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Genetic Diversity and Population Structure Analysis of Potato Somaclones

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ABSTRACT

Cultivated potato varieties are tetraploid and vegetatively propagated. Therefore genetic improvement for new traits is less effective through hybridization. The mutation is considered an alternative method for crop improvement of those varieties. This study aimed to evaluate the effects of gamma-ray irradiation on increasing genetic diversity among the somaclones and the changes in population structure. Forty-four somaclones were used as observed materials. The population was grown in a screen house using a completely randomized design with genotype (somaclone) as a single factor. Thirteen qualitatively and quantitatively morphological characters were observed. Six SSR markers were used for analyzing the population structure. The selection process was based on a weighting method divided into quartiles. Selected numbers were taken from quartile 3. Morphological analysis of stems and leaves resulted in five different somaclones, with significant differences in anthocyanin appearance and intensity of the green color of the leaflets. Diversity criteria based on quantitative characteristics showed a high level of diversity with a high heritability for tuber weight, length, and diameter and a moderate heritability for tuber number. Analyzing the population structure has offered insight into how gamma irradiation affected the somaclones. As a result of the selection, 12 somaclones met the requirements to serve as seed sources for field testing. It is expected that the results of this study will provide information about the diversity caused by gamma-ray irradiation treatment on potato somaclones and a method for improving the efficiency of the initial selection of potato populations.

1. Introduction

Potatoes have a basic chromosome number of n = 12. The majority of species are diploid (about 73%), while others are triploid (4%), tetraploid (15%), pentaploid (2%), and hexaploid (6%) (Kumari *et al.* 2018). Cultivated potatoes are usually auto-tetraploid (2n = 4X = 48) (Machida-Hirano 2015). The auto-tetraploid nature of potato plants causes sterility due to interference with the meiotic process (Suryo 1995) and rapid inbreeding depression (Ghislain and Douches 2020). Therefore, mostly cultivated potato plants are propagated vegetatively. As a result of this propagation system, the genetic diversity of potato plants is estimated to be narrowing, and it is rarely found that potato plants grow wild (Yulita *et al.* 2014). To improve the traits of vegetatively propagated plants, mutation induction is highly recommended to increase genetic diversity (Zia *et al.* 2018).

Mutations are also more efficiently used for polygenous traits than ordinary crosses because, in tetraploid plants, the inheritance pattern of a trait is more complicated than in diploid (Muthoni *et al.* 2015). Mutations can modify alleles, and their variations are not present in the germplasm pool, so they can create new genetic diversity if the genetic resources do not have the desired characteristics or

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are unsuitable for breeding purposes. The breeding time required is shorter than the conventional method because if the desired trait is detected in the M2 generation, purification of the mutant only requires 1 or 2 generations, and the resulting variety can be used because it does not require special procedures to be commercialized (Shu *et al.* 2011). This method is considered the most suitable for breeding vegetatively propagated plants such as potatoes because the mutant and original varieties have the same genetic background except for the mutated gene. Therefore, the mutant variety can be cultivated under the same environmental conditions as the original (Suprasanna and Nakagama 2011).

The use of gamma-ray irradiation to increase genetic diversity has been tested on several vegetatively propagated plants such as sweet potato (Shin *et al.* 2011), sugarcane (Suhesti *et al.* 2015), and pomelo (Mariana *et al.* 2018). Chepkoech *et al.* (2022) performed gamma-ray irradiation on three potato varieties of micro tubers. They obtained variations in the number of stems, quantity, and weight of tubers at doses of 20 and 30 gray. Gamma-ray irradiation has also produced mutants with a shelf life of 20 days longer than the control, increasing the tubers sugar content but decreasing the starch content. The phenol content in irradiated potatoes also increased, thereby increasing the resistance of potato tubers to soft rot bacteria (El-Ghany *et al.* 2017). These studies show that mutation induction by gamma-ray irradiation can cause genetic variation, so that selection can be made to obtain the desired mutant based on needs and preferences and further assist breeders in developing appropriate breeding strategies to describe potato production constraints.

The utilization of gamma-ray irradiation is frequently analyzed with respect to particular factors, but it fails to address the resultant modifications and their impact on the structure of a population. Population structure can be constructed using SSR markers because these markers are randomly distributed in the genome, have high clarity and reproducibility, have low operational cost, and can be done on low-quality DNA (Wang *et al.* 2019). SSR is a molecular marker commonly used to detect genetic diversity in any species (Islam *et al.* 2018; Trkulja *et al.* 2019; Wang *et al.* 2019; Lee *et al.* 2021; Saikia *et al.* 2021). This article discusses the effects of gamma-ray irradiation on plant morphology and the population structure of potato somaclones. Furthermore, the selection procedure that can be carried out in the initial generation (G0) is also discussed in the study to shorten the selection time. The following selection process in the field does not require too large land and can be carried out more effectively and efficiently.

2. Materials and Methods

2.1. Genetic Material

A population of somaclones was developed based on the Repita variety. The Repita variety resists late blight (Nugroho *et al.* 2015), but its productivity is still low. Stem cuttings obtained from 4 weeks old plantlets were used as material and exposed to gamma-ray irradiation with doses of 10, 20, 30, 40, and 50 grays (Munawaroh *et al.* 2018). Irradiated plantlets were maintained *in vitro* and subcultured 6 times (M1V6) to eliminate chimera. The plantlets were then acclimatized in the screen house. The planting materials used were rooted cuttings derived from plantlets that had been acclimatized. Thus, observation has been conducted at G0. Individual plants with good vigor were used as experiment materials with 45 somaclones, including the Repita variety not exposed to gamma-ray irradiation as the control (wild type).

2.2. Morphological Observation

The population was planted in a screen house at experimental station Margahayu, belonging to Indonesian Agency for Agricultural Research and Development located in Lembang, West Java (1,200 masl), in March-September 2020. The experiment used a completely randomized design (CRD) in a single factor, namely genotype (somaclone), with four replications where each replication consisted of three plants. The planting medium used was a mixture of husk charcoal and horse manure in a ratio of 2:1, which had been previously sterilized by steaming. Plants are maintained under optimal cultivation methods for potato growth (Diwa *et al.* 2015).

Morphological observations were carried out in the vegetative phase (45 Days After Planting/DAP) and the generative phase (90 DAP), using DUS Test Guidelines of Potato no. 13/3 (Pusat PVPPP 2018). Morphology observation on the vegetative stage focused on the stems and leaves. Phenotype characterization was conducted visually and presented in the form of descriptive narrative texts. The similarity analysis between the evaluated somaclones based on agro-morphological characters

was carried out by calculating the coefficient of genetic dissimilarity using the Gower method and visualizing the dendrogram using the average linkage method by PBSTAT-CL 2.1.1 (www.pbstat.com).

2.3. Analysis of Population Structure Based on SSR Markers

Sixteen SSR markers were tested for their polymorphism level, and six polymorphic markers were selected with the base arrangement presented in Table 1 (Bakker *et al.* 2011). DNA was isolated using the method of Doyle and Doyle (1990) with modifications to the use of liquid nitrogen and the composition of the buffer (2% CTAB, 5M NaCl, 1M Tris-HCl, 0.5M EDTA). The extraction results were quantified using the NanoDrop®ND-100 Spectrophotometer. PCR analysis was performed for each sample with a total reaction volume of 10 µL containing 2 µL of DNA, 1 µL of forward and reverse primers, 2 µL of ddH_2O, and 5 µL of Taq DNA polymerase (Bioline). PCR amplification was performed on Bio-Rad thermal cycler using the following steps: a 5-min denaturation step at 94°C was followed by 35 cycles of 1-min denaturation at 94°C, 1-min annealing at 55°C, and 2-min extension at 72°C. The PCR cycle was terminated with a 10-min final extension at 72°C and incubated at 10°C for 15 minutes. PCR products were separated on 3% agarose gel in 0.5xTBE. Gels were stained with ethidium bromide, then documented with a Chemi-doc transluminator.

The bands scored based on the band size detected by GelAnalyzer 2010a. Structure v2.3.4 program (Pritchard *et al.* 2000) was used for population structure analysis. Twenty independent simulations were carried out for each K (number of population), with the number of K being 1-10. Each simulation was calculated through burn-in analysis of 100,000 iterations and 100,000 repetitions of Markov Chain Monte Carlo (MCMC) (Gilbert *et al.* 2012). The optimal K value was estimated using the Structure Harvester (Earl and vonHoldt 2012). Membership coefficient q>0.8 was used to assign samples to groups. Samples in the group with a membership coefficient of q≤0.8 were considered genetically mixed (Lee *et al.* 2021).

2.4. Estimation of Genetic Parameters and Selection

Analysis of variance for quantitative characters was done using the SAS 9.1 program. Components of variance included genetic, environmental, and phenotypic variance and their heritability, which was calculated using the formula according to Singh and Chaudary (1979). The standard deviation of genetic variance ($\sigma_{\sigma}^{2}{}_{g}$) was calculated using the Anderson and Bancroft (1952) formula. The genetic diversity value of a character was determined based on the genetic variance (σ^2_g) and the standard deviation of the genetic variance ($\sigma_{\sigma}^{2}{}_{g}$). If $\sigma^2_g > 2\sigma_{\sigma}^{2}{}_{g}$, then the genetic diversity was broad, while if $\sigma^2_g \leq 2\sigma_{\sigma}^{2}{}_{g}$, then the genetic diversity was narrow (Pinaria 1995). The estimation of the overall heritability value (h_{bs}^2) was according to Hallauer and Miranda (1995). In this context, the criteria, according to Stanfield (1991), were high if h_{bs}^2>50, moderate if 20 ≤ h_{bs}^2≤50, and low if h_{bs}^2< 20.

The selection was made through the weighting method by sorting the characters considered more important to gain the highest weight value. In this study, the character of the number of tubers had a value of 4, followed by a tuber weight of 3, and both length and diameter had the same value of 2. The number of tubers was considered the most important because the G0 generation is seedling. Genotypes with lots of tubers will be candidates for testing in further generations. Tuber weight and tuber size (length and diameter) were the next important characteristics related to the seed tuber size desired by farmers. The total number of weights was then divided into quartiles using Ms. Excel. The somaclones included in quartile 3 were determined as the selected somaclones. The somaclones in

Table 1. Primary base arrangement of SSR markers used in research

Marker	Sequences		Annealing (°C)
	F	R	
SSR35	GCCAGACAGCAGATGAAAGC	CCTTCAAGAATTGCAGAAACAG	55
SSR16	CATCTGCTTGAATGATTATCGC	CTCTTCCTCGTTCCTTTCCC	55
SSR50	TCATTTACGTGATACTTTGGGC	ATCACCTGGGTCGGATCTAC	55
SSR13	TCCGTTGGTCTATCGGAAAC	TCCATGGGGTATTTGCTACC	55
SSR4	GGGTCGATGATCCATTTATTG	CCCTTTTGTTCCATATCAGTTG	55
SSR3	AGTTGTCCTGATATTGGCGG	TTTTGAGTTTGTCCCCTTCC	55

quartile 3 have a higher value than the population means, indicating an advantage in the observed characters compared to other genotypes.

3. Results

3.1. Phenotypic Diversity of the Somaclone Population

Morphological variations appeared on the character of anthocyanin coloration on the stem, pubescence on the upper side of leaflets, and leaf color (Table 2). Those observations obtained 5 somaclones from 44 somaclones tested (9.09%), with different characters from the wild type. Somaclone R40 and R41 showed the same morphological variations; the most prominent was the appearance of anthocyanins on the stems and lower side of the leaflets (Figure 1A). The second group of variations was R49 and R76, which were pubescence on the upper side of leaflets and were less frequent than wild-type with an uneven green leaf color (Figure 1B). Meanwhile, in somaclone R10, variation was seen in the pubescence on the upper side of leaflets and wavy leaf edges (Figure 1C). Morphological observations in the generative phase were carried out on tuber characters. In general, the character of the tuber did not change. The difference was seen in the uniformity of tuber size, where the somaclone population was more varied than the wild type (Figure 1D).

The qualitative and quantitative data were then processed using cluster analysis, resulting in three groups (Figure 2). Most of the somaclones clustered in cluster I with 30 somaclones, 13 somaclones were clustered in cluster 2, and cluster 3 only consisted of two somaclones, namely R40 and R41. Somaclones in cluster 3 had significant differences, mainly due to qualitative characters that appeared different from the wild type. The most similar somaclone to the wild type was R75, with a coefficient of dissimilarity of 0.096, where this value indicated the least genetic change in the somaclone. The largest genetic change due to irradiation was found in somaclone R40, with a dissimilarity value of 0.386, or about 38% of the wild type based on the observed characters.

Groups I and II had similar qualitative characteristics but differed in quantitative characteristics. The wild-type R0 was clustered with 29 somaclones from different radiation doses. This group had a higher average tuber length, tuber diameter, tuber weight, and number of tubers than group II, which were 21.13 mm, 20.67 mm, 8.68 g, and 15.37, respectively. Group II was 14.96 mm, 14.15 mm, 2.84 g, and 9.03.

3.2. Grouping Analysis Based on Population Structure

Population structure analysis was conducted to determine the pattern of genetic structure based on SSR markers. The analysis results show that the optimal population structure was obtained at K=3 based on the value of the K delta (Figure 3). This value indicates that the mutant population can be genetically grouped into three subpopulations. Subpopulation I and III consisted of 17 somaclones; meanwhile, subpopulation II consisted of 11 somaclones. One somaclone from group I and two somaclones from group II were classified as mixed somaclones because they had membership probability <0.8, namely R13, R76, and R58. Further investigation showed the fact that the grouping of somaclones based on population structure was related to the treatment of gamma-ray irradiation dose, where group I consisted of somaclones in which the majority received a dose of 10 grays, group II received a dose of 20 gray and group III received a dose of 40-50 gray (Figure 3).

Table 2. Variation of morphological characters in the vegetative phase

Accession	ACS	OL	PSL	IGL	ACM	PUL	FC	LC
R0 (wild-type)	absent	open	very weak	weak	absent	medium	absent	green
R10	absent	open	very weak	weak	absent	sparse	absent	green
R40	medium	open	very weak	very weak-weak	absent	dense	absent	green (upper), purple (lower)
R41	medium	open	very weak	very weak-weak	absent	dense	absent	green (upper), purple (lower)
R49	absent	open	very weak	very weak-weak	absent	sparse	absent	light green
R76	absent	open	very weak	very weak-weak	absent	sparse	absent	light green

ACS = anthocyanin coloration on stem, OL = openness of leaflets, PSL = presence of secondary leaflets, IGL = intensity of green color of leaflets, ACM = anthocyanin coloration on midrib, PUL = pubescence on the upper side of leaflets, FC = frequency of coalescence, LC = leaf color

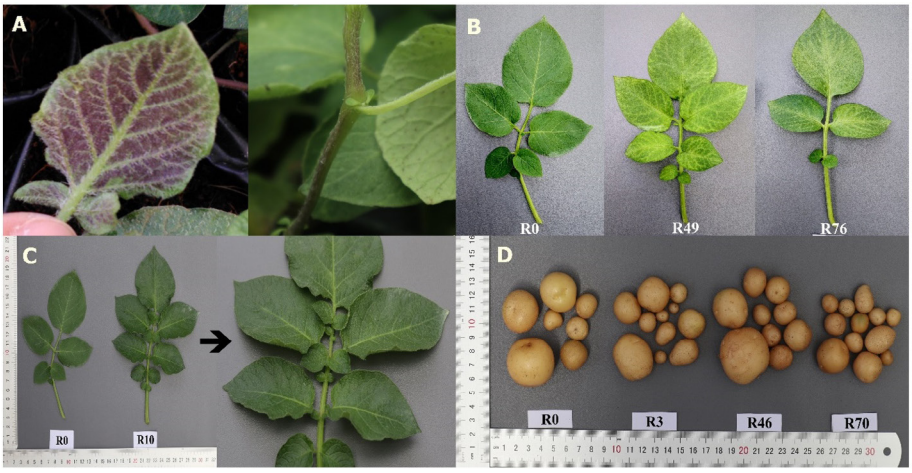

Figure 1. Morphological variation of mutant population versus wild-type R0; Changes in leaf size, the green color intensity of the upper side of the leaflets, the presence of anthocyanins on the lower side of leaflets and stems in somaclones R40 and R41 (A); variation of leaf color in somaclones R49 and R76 (B); leaf margins waviness in accession R10 (C); variation in tuber size uniformity (D)

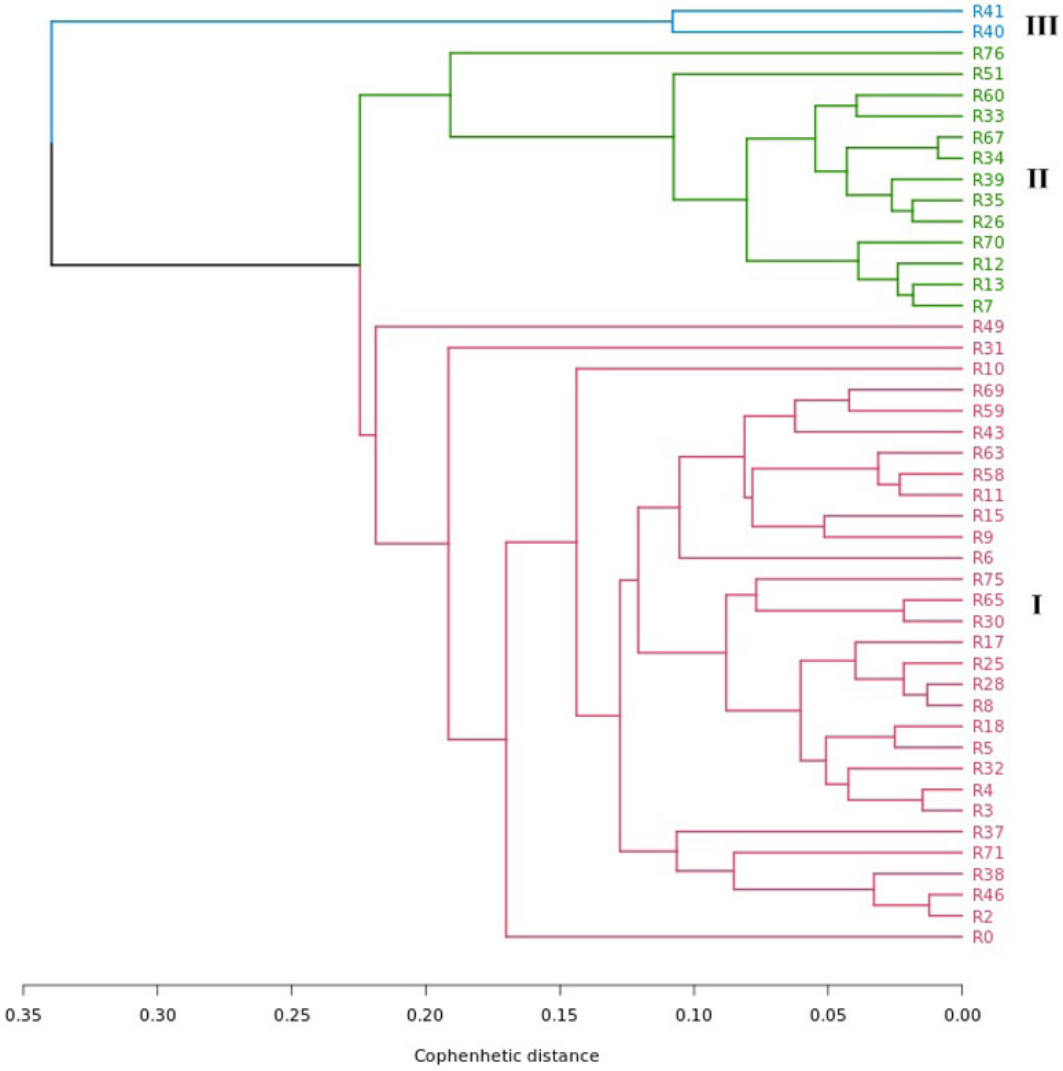

Figure 2. Genetic distance dendrogram of 44 somaclones of Repita mutants with wild-type R0

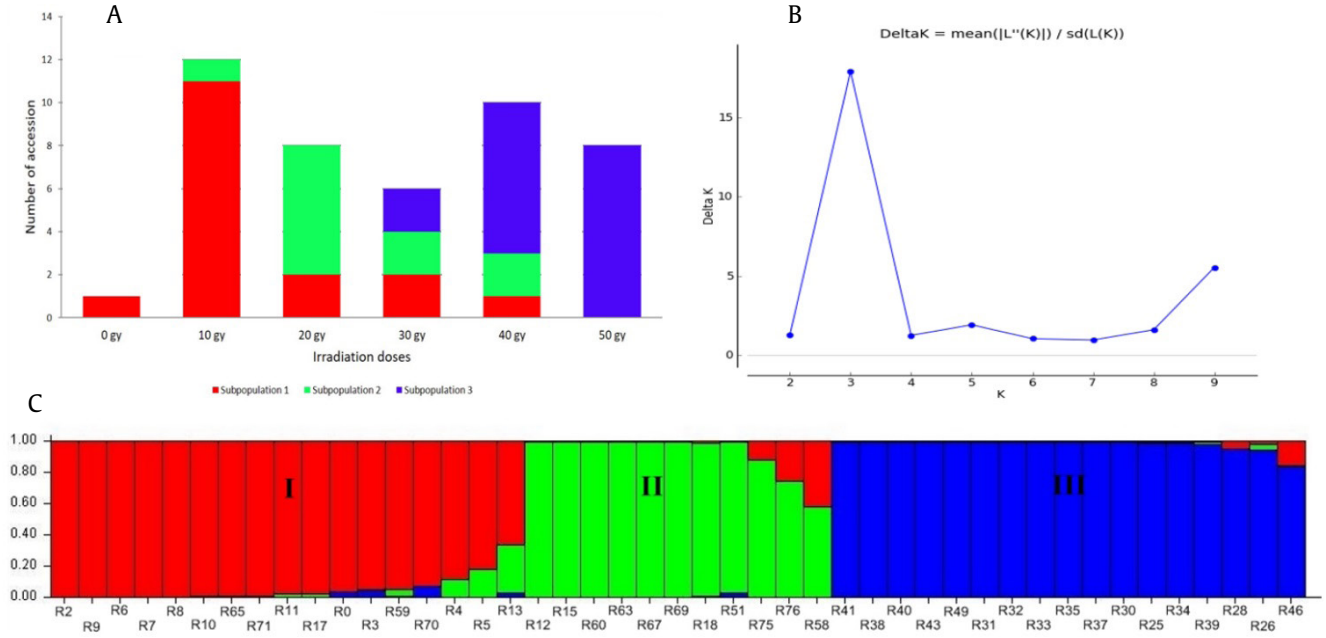

Figure 3. The number of somaclones for each radiation dose and their distribution in groups based on population structure analysis (A); the value of deltaK (B); population structure based on the Q value of 44 potato mutant somaclones and their control (wild type) (C). The colored regions grouped somaclones into 3 subpopulations

3.3. Estimation of Quantitative Character Genetic Parameters and Selection

The observed material was G0; then, the genetic diversity assessment was carried out on four important characteristics of potato seed tuber production, i.e., tuber length, diameter, weight, and number of tubers. The genetic diversity of the four morphological characters of tubers showed all characters included in the wide diversity criteria (Table 3). Broad heritability values ranged from 40.07-82.52%. All characters had high heritability criteria except for the number of tubers with moderate heritability values (Table 3). Selection based on quartile analysis showed that the 12 somaclones had the highest quartile values according to character weighting, which ranged from 172.83-267.33 (Table 4), i.e., R6, R17, R15, R69, R51, R63, R58, R11, R59, R71, R43 and R31 which had the potential to be used as seed sources for further evaluation in the field. The characters of the 12 somaclones are summarized in Table 4.

4. Discussion

One of the advantages of mutation treatment is that it can produce new genetic diversity. However, the resulting variability is random and unpredictable (Shu *et al.*2011). The low variation in the population of Repita somaclones indicates that the effect of irradiation was not too large on potato plants. This is in line with Suharsono *et al.* (2020), who observed changes in the morphology of potato mutants of the Kennebec variety, where the variation obtained was only in plant height characters, while other vegetative characters remained unchanged. The appearance of anthocyanin on leaflet stems, and lower sides indicated that irradiation treatment increased the production of anthocyanin pigments in genotypes R40 and R41.

Similarly, based on the research results by Suharsono *et al.* (2020), anthocyanins appeared in tuber shoots of the 53.1 mutant clone and looked different from the control. Changes in leaf color in R49 and R76 were caused by the disruption of chlorophyll formation on the leaf surface due to the influence of gamma-ray irradiation. Mutations in color are a common phenomenon that is easily recognized in higher plants. Mutated leaf color genes can directly or indirectly affect the synthesis of pigments (such as chlorophyll and anthocyanins), degrades, and affects the content and proportion of pigments, which can block photosynthesis and cause abnormal leaf color (Zhao *et al.* 2020).

Morphological observations on tuber characters showed a slight variation in the size of tubers of somaclones compared to the wild type. Variations in

Table 3. Estimation of genetic parameters of morphological characters of 45 potato somaclones

Characters	MS_g	σ^2_g	σ^2_p	$2\sigma_{\sigma g}^2$	DC	h^2_{bs}	Ch^2_{bs}
Tuber length	47.98	8.49	12.00	0.77	B	70.76	H
Tuber diameter	62.18	12.83	15.55	0.87	B	82.52	H
Tuber weight	27.44	4.74	6.86	0.59	B	69.13	H
Number of tubers	223.36	22.38	55.84	1.75	B	40.07	M

MS_g: genotype mean square, σ^2_g: genetic variance, σ^2_p: phenotype variance, $2\sigma_{\sigma g}^2$: 2 x standard deviation of genetic variance, DC: diversity criteria (B = Broad, N = Narrow), h^2_{bs}: broad sense heritability, Ch^2_{bs}: broad sense heritability criteria (H = High, M = Moderate, L = Low)

Table 4. Quantitative characters of selected accession tubers compared to control somaclones

Accession	Value	NT	TW (g)	TL (mm)	TD (mm)
R0	144.8	1.67	9.50	23.85	24.30
R6	172.83	5.67	6.53	20.38	22.25
R17	173.90	6.92	4.15	20.95	18.28
R15	187.63	6.17	8.53	21.93	22.10
R69	187.78	5.08	8.82	26.08	24.08
R51	189.05	10.08	2.35	16.73	13.77
R63	190.23	7.93	5.43	18.90	20.57
R58	192.85	8.33	5.10	18.68	20.10
R11	198.08	7.1	8.53	21.48	22.27
R59	200.28	6.92	8.22	22.38	23.93
R71	205.63	7.08	8.72	23.02	24.20
R43	216.63	7.83	9.43	22.27	24.90
R31	267.33	12.60	8.78	22.15	22.85

NT: number of tubers, TW: tuber weight, TL: tuber length, TD: tuber diameter

tuber size in the somaclones indicated the influence of gamma-ray irradiation on the character of potato tubers. Ibadullah *et al.* (2018) reported a significant interaction between irradiation dose and tuber growth, as indicated by the number of tubers, tuber weight, and tuber diameter.

The main characteristic distinguishing groups I and II was the quantitative character of the tubers. This indicates that the yield component was a significant distinguishing character. Group III consisted of two somaclones that showed the presence of anthocyanins on the lower side of the leaflets and stem. Meanwhile, groups I and II scattered the other three somaclones that showed differences in upper leaf color and leaf margins. The appearance of anthocyanins in plant organs was an important characteristic distinguishing somaclone from wild type.

This study showed that population structure analysis could classify somaclones based on the treatment of gamma-ray irradiation dose. The population structure analysis conducted by Lee *et al.* (2021) and Wang *et al.* (2019) could classify potato germplasm into two subpopulations distinguishing between modern cultivars or varieties and wild-type or landraces groups. In line with the grouping based on morphology, somaclone R40, and R41 were included in subpopulation III, indicating a large genetic change due to high irradiation doses, causing morphological changes in somaclone. Based on morphological observations, a dose of 50 grays caused a significant difference in the population, especially in the presence of anthocyanins. However, based on molecular analysis, the highest diversity was obtained from the dose of 30 grays because, at that dose, the number of somaclones from each subpopulation was fairly even (Figure 3A).

The four morphological characters of tubers had wide diversity to provide flexibility in the selection of diverse genotypes to be selected and planted for the next generation. All characters had high heritability criteria except for the number of tubers with moderate heritability values. This is different from the results of Pangemanan *et al.* (2013), where the characteristics of the number of harvested tubers per plant and weight per harvested tuber had extensive genetic variability and high heritability. The planting materials used in this experiment were cuttings from acclimatized somaclones, while Pangemanan *et al.* (2013) used G0 tubers as planting material. Based on these results, the possibility of using tubers as planting material will get more stable results than

cutting propagules because potato plantlets still need time to adapt from a heterotrophic environment to an autotrophic environment, so the yield was still unstable.

The selection of superior somaclones was based on four important characteristics of the tuber. Although having a moderate heritability value, the character of tuber number is essential because potato G0 is a source of seeds for the next generation of planting. The Tuber weight character is also important. The results of Fatchullah's research (2017) showed that the seed weight of G0 significantly affected the growth and production of tubers in G2. The 12 selected somaclones had a high morphological similarity as indicated by their inclusion in the same group, namely group 1 (Figure 2). In addition, based on their genetics, the 12 selected somaclones fell into two population groups, namely subpopulations 1 and 2 (Figure 3C).

In conclusion, gamma ray irradiation treatment on potato variety Repita caused significantly increased genetic diversity. Population structure analysis could classify somaclones based on the magnitude of the genetic change. The selection in this study resulted in 12 somaclones that have the potential to be evaluated for morpho agronomical characters in the field based on the criteria for a large number of tubers, as well as a fairly large tuber weight and size.

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