change (diversity) happened to the Bogor taro apical bud culture sample. According to the dendogram on this research, it is seen that induction of somaclonal variation of Bogor taro with gamma ray irradiation yields diversity up to 51% in comparison to the parents. The result is differ from Malamug et al. (1994) that observed 12% rate variation of taro with gamma irradiated evaluated in morphological level. AFLP analysis is also used to analyze the geographical differentiation, phylogenetic relationships and distinguish Indian taro cultivars by their unique and different banding patterns.

Phenotype changes which can be already seen upon micropropagation is on sample B1041 which is the forming of 2 sucker (Figure 3) and said sample is at sub-cluster II which had been separated from its parent in sub-cluster I, whereas on sample B1542 which showed curled leaves and midrib phenotype (Figure 3) while being in sub-cluster I together with its parent plant, yet it separated on sub-cluster B.

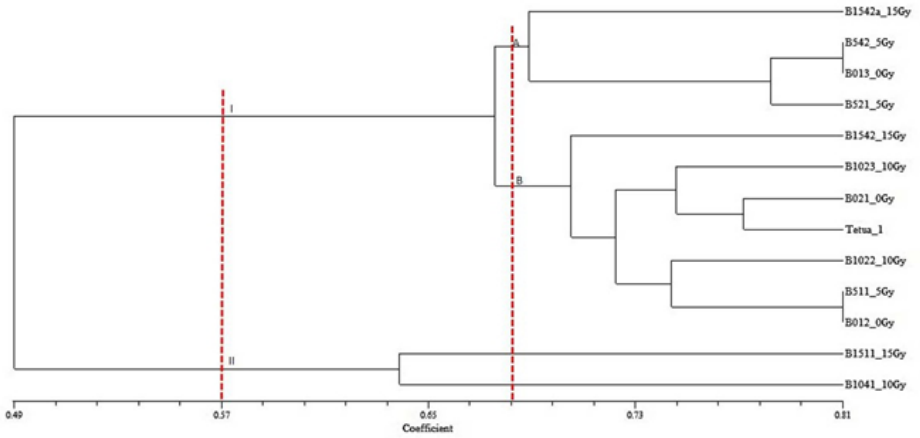

Figure 5: Dendogram of 13 samples of Bogor taro based on 235 locus of AFLP data.

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