Cytological Differences of MV₃ Patchouli Plants (Pogostemon cablin Benth.) Derived From Gamma Ray-Irradiation

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Mutation breeding is a tool to create significant genetic variability for crops that reproduce vegetatively. In this study, morphological variability has been determined to confirm that mutational effect on desirable traits were inherited on vegetatively propagated plants. Research was conducted with randomized block design in three replications from 25 MV₃ of patchouli clones. Chromosomes of all patchouli mutants were examined by simple somatic cytological method, and morphological data were collected at maturity. The experiment revealed that cytogenetical changes on chromosome structure based on cytology test were occurred on a dose of 60 Gγ, 70 Gγ, 75 Gγ, 80 Gγ, 85 Gγ, 90 Gγ and 100 Gγ. Based on the analysis of agronomical variability traits, several parameters indicate wide range of variability, such as leaf angle, plant height, and branch length. The fresh herbage yield obtained from total 25 clones ranged from 15.21 tonnes / ha⁻¹ to 22.77 tonnes / ha⁻¹. This report confirms that patchouli mutants can effectively maintain its morphological variability on their vegetative progenies after propagated for two generations. This current study discovered chromosomal changes still occur on vegetatively-propagated progenies (MV₃) of patchouli plants, that can be potential as breeding source of new superior commercial clones.

Keywords: Chromosomal changes, cytogenetical, mutations, Patchouli, variability.

INTRODUCTION

Patchouli plant (Pogostemon cablin Benth.) is commercially important and widely known for its aromatic and essential oil use by perfume industries, medicinal purposes and antiseptic (Huang et al., 2016; Wu et al., 2011; Misra, 1996). Due to its characteristic that easily evaporate on room temperature, patchouli oil is insoluble on water and has a sweet-scent fragrance. As the year of 2015, patchouli plant is extensively cultivated in Indonesia (Ministry of Agriculture, 2017), India, Vietnam, and China (Ramya et al., 2013 and We et al., 2011).

Currently known that the demand of its important essential oil slightly rising over the year, serious efforts are needed in order to maintain patchouli’s high productivity, and high quality as well. However, development of hybrid or superior patchouli plants has some limited factors. As widely known as fact that patchouli plant is generally propagated by cutting propagation since their lack of blooming due to photoperiodism sensitivity. This method of reproduction leads to weaker resistance of progeny, and a decline in variability and unimproved oil quality (Kalra et al., 2006). Furthermore, simultaneous cutting propagation in traditional patchouli farming leads to declining of variability (Swamy et al., 2016), thus a level of diversity in certain area were found to be narrow. Certainly, those mentioned
problems in the development of high yielding varieties of patchouli were resulting to complicate the selection process, which is caused by the process of no hybridization (Wei et al., 2003).

Some reports related to the effort to broaden the genetic variability of patchouli has been conducted by Nuryani et al., (2004), Mo et al., (2012), and Yan et al., (2016). Expansion of genetic variability was conducted by protoplast fusion and in vitro induction of colchicine, whereas the mutation induction by gamma ray irradiation techniques, the information is still not yet widely available, only by Mohan et al., (2018), that currently reported an effort to increase fresh herbage yield in patchouli mutants. As previous studies have been conducted in mutation induction of gamma irradiation with various doses on several clones of patchouli (Tahir and Rofiq, 2013) also known that mutation breeding resulted in many varieties and provide broad benefits for the global agriculture (Ahoowalia et al., 2004).

As the gamma ray mutation target is random, a validation at through molecular level is indispensable. Cytological test were previously use in many crop plants, such as Arabidopsis (Mokros, 2007), Tomato (Ding et al., 2016) and rose (Shearer et al., 2014). While the reports regarding cytological test on patchouli plants remains insufficient. Phenotypic differences due to chromosomal changes can affecting the genetic diversity within the population, it creates changes in crop genotypes. Many studies documented that variation in patchouli genotypes through morphological criteria could be utilized as a tool in clonal selection. Selection on quality and yield of patchouli plant requires a broad genetic variability. This study was conducted to investigate the cytological effect and agronomical diversity on patchouli mutants at MV generations and to determine the proper doses for gamma-ray radiation in patchouli plants.

MATERIALS AND METHODS

Gamma ray induced mutations

This trial was conducted at Field Experimental Station and Lab. Plant Analysis, State Polytechnic of Lampung, Indonesia from March to November 2017. Gamma ray irradiations (Gamma Chamber 4000 A) were performed at National Nuclear Energy Agency of Indonesia, Jakarta. Patchouli genotypes (Sidikalang) were exposed to a 25 different doses of gamma ray, as follow (1) 0 Gy, (2) 5 Gy, (3) 10 Gy, (4) 15 Gy, (5) 20 Gy, (6) 25 Gy, (7) 30 Gy, (8) 35 Gy, (9) 40 Gy, (10) 45 Gy, (11) 50 Gy, (12) 60 Gy, (13) 70 Gy, (14) 75 Gy, (15) 80 Gy, (16) 85 Gy, (17) 90 Gy, (18) 95 Gy, (19) 100 Gy, (20) 105 Gy, (21) 110 Gy, (22) 115 Gy, (23) 120 Gy, (24) 125 Gy, and (25) 130 Gy. 1 Gray (Gy) is equal to 0,10 k rad/100 rad or 1 J kg-1 of exposed released energy. The vegetative mutants were grown in polyethylene bag at optimum growth conditions.

Cytological analysis

A total of 25 patchouli mutant samples derived from previous work (Tahir and Rofiq, 2013) were collected during active roots development. The tip of the roots were used as a sample preparations for observation of chromosomes. Preparations were made using squash method, patchouli mutants were taken its root tip with a length of about 5 mm, and then soaked in distilled water for 24 hours at a temperature of 50°C - 80°C.

After soaking, the samples were subsequently fixed with Carnoy solution for 24 hours at room temperature. Once fixed, then hydrolysed with 1N HCl solution for 5-10 minutes at room temperature, and then washed with distilled water. Next, the sample root tip soaked in a solution of 2% aceto-orcein for 24 hours at room temperature, with the aim of staining chromosomes. Once colored, apex sections separated by needle preparations, then meristematic part were taken and placed on a glass preparations. Further material on the preparations glass etched with 45% acetic acid, and then covered with a glass lid, and then pressed.

Observations were made using a microscope. Parameter observations conducted on the frequency of total abnormalities, chromosomal bridge, fragmented chromosome, and scattered chromosome, and estimated based on a method described by Fiskesjo (1985). Results were obtained by chromosomal structural changes that ultimately improve the quality of patchouli oil. Observation of the chromosome photographed through a microscope using a high-resolution camera that produces good images.

Agronomical trait analysis

The agronomic components of P. cabilin mutants were examined during the harvesting stage. All agronomic practices were kept same for all the replications and treatments. Twenty-five plants were selected from each replication of each accession and data were recorded. Those variables were chlorophyll content, leaf angle (θ),
total primary branch, branch angle (°), plant height (cm), stem diameter (cm), branch diameter (cm), branch length (cm), fresh herbage yield/plant (kg), and total yield (ton/ha⁻¹). Experiment were conducted with Randomized Block Design with three replications on each variables.

Data results of cytological effects were presented descriptively based on the percentage of observations. Furthermore, homogenized data were subjected to one-way analysis of variance (ANOVA) with Tukey’s HSD significant test at 5% level. Furthermore, dendrogram analysis for agronomical traits were calculated by using software NCSS ver. 11 (2016).

RESULTS

Cytological effects
Cytological data showed that there was a significant change in the mitotic processes. As could be seen in the Table 1, the total abnormality increased significantly with gamma ray irradiation dose, when compared to control. It can be seen that chromosomal damage began to occur upon exposure to irradiation with dose of 50 Gy. Each type of damage has consistently increased in line with increasing doses of irradiation. Total abnormality shows nine different irradiation treatments was significant, i.e. a dose of 75 Gy to 115 Gy, respectively. Chromosomal bridging showed significant differences in the doses of 60 Gy and 70 Gy to 115 Gy. There are five doses of irradiation dose treatment were significantly different and lead to fragmented chromosomes, i.e. at a dose of 90 Gy to 110 Gy. While scattered chromosomes only showed four different doses of irradiation real, i.e at a dose of 100 Gy, 115 Gy, 120 Gy and 125 Gy. The real differences began to appear at dose of 60 Gy, in which the type of chromosomal abnormality that occurred was bridging. At high doses of irradiation, there is no significant differences in the total chromosomal abnormalities, chromosomal bridging and fragmented chromosomes.

Agronomical variability
Based on the result of clustering analysis, there are 5 main clusters (Figure 1) of 25 MV₃ patchouli plants. Group 1 consisted of four treatment plants from 5 Gy, 15 Gy, 115 Gy and 120 Gy. Chlorophyll content ranged from 15.6 to 45.7. Wide range of chlorophyll content were occur probably as the residual effect of gamma-ray, which resulted to chlorophyll deficiency from several samples.

Similar report were explained by Wani et al., (2011), that certain doses of chemical of physical mutation can increase or decrease the level of chlorophyll. Leaf angle ranged from 20.6⁰ to 27.6⁰. Total primary branch as much as 15.6 to 19.3. Branch angle ranged from 37.3⁰ to 47.3⁰. Plant height ranged from 73.3 cm - 92.3 cm. Stem diameters ranging from 0.87 cm - 1.01 cm. Branch diameter ranging from 0.42 cm - 0.51 cm. Branch length ranged from 30.6 cm - 39 cm. Fresh herbage yield / plant ranged from 0.38 kg - 0.55 kg. Total yield ranged from 15.21 tonnes / ha⁻¹ to 17.14 t / ha⁻¹.

Group 2 consisted of five treatment plants from 25 Gy, 50 Gy, 80 Gy, 95 Gy and 100 Gy. Chlorophyll content ranged from 39.9 to 44. Leaf angle ranged from 15⁰ to 19⁰. Total primary branch as much as 17.6 to 18.6. Branch angle ranged from 41.6⁰ to 45.3⁰. Plant height ranged from 74 cm - 77 cm. Stem diameters ranged from 0.98 cm - 1.17 cm. Branch diameter ranged from 0.42 cm - 0.47 cm. Branch length ranged from 30.3 cm - 40 cm. Fresh herbage yield / plant ranged from 0.38 kg - 0.55 kg. Total yield ranged from 15.21 tonnes / ha⁻¹ to 17.14 t / ha⁻¹.

Group 3 consisted of five treatment plants from 10 Gy, 20 Gy, 40 Gy, 45 Gy and 75 Gy. Chlorophyll content ranged from 42.7 to 45.9. Leaf angle ranged from 15⁰ to 21.3⁰. Total primary branch as much as 20 to 22.6. Branch angle ranged from 43.3⁰ to 46⁰. Plant height ranged from 82.3 cm – 96.6 cm. Stem diameters ranging from 0.83 cm - 1.06 cm. Branch diameter ranging from 0.40 cm - 0.56 cm. Branch length ranged from 36.6 cm – 41.6 cm. Fresh herbage yield / plant ranged from 0.50 kg - 0.63 kg. Total yield ranged from 15.62 tonnes / ha⁻¹ to 19.29 t / ha⁻¹.

Group 4 and 5 consisted of two treatment plants of each clusters from 35 Gy, 85 Gy, 90 Gy, and 125 Gy. Chlorophyll content ranged from 42 to 46.2. Leaf angle ranged from 13.3⁰ to 20⁰. Total primary branch as much as 18.6 to 20.6. Branch angle ranged from 38⁰ to 45.3⁰. Plant height ranging from 83.3 cm – 97.7 cm. Stem diameters ranging from 0.90 cm - 1.02 cm. Branch diameter ranging from 0.43 cm - 0.50 cm. Branch length ranging from 28 cm – 40.3 cm. Fresh herbage yield / plant ranged from 0.48 kg - 0.76 kg. Total yield ranged from 15.20 tonnes / ha⁻¹ to 22.77 t / ha⁻¹.

DISCUSSION
The foregoing results showed an increase chromosomal change in mitotic activity at certain dose, and surprisingly decrease while gamma-ray
dose increasing. The effect of mutations on various plants through gamma-ray induction were reported by Melki et al., (2010), Rashid et al., (2013), and Badr et al., (2014).

Table 1. Effect of Gamma-ray Irradiation on Chromosomal Abnormalities

<table>
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<tr>
<th>Dose (Gy)</th>
<th>Total Abnormalities (%)±SE</th>
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* = significantly different at 5% of Tukey’s test

Figure 1. Dendrogram analysis of 25 Patchouli mutants

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Change in a chromosome can affect the phenotypic performance of patchouli plant. Despite there are reports revealed an increasing yield on advance progenies of patchouli mutants (Rekha et al., 2009), no specific findings concerning the inheritance of mutational effect on vegetatively-propagated plants. This study revealed that chromosomal changes were still occur in vegetative progenies.

On this work, specific dose to broaden genetic variability on patchouli plant has been determined. High chromosomal abnormalities were spotted on 90 Gy to 110 Gy, assuming there were several peak of abnormalities on certain doses before it begin to decrease. The level of abnormalities on Vigna species were reported has a similar pattern with this study (Girija et al., 2013), beside this report, further recommendation regarding gamma-ray dose remain insufficient. Different dose on gamma-ray with krypton radioisotope ranged from 5 – 70 60Kr resulting various fresh herbage yield and oil yield (Mohan et al., 2018). Proper doses of gamma-ray radiation can be utilized for industrial purposes to produce parental patchouli plants in a large scale.

Genetic variability is essential for natural selection because natural selection can only increasing or decreasing frequency of alleles that already exist in the population. Genetic variation is caused by mutation as well as random mating between organisms. The range of genetic variability in this experiment were important substance in order to perform selection, since the heritability on each generation will be calculated to gain superior clones. Chromosomal change in present study affected the phenotypic performance, and separate them into a different group (Fig. 1). This study clearly shows that gamma-ray mutation can continuously broaden the genetic and phenotypic variability on vegetative progenies of patchouli plants.

CONCLUSION

In conclusion, the effect of gamma radiation drastically influence all aspects of cytological and agronomical in patchouli plants, at MV3 generation in particular. After two generation of cutting propagation, mutational effects were still occur in chromosomal level and phenotypical level. The present investigation clearly showed a change of phenotypical performance with specific gamma-ray dose. Chromosomal abnormalities were found to be increased as the concentration of the irradiation escalate when compare to control as well as found to be correlated with agronomical traits. This current study discovered chromosomal changes still occur on vegetatively-propagated progenies (MV3) of patchouli plants, that can be potential as breeding source of new superior commercial clones. Results of this study will help patchouli breeders to uncover the appropriate doses of physical mutations to broaden genetic diversity.

CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

ACKNOWLEDGEMENT

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AUTHOR CONTRIBUTIONS

MT designed and performed the whole experiments. DR and E performed agronomical analysis, tissue collection, and provide data analysis. JK and MT designed and performed cytogenetic experiments, wrote the manuscript, as well as reviewed the manuscript. All authors read and approved the final version.

REFERENCES


