Morphological variations of patchouli putative mutants

S Suhesti*, M Susilowati2, N Sirait2, Amalia2 and E Hadipoentyanti2

1 Indonesian Center for Estate Crops Research and Development, Indonesian Agency for Agricultural Development, Jalan Tentara Pelajar No. 1, Cimanggu, Bogor 16111, West Java, Indonesia.

2 Indonesian Spice and Medicinal Crops Research Institute, Indonesian Agency for Agricultural Development, Jalan Tentara Pelajar No. 3, Cimanggu, Bogor 16111, West Java, Indonesia.

*Email: hesti.khrisnawijaya@gmail.com

Abstract. World patchouly oil need is almost 90% fulfilled by Indonesia as the main producer, however, their narrow genetic variation is a major obstacle in plant breeding. Chemical mutation such as colchicine is believed to be able to wider patchouly genetic variation. The aim of this study was to increase patchouly genetic variation using chemical mutagen. The variety of Patchoulina 2 was treated by colchicine on callus stage. Callus irradiated was grown into regeneration medium until formed plantlet and ready to acclimatization. The results showed that there were 15 different morphological putative mutants with different characters. The mutants varied in qualitative characters (leaf shape, leaf color, leaf base, and leaf edge) and quantitative characters (plant height, number of leaf stalks). Two distinguishable main groups of mutants shared 62.62% genetic similarity level based on morphology. These new mutants in this study would be potential to be developed as new varieties along with the morphological characters as their specific identities.

Keywords: genetic variation, mutation, colchicine, qualitative characters, genetic similarity

1. Introduction

Patchouli (Pogostemon cablin Benth.) is one of the important plants that produce essential oils. The essential oil is the raw material that functions as a binding material (fixative) in making perfumes and food flavoring products. Most of patchouli is cultivated in Indonesia and almost 90% of the world's patchouli oil needs are supplied from Indonesia [1]. Based on tree crop estate statistics, the area of patchouli in 2017 was 18.841 hectares with the production of 2.115 tons of patchouli oil. Improving patchouli possessing high oil should be prioritized through breeding program. High genetic diversity will increase the chances of successful plant breeding [2] including patchouli. However, the patchouli plant breeding program is limited by their less diversified genetic source because patchouli does not flower, as consequence, hybridization are not possible for this plant species.

As an alternative to broader patchouli diversity is through induced mutation techniques. Physical or chemical mutagens are commonly used for induction of mutation in plant. Physical mutagen is performed using Gamma rays, X rays or neutrons. This mutagens is ionizing radiation ands capable of causing ionization, releasing ionizing energy when passing through or penetrating material [3]. The use of chemical mutagens is also often used to increase plant genetic diversity. Colchicine is one of the chemical mutagens that is often used. The colchicine does not cause spindle formation during the metaphase so that the number of doubling chromosomes is often referred to as polyploidy induction.
which allow to increase level of polyploidy. Polyploidy induction is reported as an effective way to improve productivity in several plants species, and could be applied to patchouli for various important traits. Thus, mutation will increase the chances of obtaining new varieties according to the purpose of plant breeding [4]. The aim of this study was to evaluate the morphological diversity of the patchouli putative mutants produced from colchicine treatment

2. Materials and methods
The study was conducted at the tissue culture laboratory of the Agricultural Superior Seed Management Unit, Indonesian Center for Estate Crops Research and Development, Indonesian Agency for Agricultural Research and Development, Bogor from August 2018 to April 2019. The observed mutants were 14 genotypes (four months after acclimatization) obtained M1 to M10 were treated with 0.02% colchicine and the rest with 0.06%. and Patchoulina 2 as a control variety. Patchoulina 2 was a parental of mutants. This variety had high productivity and resistance to bacterial wilt disease. The treatment of colchicine by immersing callus variety P2 into colchicine at a concentration of 0.02% and 0.06% for 2 hours. The mutant callus was then regenerated into MS medium with starting growth regulator 2,4-D 0.1 mg/l and BAP 0.5 mg/l.

Observations were made on leaf and stem morphological characters [5]. Observation of leaf morphology characters consisted of eight characters (leaf shape, leaf base, leaf tips, leaf edges, leaf veins, leaf surface, top leaf surface color, and bottom leaf surface color), while observation of stem morphology characters consisted of seven characters (type of growth, base color, middle color, shoot color, stem shape, branching, and stem surface). Morphological descriptive character data is transformed into a scoring data. Data were analyzed by grouping using multivariate cluster observation with a single linkage method, euclidean distance using Minitab 1.5.

3. Results and discussion
A total of 15 morphological characters on the leaves and stems were characterized in the putative mutants in this study, of which, 10 out of 15 were diverse on the mutants compared to Patchoulina 2 as their parental parental line. These characters were as followed:

3.1. Leaf character
Leaf is one of the important characters that determine the productivity of patchouli. This observation showed that character of putative mutant leaves changed from their parental. These changes were found in six leaf characters from the eight leaf characters observed. The mutant leaf characters changed were leaf shape, leaf base shape, leaf tip shape, leaf edge, leaf surface, and leaf under surface color, while leaf characters on putative mutants that were the same as their parental i.e. leaf veins and surface color upper leaf (Table 1).

Patchoulina 2 (P2) variety as parental has deltoidus leaves. All mutants have the same leaf shape as their parental except for three mutants which have triangularis leaves. P2 as a control has leaf shape base is acutus, but some mutants have changed from their parental in the leaf shape base from acutus become acuminatus, truncatus, obtusus. Leaf tip shape’s P2 is acutus. There are 10 mutants whose leaf tip shape are acutus like their parental, while some mutants change leaf tip shape become acuminatus, obtusus and rotundatus. The same thing is also seen in leaf edge. The Patchoulina 2 variety has biserratus, but all putative mutants leaf edge changes become repanda, crenatus and serratus. There is no mutant that has the same leaf edge shape as their parental.

All mutants have leaf vein characters the same shape as their parental i.e. penninervis. Leaf surface’s P2 is hispidus, but some mutants have changed to pilosus. However, most of the mutants still have the same leaf surface as their parental. All mutants have top leaf surface color the same yellowish green as their parental. This is different from bottom leaf surface color. All mutants change their color. Patchoulina 2 as parental has purplish green but the mutants have yellowish green. Apart from these mutants, most of the mutants are dominated by green color (Table 1).
Table 1. Morphological diversity based on leaf character for 14 putative mutants and parental line

| Morphological character | Parental | Mutants |
|-------------------------|----------|---------|
|                         | P2      | M1  | M2  | M3  | M4  | M5  | M6  | M7  | M8  | M9  | M1  | M1  | M1  | M1  |
| **Leaf shape (D1)**     |          |      |     |     |     |     |     |     |     |     |     |     |     |
| 1. Ovalis               | v        | v    | v   | v   | v   | v   | v   | v   | v   | v   | v   |
| 2. Triangularis         |          |      |     |     |     |     |     |     |     |     |     |     |     |
| 3. Deltoideus           | v        | v    | v   | v   | v   | v   | v   | v   | v   | v   | v   |
| 4. Rhomboideus          |          |      |     |     |     |     |     |     |     |     |     |     |     |
| 5. Others               |          |      |     |     |     |     |     |     |     |     |     |     |     |
| **Leaf base shape (D2)**|          |      |     |     |     |     |     |     |     |     |     |     |     |
| 1. Acutus               | v        | v    | v   | v   | v   | v   | v   | v   | v   | v   | v   |
| 2. Acuminatus           |          |      |     |     |     |     |     |     |     |     |     |     |     |
| 3. Obtusus              | v        | v    | v   | v   | v   | v   | v   | v   | v   | v   | v   |
| 4. Rotundatus           |          |      |     |     |     |     |     |     |     |     |     |     |     |
| 5. Truncatus            | v        | v    | v   | v   | v   | v   | v   | v   | v   | v   | v   |
| **Leaf tip shape (D3)** |          |      |     |     |     |     |     |     |     |     |     |     |     |
| 1. Acutus               | v        | v    | v   | v   | v   | v   | v   | v   | v   | v   | v   |
| 2. Acuminatus           |          |      |     |     |     |     |     |     |     |     |     |     |     |
| 3. Obtusus              | v        | v    | v   | v   | v   | v   | v   | v   | v   | v   | v   |
| 4. Rotundatus           |          |      |     |     |     |     |     |     |     |     |     |     |     |
| 5. Truncatus            | v        | v    | v   | v   | v   | v   | v   | v   | v   | v   | v   |
| 6. Retusus              |          |      |     |     |     |     |     |     |     |     |     |     |     |
| 7. Macronatus           |          |      |     |     |     |     |     |     |     |     |     |     |     |
| **Leaf edge (D4)**      |          |      |     |     |     |     |     |     |     |     |     |     |     |
| 1. Integer              |          |      |     |     |     |     |     |     |     |     |     |     |     |
| 2. Serratus             | v        | v    | v   | v   | v   | v   | v   | v   | v   | v   | v   |
| 3. Biserratus           | v        | v    | v   | v   | v   | v   | v   | v   | v   | v   | v   |
| 4. Dentatus             |          |      |     |     |     |     |     |     |     |     |     |     |     |
| 5. Crenatus             | v        | v    | v   | v   | v   | v   | v   | v   | v   | v   | v   |
| 6. Repandus             | v        | v    | v   | v   | v   | v   | v   | v   | v   | v   | v   |
| **Leaf veins (D5)**     |          |      |     |     |     |     |     |     |     |     |     |     |     |
| 1. Penninervis          | v        | v    | v   | v   | v   | v   | v   | v   | v   | v   | v   |
| 2. Palminervis          | v        | v    | v   | v   | v   | v   | v   | v   | v   | v   | v   |
| **Leaf surface (D6)**   |          |      |     |     |     |     |     |     |     |     |     |     |     |
| 1. Pilosus              | v        | v    | v   | v   | v   | v   | v   | v   | v   | v   | v   |
| 2. Villosus             | v        | v    | v   | v   | v   | v   | v   | v   | v   | v   | v   |
| 3. Hispidus             | v        | v    | v   | v   | v   | v   | v   | v   | v   | v   | v   |
| 4. Villosus             | v        | v    | v   | v   | v   | v   | v   | v   | v   | v   | v   |
| **Top leaf surface color (D7)** |          |      |     |     |     |     |     |     |     |     |     |     |     |
| 1. Green                | v        | v    | v   | v   | v   | v   | v   | v   | v   | v   | v   |
| 2. Purplish green       | v        | v    | v   | v   | v   | v   | v   | v   | v   | v   | v   |
| 3. Yellowish green      | v        | v    | v   | v   | v   | v   | v   | v   | v   | v   | v   |
| **Bottom leaf surface color (D8)** |          |      |     |     |     |     |     |     |     |     |     |     |     |
| 1. Green                | v        | v    | v   | v   | v   | v   | v   | v   | v   | v   | v   |
| 2. Purplish green       | v        | v    | v   | v   | v   | v   | v   | v   | v   | v   | v   |
| 3. Yellowish green      | v        | v    | v   | v   | v   | v   | v   | v   | v   | v   | v   |
3.2. Stem character

The stems are also one of the important characters that determine the productivity of patchouli plants. Patchouli oil production is obtained from leaves and stems. Based on observations, there was diversity in the 4 stem characters between putative mutants from 7 observed stem characters (Table 2). The diversity of stem characters includes the type of growth, base color, middle color, and shoot stem color, while the stem characters on putative mutants that are the same as their parental or do not change are the shape of the stem, branching, and stem surface.

Table 2. Morphological diversity based on stem character for 14 putative mutants and parental

| Morphological character | Parental | Mutant |
|-------------------------|----------|--------|
|                         | P2 | M1 | M2 | M3 | M4 | M5 | M6 | M7 | M8 | M9 | M10 | M11 | M12 | M13 | M14 |
| Growth type (B1)        |   |    |    |    |    |    |    |    |    |    |     |     |     |     |     |
| 1. Erectus              | v |    |    |    |    |    |    |    |    |    |     |     |     |     |     |
| 2. Dependens            |   | v |    |    |    |    |    |    |    |    |     |     |     |     |     |
| 3. Humifusus            |   |   |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 4. Repens               |   |   |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 5. Ascendens            |   |   |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 6. Nutans               |   |   |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 7. Scandens             |   |   |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 8. Volubilis            |   |   |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Base color (B2)         |   |    |    |    |    |    |    |    |    |    |     |     |     |     |     |
| 1. Brown                | v |    |    |    |    |    |    |    |    |    |     |     |     |     |     |
| 2. Purplish brown       |   | v |    |    |    |    |    |    |    |    |     |     |     |     |     |
| 3. Greenish brown       |   |   |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 4. Purplish             |   |   |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 5. Greenish             | v |    |    |    |    |    |    |    |    |    |     |     |     |     |     |
| Middle color (B3)       |   |    |    |    |    |    |    |    |    |    |     |     |     |     |     |
| 1. Brown                | v |    |    |    |    |    |    |    |    |    |     |     |     |     |     |
| 2. Purplish brown       |   | v |    |    |    |    |    |    |    |    |     |     |     |     |     |
| 3. Greenish brown       |   |   |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 4. Purplish             |   |   |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 5. Greenish             | v |    |    |    |    |    |    |    |    |    |     |     |     |     |     |
| Shoot color (B4)        |   |    |    |    |    |    |    |    |    |    |     |     |     |     |     |
| 1. Brown                | v |    |    |    |    |    |    |    |    |    |     |     |     |     |     |
| 2. Purplish brown       |   | v |    |    |    |    |    |    |    |    |     |     |     |     |     |
| 3. Greenish brown       |   |   |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 4. Purplish             |   |   |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 5. Greenish             | v |    |    |    |    |    |    |    |    |    |     |     |     |     |     |
| Stem shape (B5)         |   |    |    |    |    |    |    |    |    |    |     |     |     |     |     |
| 1. Teres                | v |    |    |    |    |    |    |    |    |    |     |     |     |     |     |
| 2. Quadrangularis       |   | v |    |    |    |    |    |    |    |    |     |     |     |     |     |
| Branching (B6)          |   |    |    |    |    |    |    |    |    |    |     |     |     |     |     |
| 1. Monopodial           | v |    |    |    |    |    |    |    |    |    |     |     |     |     |     |
| 2. Simpodial            | v |    |    |    |    |    |    |    |    |    |     |     |     |     |     |
| Stem surface (B7)       |   |    |    |    |    |    |    |    |    |    |     |     |     |     |     |
| 1. Laevis               | v |    |    |    |    |    |    |    |    |    |     |     |     |     |     |
| 2. Costatus             | v |    |    |    |    |    |    |    |    |    |     |     |     |     |     |
| 3. Sulcatus             |   | v |    |    |    |    |    |    |    |    |     |     |     |     |     |
| 4. Alatus               |   | v |    |    |    |    |    |    |    |    |     |     |     |     |     |
| 5. Pilosus              |   |   |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 6. Pilosus              | v |    |    |    |    |    |    |    |    |    |     |     |     |     |     |
| 7. Spinosus             | v |    |    |    |    |    |    |    |    |    |     |     |     |     |     |
Figure 1. Leaf morphological diversity of putative mutant i.e. a. Deltoideus, obtusus, obtusus, serratus, 3 leaves per node; b. Ovalis, acutus, acutus, serratus, 3 leaves per node; c. Deltoideus, obtusus, obtusus, repandus; d. Ovalis, truncatus, rotundatus, repandus; e. Ovalis, obtusus, obtusus, serratus; f. Deltoideus, truncatus, obtusus, serratus, variegata; g. Deltoideus, truncatus, acuminatus, biserratus; h. Deltoideus, acuminatus, rotundatus, repandus; i. Deltoideus, acuminatus, obtusus, creanatus; j. Deltoideus, truncatus, acutus, biserratus; k. Ovalis, acuminatus, rontudatus, repandus.

The growth type of Patchoulina 2 (P2) variety as parental is erectus. Most mutants undergo changes in growth type character from erectus to ascedens. However, there is one M14 mutant that still same with parental. P2 as a control has base color is purplish brown. There are 3 mutants that have the same base color stem as P2 namely M2, M3 and M14. Some mutants that experienced stem color changes into brown, greenish brown, and greenish. The color of the middle stem is quite varied from their parental to purplish brown to brown, greenish brown, purplish and greenish. Mutants that do not change color in the middle stem are M4 and M5 which have the same purple-brown color as their control. The color at the top of the stem also has a high diversity. P 2 has a greenish stem shoots but some mutants are purplish and greenish brown. Even so, most of the mutants still have the same shoots color as their control. In the stem shape, all mutants have the same shape as their parental, which are quadrangularis. It is also the same for character branching forms where all mutants have the same sympodial branching as their parental. In addition, the character of the stem surface also did not change. All mutants has the same pilosus stem surface as their parental (Table 1). The morphological characters are specific characteristics of the new mutant in the patchouli mutants that are useful for differentiating the mutants produced from their parental and between the mutants produced.
A morphological diversity of leaves was found on 14 patchouli genotypes. The noticeable morphology could be observed on number of leaf per node, leaf shape, leaf surface etc, branched leaf bone, (Figure 1). Based on the optimal concentration, colchine 0.02% and 0.06% produced high morphological diversity in patchouli. The mutagent induced patchouli mutant lines with greater variation and significantly differed from the parent. For example, these mutants leaves tend to be with larger in size, thicker and darker in color than their parental line. Number of leaves from 2 leaves per node to 3 leaves per node was changed on the parental line to mutants, respectively, indicating their increased biomass (Figure 1a and 1b). The greater on number and size of leaves as a component of production could directly increase crop productivity. Notable, these putative mutants have the potential to be developed as candidates for high-yielding patchouli varieties.

Cluster analysis based on morphological characters of leaves and stems on 14 putative mutants resulting from polyploidization generated two major groups which differentiated between putative mutants and P2 as parental line with genetic similarity of 62.62% (Figure 2). Twelve mutants (M1, M4, M5, M7, M9, M12, M13, M10, M11, M8, M6, and M14) were in the same group as their parent (Group I), their closer relation with the parent compared to others. Mutants that have the farthest genetic distance from the parent M2 and M3 which were produced from the 0.02% colchicine. In contrast, M12 and M13 had the closest genetic similarity (80%). This result suggested that the mutant lines produced in this study revealed a high diversity as expected.

As reported that genetic diversity is the basis in plant breeding activities to get the expected character. The higher genetic diversity will provide a higher opportunity for breeders to get new superior varieties according to the purpose of plant breeding.

4. Conclusion
Colchine with concentrations of 0.02% and 0.06% can produce mutants of patchouli with high morphological diversity. Ten mutants were changed their phenotypes and differed from the parental line (Patchoulina 2). The changed characters in the mutants included six-leaf morphology characters (leaf shape, leaf base, leaf tip, leaf edge, leaf surface, and leaf surface color) and four-stem morphological characters (growth type, base color, middle color, and shoot color). Cluster analysis generated two major groups which were distinguished the parent from the mutants.
Acknowledgment
We thank Lusia Seti Palindung, Totong Sugandi, Abdul Miftah, and Dewi Yuliyanti for technical assistance in the greenhouse and laboratory. This research was funded by APBN T.A. 2018, Indonesian Research Institute for Spice and Medicinal Crops.

References
[1] Sugimura Y, Padayhag B F, Cenizat M S, Kamataf N, Eguchit S, Natsuaki T and Ekuda S 1995 Essential oil production increased by using virus-free patchouli plants derived from meristem-tip culture Plant Pathol. 44 510–5
[2] Jayasankar S, Z. Li and Gray D J 2000 In vitro selection of vitis vinifera chardonnay with elsinoe ampelina culture filtrates is accompanied by fungal resistance and enhanced secretion of chitinase Planta 211 200–8
[3] Medina F I S, Amano E and Tano S 2005 Mutation Breeding Manual (Tokyo: Forum for Nuclear Cooperation in Asia (FNCA))
[4] Husni A, Kosmiatin M and Mariska I 2006 Peningkatan Toleransi kedelai sindoro terhadap kekeringan melalui seleksi in vitro Bul. Agron. 34 25–31
[5] Tjitrosoepomo G 2005 Morfologi Tumbuhan (Yogyakarta: Gadjah Mada University Press) p 266