The effects of colchicine concentration and soaking time on formation of leaves and roots of katokkon (*Capsicum chinense* Jacq.) in vitro

Kasmiati¹, R Sjahri³, M Riadi³, I Ridwan⁴ and Trisnawaty AR⁵

¹Master Student, Agrotechnology Program Study, Universitas Hasanuddin, Makassar, 90245, Indonesia
²Laboratory of Plant Bio-science and Reproduction Biotechnology, Faculty of Agriculture, Universitas Hasanuddin, Makassar, 90245, Indonesia
³Laboratory of Plant Breeding and Seed Science Faculty of Agriculture, Universitas Hasanuddin, Makassar, 90245, Indonesia
⁴Department of Agronomy, Faculty of Agriculture, Universitas Hasanuddin, Makassar, 90245, Indonesia
⁵Department of Agrotechnology, Faculty of Science and Technology, University of Muhammadiyah Sidenreng Rappang, South Sulawesi

E-mail: rinaldi.sjahril@gmail.com

Abstract. Katokkon chili is a plant of the Solanaceae family which has a unique characteristic of fruit shape, spiciness level and also has a sweet taste. In addition, it is a diploid plant with 12 pairs of chromosomes (2n=24). Quality improvement and production of katokkon chili can be done by doubling the chromosomes. This research aimed to study the effect of several concentrations and time of immersion of colchicine on the buds of the katokkon chili shoots. Research activity was carried out by soaking the buds of the katokkon at several concentrations of colchicine (0.25%, 0.50%, 0.75%, and 1.00%) for some immersion times (1.5 hours, 3 hours and 4.5 hours) resulted in a total of 12 treatment combinations and 1 control. The shoots were grown in vitro on MS media until the roots and leaves grow. The characters observed were the formation of roots and leaves which were then compared to controls. The results show that the concentrations of colchicine (0.25%, 0.50%, 0.75%, and 1.00%) had a negative effect on the katokkon chili which was characterized by no leaf formation as in control plants (0%). The application of colchicine did not affect root formation, it can be seen from the emergence of roots in shoots which are induced by colchicine similar to control plants without colchicine treatment.

1. Introduction

Chili is a vegetable commodity that is very useful, has a high economic value, and has become a daily need of the Indonesian people, both in the scope of households and industries so that demand is increasing which needs to be accompanied by efforts to increase production. Katokkon chili (*Capsicum chinense* Jacq.) is a special chili from Tana Toraja and North Toraja regencies in the Southern part of Sulawesi Island in Indonesia. The plant is much in demand by the local community because it has a distinctive aroma and specific taste with a very spicy taste and also has a sweet taste in
the fruit flesh. The shape of this type of chili is also unique, which is similar to paprika but with a smaller size.

Katokkon chili belongs to the family of Solanaceae. Most families of Solanaceae are diploid plants with 12 pairs of chromosomes (2n = 24) [1]. Polyploidization is one of the methods used to duplicate plant chromosomes to improve the quality of plants such as larger fruits. Besides polyploidization fruit can also produce larger leaves, darker green color, thicker and better tolerance to abiotic pressure [2]. Plant polyploidy can be used by various methods such as the use of colchicine and oryzalin which have been widely reported to be successful in inducing polyploidy in some plant species [3,4]. Chromosome multiplication in vitro can be induced by several antimitotic agents and the most commonly used are colchicine, oryzalin and trifluralin [5].

Colchicine is the most widely used agent for chromosome multiplication [5,6]. The number of chromosomes doubled by colchicine inhibits the formation and activity of spindle threads during mitotic cell division and prevents nuclei and cells from defending [7]. Doubling the number of chromosomes using colchicine is highly dependent on the concentration of colchicine given [5]. According to Syafifudin [8], colchicine will work effectively at concentrations of 0.01-1.00% or 0.001-1.00% with the duration of soaking colchicine ranging from 3-24 hours. Concentration and induction time are important parameters and clear interactions between the two. Doses that are too low will not give results and too high a dose can kill plants. Concentrations or induction times that are too high can give multiplied results with higher ploidy levels than desired [9].

In vitro polyploidization has a greater potential for success with a controlled and standardized environment [5]. The induction of colchicine in vitro gives better results [10]. In vitro induction was successful in producing tetraploid plants in cassava at a concentration of 0.10% for 96 hours [11], concentrations of 0.05 - 0.1% for 24 - 72 hours in shoots of Capsicum annuum (L.) producing tetraploid plants [10], in paprika plants (Capsicum annuum cultivar 'Shishito No.562') [12].

Until now there has not been found any research on polyploidy induction in katokkon chili of Tana Toraja and North Toraja regencies by using high concentrations of colchicine with shorter immersion time. This study aims to determine the effect of high concentrations of colchicine with shorter soaking in shoots of the katokkon chili shoots.

2. Methodology
Research activities were carried out at the Laboratory of Biosciences and Plant Reproductive Biotechnology, Teaching Industry, Universitas Hasanuddin from July to September 2019. The source of the explants was taken from the katokkon chili seeds from the survey results in Tana Toraja and North Toraja Regencies which were then germinated using ½ MS media in the Laminar Air Flow Cabinet (LAFC).

The induction of colchicine was conducted on shoot buds from 2 weeks after planting seedling. The shoots were cut from the plantlet using a scalpel knife by removing the stems and all the leaves then soaked and shaken using a shaker in a colchicine solution that has been diluted previously using distilled water and sterilized using millipore 0.22 µm in the Laminar Air Flow Cabinet (LAFC). The concentration of colchicine used was 0%, 0.25%, 0.50%, 0.75% and 1.00% and soaking time was 1.5 hours, 3 hours and 4.5 hours. The shoots from the colchicine induction were rinsed with distilled water and grown on MS media until the roots and leaves grew and then observed.

3. Results and discussion
The treatment of colchicine has no noticeable effect on the buds of the katokkon chili shoots (Capsicum chinense Jacq.). Growth of colchicine-induced katokkon chilies (0.25%, 0.50%, 0.75% and 1.00%) were found to be inhibited, indicated by leaves that are not perfectly formed, curled, and stunted while control plants (A) grow perfectly (Figure 1). On the other hand, roots of the colchicine-induced plants were grown normally as control plants.

The incomplete formation of leaves on colchicine induced katokkon chili is possible because the concentrations given are too high so that it is inhibiting growth. Research from Kulkani and Borse [10]...
showed that at concentrations above 0.2% in *Capsicum annuum* L. cotyledon germination is thicker and the hypocotyl swells and at a concentration of 0.4% resulting in germination of death.

**Figure 1.** Katokkon chili growth at 5 weeks after colchicine treatment and soaking time: A (control), B (0.25%, 1.5 hours), C (0.25%, 3 hours), D (0.25%, 4.5 hours), E (0.50%, 1.5 hours), F (0.50%, 3 hours), G (0.50%, 4.5 hours), H (0.75%, 1.5 hours), I (0.75%, 3 hours), J (0.75%, 4.5 hour), K (1.00%, 1.5 hours), L (1.00%, 5 hours), M (1.00%, 4.5 hours)
Stunted growth and thicker leaves can be characterized as polyploidy plants [13], but with too high a dose of colchicine can cause the plant to die. Higher concentration colchicine and longer soaking time will result in the obnoxious appearance of plants, cell damage or even death.

4. Conclusion
Concentration of 0.25% - 1.00% with a soaking time of 1.5 - 4.5 hours was found to exceed the concentration required, hence had a negative impact on the plants indicated by inhibition of leaf formation in the katokkon chili plants grown in vitro.

Acknowledgment
This research was supported by the Ministry of Research, Technology and Higher Education of Republic Indonesia, therefore authors would like to show appreciation for the support in funding the research.

References
[1] Aristya G R, Zuyyina C, Febiansi D, Ayuningsih R, Prasiwi K D, Nurwijayanti T A, and Renaldi B 2019 Karakterisasi kromosom spesies anggota familia solanaceae Biotropic: The J. of Trop. Biol. 3 24-38.
[2] Shao J, Chen C, and Deng X 2003 In vitro induction of tetraploid in pomegranate (Punica granatum) Plant Tissue Organ Cult. 75 241–246.
[3] Pliankong P, Ard P S, and Wannakairoj S 2017 Effects of colchicine and oryzalin on polyploidy induction and production of capsaicin in Capsicum frutescens L Thai J. of Agricult. Sci. 50 108-120.
[4] Rey H Y, Sansberro P A, Collavino M M, Daviña J R, González A M, and Mroginski L A 2002 Colchicine, trifluralin, and oryzalin promoted development of somatic embryos in Ilex paraguariensis (Aquifoliaceae) Euphytica 123 49–56.
[5] Dhooghe E, Van Laere K, Eeckhaut T, Leus L, and Van Huynlenbroeck J 2011 Mitotic chromosome doubling of plant tissues in vitro Plant Cell, Tissue and Organ Cult. (PCTOC) 104 359-373.
[6] Takahira J, Cousin A, Nelson M N, and Cowling W A 2011 Improvement in efficiency of microspore culture to produce doubled haploid canola (Brassica napus L.) by flow cytometry. Plant Cell, Tissue and Organ Cult. (PCTOC) 104 51-59.
[7] Ascough G D, Van Staden J, and Erwin J E 2008 Effectiveness of colchicine and oryzalin at inducing polyploidy in Watsonia lepida NE Brown HortScience 43 2248-2251.
[8] Syaifudin A, Ratnasari E, and Isnawati I 2013 Pengaruh pemberian berbagai konsentrasi kolkhisin terhadap pertumbuhan dan produksi tanaman cabai (Capsicum annuum) varietas lado F1 LenteraBio 2 167-171.
[9] Allum J F, Bringloe D H, and Roberts A V 2007 Chromosome doubling in a Rosa rugosa Thunb. hybrid by exposure of in vitro nodes to oryzalin: The effects of node length, oryzalin concentration and exposure time Plant Cell Rep. 26 1977–1984.
[10] Kulkarni M, and Borse T Induced polyploidy with gigas expression for root traits in Capsicum annuum (L.) Plant Breed. 129 461–464.
[11] Mondin M, De Mello e Silva P A K X, Latado R R, and Mourão Filho F D A A 2018 In vitro induction and regeneration of tetraploids and mixoploids of two cassava cultivars Crop Breed. Appl. Biotechnol. 18 176–183.
[12] Takizawa K, Ishikawa K, Nunomura O, and Ito T Ploidy level effect on physiology of pepper plant as affected by fruit loading Acta Hortic. 779 689–697.
[13] Indira C, and Abraham S 1977 Morphological Induced and Cytological Studies on a Radiation Polyploid in Capsicum annuum of Botany, of Kerala Cytologia (Tokyo) 42 371–375.