Physiological Effect of Colchicine Treatment to Garlic (*Allium sativum* L.) cv. Doulu

G M Ayu, Elimasni*, I Nurwahyuni

Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, Indonesia

*Email: elimasni@usu.ac.id

**Abstract.** Productivity of garlic from Doulu cultivar is still considered low and has not yet been able to fulfill local needs, especially in North Sumatera. Meanwhile, the public interest of local garlic was low due to its tiny size and high cost compared to imported garlic. The aim of this study was to determine the effect of colchicine concentration during immersion treatment towards physiological performance of Doulu cultivar. Experimental design used in this study was randomized block design with two factorials comprised of 20 combinations and three replications. The first factor was colchicine concentration (D): 0% (D0), 0.1% (D1), 0.2% (D2), and 0.3% (D3). The second factor was immersion durations: 0 (T0), 6 (T1), 12 (T2), 18 (T3), 24 (T4) hr. The results showed that control group exhibit higher value in terms of plant height, number of leaves, dry and fresh weight of bulbs compared to treatment groups. Concentration of 0% (control) within 12 hr of immersion duration (D0T2) showed the highest plant height of 22.38 cm, producing 5.05 leaves and dry weight of 1.57 g. The heaviest weight of fresh bulbs was observed from treatment group, in concentration 0.3% colchicine within 0 hr of immersion duration (D3T0) yielding 4.58 g.

1. **Introduction**

Significant changes to garlic breeding has occurred a long time ago. Garlic cultivars are rarely abundant nowadays, especially the local cultivars from farmers and domestic markets. The lack of interest from farmers to breed the local cultivars is due to its tiny bulbs and high costs compared to imported garlics [1]. Garlic (*Allium sativum* L.) is one of the popular horticulture plant, known by people which have lots of benefits from its bulbs. Phytochemical constituen ts of its bulbs are commonly used as flavors and drug materials containing essential oils and organosulphures [2,3].

In 2012, production of garlic in Indonesia was about 296,500 tons, while national needs about 400,000 tons. Production of garlic in Indonesia still fulfill national needs until a significant gap occurs between consumption and production [4]. The condition supports government to import garlic from China ± 320 tons on 2013 to fulfill national demands [5]. In 2016, local garlic productions are estimated ± 18,000 tons, while imported ± 117,000 tons from China and India.

A cultivar from North Sumatra named Doulu is recognized by society due to its spicy taste and significant smell [6]. However, information regarding its breeding is still limited. In addition, its abundance in the field is currently facing a limit due to less demand to breed this garlic cultivar. Effort to improve productivity based on laboratory studies, is reported in this study. We treated Doulu cultivar with various colchicine concentration as one of chemical mutagen. Various physiological effects are reported in this study.
2. Materials and Methods
This research has been conducted in the Doulu village, Berastagi, North Sumatra from May to August 2018. Materials used in this study were garlic seeds, colchicine, distilled water, tap water, manure, mulch plastic, hoes, and ruler. Experimental design used in this study was Complete randomized block design with two factorials consisted of 20 combinations and three replications. The first factor was colchicine concentration (D): 0% (D0), 0.1% (D1), 0.2% (D2), and 0.3% (D3), while second factor was immersion durations: 0 (T0), 6 (T1), 12 (T2), 18 (T3), 24 (T4) hr.

Planting plots were made sizing 4 × 1 m. Distance between plots was arranged in 11 cm while between replications in 5 cm. Weeding was done manually and periodically two weeks. Observable parameters were plant height, number of leaves, dry and fresh weight of bulbs. Observation data were analyzed by ANOVA with significant level of 5% followed by Duncan’s new Multiple Range Test as post-hoc test.

3. Results and Discussions
3.1. Plant height
The results showed that colchicine concentrations and immersion durations significantly affected plant height, number of leaves, dry and fresh weight bulbs. The results are shown in consecutive manner.

Based on Figure 1, the highest plant height was observed from D0T2 with 22.47 cm, similarly to D3T0 with 22.38 cm. The lowest plant height was observed from D3T4 with 15.93 cm. Several studies have reported a significant shortening effect of colchicine to plant height in certain species. In accordance with previous study using Citrus Nobilis Lour, the plant also experienced a decreased in plant height of plants after 0.3% colchicine treatment compared to control [7]. Other study also reported a decreased plant height of Plox drummondii after 0.5% colchicine treatment within immersion duration for 36 hr [8]. The similar effects were also observed to Lagerstroemia indica L., after 0.5% colchicine treatment for 72 hr and to Artemisia annua L. after 0.2% colchicine treatment [9,10].

3.2. Number of leaves
The results of colchicine treatment to number of leaves are presented in Table 1. The highest number of leaves was observed from D0T2 with 5.05 leaves while the lowest was observed from D3T2 with only 3.95 leaves.
Table 1. Effect of colchicine treatment to number of leaves

| Colchicine Concentration (%) | Duration (hr) | Mean Number of Leaves |
|-----------------------------|---------------|-----------------------|
|                             | 0 (T0)        | 6 (T1)  | 12 (T2) | 18 (T3) | 24 (T4) |
| 0.0 (D0)                    | 4.38<sup>a</sup> | 4.67<sup>a</sup> | 5.05<sup>a</sup> | 4.14<sup>a</sup> | 4.14<sup>a</sup> |
| 0.1 (D1)                    | 4.38<sup>a</sup> | 4.09<sup>a</sup> | 4.48<sup>a</sup> | 4.29<sup>a</sup> | 4.05<sup>a</sup> |
| 0.2 (D2)                    | 4.05<sup>a</sup> | 4.62<sup>a</sup> | 4.19<sup>a</sup> | 4.33<sup>a</sup> | 4.24<sup>a</sup> |
| 0.3 (D3)                    | 4.57<sup>a</sup> | 4.19<sup>a</sup> | 3.95<sup>a</sup> | 4.09<sup>a</sup> | 4.33<sup>a</sup> |

The decrease in the number of leaves was also reported in *Artemisia annua* after 0.2% colchicine treatment [10], *Plox drummondi* after 0.3% colchicine treatment, and *Phalaenopsis pulcherrima* after 3,000 ppm colchicine treatment [8,10,11]. In contrary, lower colchicine concentrations (0.05 – 0.2%) also decreased number of leaves in *Centella asiatica* after immersion duration from 12–24 hr [12]. In general, high colchicine concentration will decrease number of leaves to almost plant species. Colchicine may affect cell divisions of plant tissue region and spread through cells, interfere with cellular mechanisms and causes toxicity at high concentration, leading to slow growth and tissue damages [13,14,15].

3.3. Dry and fresh weight of bulbs

The results of colchicine treatment to dry and fresh weight of bulbs is presented in Figure 2 and 3. The highest fresh weight of bulbs was observed from D3T0 yielding 4.58 g in similar to D0T2 and D0T3. The lowest fresh weight was observed from D1T1 yielding 0.59 g. In contrary, other studies reported an increased fresh weight of *Fagopyrum tataricum* and [16] and *Echinacea purpurea* after 0.25% colchicine treatments [16,17]. In *Tanacetum paerthenium*, the fresh weight was increased after 0.05% colchicine treatment [18]. In *Centella asiatica*, the fresh weight was also increased after 0.05 – 0.2% colchicine treatments for 12–24 hr. However, we obtained a negative effect of colchicine to Doulu cultivar. Low moisture and other cell components in plants may contribute to decreased fresh weights [19].

![Figure 2. Effect of colchicine to fresh weight of bulbs](image-url)
The highest dry weight was observed from D0T2 yielding 1.57 g in similar to D0T3. The lowest dry weight was observed from D1T1 yielding 0.19 g. Previous studies have reported a reduced dry weight of fruits after colchicine treatment. Treatment of 0.16% colchicine for 10 hr reduced the dry weight of dried legumes yielding 6.35 g lower than control [20]. Low dry weight is a consequence of high colchicine concentration due to sub-optimal photosynthesis generated by a reduced number of leaves [19]. A reduced number of leaves means a lower level of chlorophyll which render plant to synthesize nutrients needed for growth and development [21].

Figure 3. Effect of colchicine treatment to dry weight of bulbs

Generally, the results between control and treatment groups were not significantly different. Delayed growth may cause microtubule disruption and polymerization, disrupting the formation of mitotic spindle leading to changes in chromosome numbers [11,22,23]. Other negative effects of colchicine to plants are irregular mitosis, stunted appearance and abnormal sizes [20,24,25]. A high colchicine concentration will lead to lower seedling viability so for application, certain low concentrations and immersion durations are needed to evaluate.

4. Conclusion
Growth of Garlic (*Allium sativum* L.) from Doulu cultivar is delayed due to higher colchicine concentration and longer immersion durations. The 0% colchicine treatment for 12 hr affected significantly on plant height, number of leaves and dry and fresh weight of bulbs of Doulu cultivar. The treatment of 3% colchicine for 0 hr also resulted in a significant effect to fresh weight of bulbs yielding 4.58 g.

Acknowledgement
The authors would like to express our gratitudes to Rizky Yudha Pratama, S.Si as the laboratory assistant of Biology that have helped in the implementation of the research in the laboratory of Physiology and Tissue Culture Plants, Universitas Sumatera Utra and local farmers who have helped in the implementation of the research in the field.

References
[1] Hardiyanto, Devy, NF, Supriyanto 2007 *J. Hort.* 17(4) 307 – 313.
[2] Rismunandar, 1989, *Membudidayakan 5 Jenis Bawang*, Bandung.
[3] Wibowo S, 2008, Budidaya Bawang Putih, Merah, dan Bombay, Jakarta.
[4] Wijaya MA, Anindita R, Setiawan B 2014 AGRISE14(2) 128 – 143.
[5] Badan Pusat Statistika, 2012, Laporan Perekonomian Indonesia, Jakarta.
[6] Gultom T 2016 Jurnal Biosains2(3) 165 – 172.
[7] Yulianti F, Purwito A, Husni A, Dinarti D 2015 J. Agron. Indonesia43(1) 66 – 71.
[8] Tiwari AK and Mishra SK, 2012 African Journal of Biotechnology11(39) 9336 – 9342.
[9] Ye YM, Tong J, Shi XP, Yuan W, Li GR 2010 Scientia Horticulturae124 95 – 101.
[10] Yunus A, Parjanto, Samanhudi, Hikam MP, Widyastuti 2018 IOP Conf. Series: Earth and Environmental Science 142 1-7.
[11] Soetopo L and Hosnia 2018 Bioscience Research15(2) 941 – 949.
[12] Kaensaksiri T, Soontornchainaksang P, Soonthornchareonnon N, Prathanturarug S 2011 Plant Cell Tiss Organ107 187–194.
[13] Dermen H 1940 The Bot. Rev.6 599 – 635.
[14] Damayanti F, Mariska I 2003 J. Ilmiah Mulawarman Scientifie2 12 – 17.
[15] Vichiato MRM, Vichiato M, Pasqual M, Rodrigues FA, Castro DM 2014 Crop Breeding and Applied Biotechnology14 154 – 159.
[16] Wang LJ, Shen MY, Wen PC, Du JY 2017 Botanical Studies58(2) 1 – 12.
[17] Abdoli M, Moeini A, Badi HN 2013 Acta Physiol Plant35 2075 – 2083.
[18] Majdi M, Karimzadeh G, Malboobi MA, Omidbaigi R, Mirzaghaderi G 2010 Hort. Science 45(2) 16 – 21.
[19] Haryanti S, Hastuti RB, Setiari N, Banowo A 2009 Jurnal Penelitian Sains & Teknologi10 11-120.
[20] Sinaga E J, Bayu E S, Hasyim H 2014 J. Online Agroekoteknologi2(3) 1238 – 1244.
[21] Loveless A.R, 1991, Prinsip-Prinsip Biologi Tumbuhan untuk Daerah Tropik, Jakarta.
[22] Suryo, 2007, Sitogenetika, Yogyakarta.
[23] Yan HJ, Xiong Y, Zhang HY, He ML 2016 Breeding Science66 169 – 174.
[24] Ascough GD, Staden JV, Erwin JE 2008 HortSci.43 2248 – 2251.
[25] Jensen CJ 1974 Proceedings of the First International Symposium 153 – 190.
[26] Trojak-Goluch A and Skomra U 2013 Breed. Sci.63(4) 393 – 399.