In vitro polyploidy induction of foxtail millet (*Setaria italica* (L) beauv) cv. buru hotong using colchicine treatment

Asep Rodiansah¹*, Melisa Ika Puspita² & Iriawati²

¹Department of Agrotechnology, Faculty of Agriculture, Medan Area University, Jl. Kolam no.1 Medan Estate 20223, Indonesia
²School of Life Sciences and Technology, Institut Teknologi Bandung, Jalan Ganesha 10, Bandung 40132, Indonesia

*Email: asep343@gmail.com

Abstract. In vitro polyploidy induction of foxtail millet (*Setaria italica* (L) Beauv) was done using callus from the basal leaf of 10-day old seedlings. Callus was treated using three different concentrations of colchicine (0, 125, 250 and 500 ppm) and two exposure times (24 and 48 hours). Results showed that colchicine declines the survival rate and the number of shots on all combinations. Putative polyploidy plant was produced on the combination of K3T1 (500 ppm colchicine; 24 hour exposure time). Based on field experiment data, putative polyploid plant has smaller characteristics in the term of plant height, the number of tillers, and number of seed per panicle than the diploid plant. However, leaf width, panicle length and diameter, seed size, and weight of 100 seeds of putative polyploid plant bigger than the diploid plant.

1. Introduction

Foxtail Millet (*Setaria italica* (L) Beauv.) is an annual and self-pollinating crop of the Setaria genus. This plant has many ranges of benefits. In China and India, the seeds are used as food. It seeds also used as feed for poultry while the green straw used as feed for stock, moreover foxtail millet seeds are used for mine land recovery due to its good adaptability in dry and heavy metals soil [1]. In Indonesia, the use of foxtail millet seeds as a food source is still limited. Whereas the foxtail millet seeds nutrition's contents are better than rice. 100 g of dried foxtail millet seeds contain 351 kcal, 6.7 g crude fiber, 11.2 g protein, 4.0 g fat, 63.2 g carbohydrates, 31 mg calcium (Ca), 2.8 mg iron (Fe), 0.59 mg B1 vitamin (thiamine), 0.11 mg B2 vitamin (riboflavin), and 3.2 mg niacin [2].

In Indonesia, foxtail millet’s cultivation has a lot of problems. Conventional breeding is not to develop due to limited genetic diversity. To solve this problem, in vitro breeding is an efficient alternative solution that would result in crop improvement. The mutation breeding method using chemical mutagen application can be used for increasing the genetic diversity in this plant. One of the chemical mutagens is colchicine. Colchicine is a chemical mutagen used for the induction of polyploidy in many plants [3]. Polyploidy would not only improve the quality and quantity of crop yields but also produce a unique plant which has a resistance to pests and others abiotic stress.

In vivo polyploidy induction of foxtail millet using colchicine has been done by several breeders. Tetraploid foxtail millet was obtained though seeds in 0.25% colchicine for 4 hours exposure in yellow sand cultivar [4]. However, in vitro polyploidy induction in foxtail millet cultivar Buru Hotong
never been done. Therefore, this research is aims to obtain the different polyploid foxtail millet as a genetic resource that be used in the foxtail millet crop improvement program.

2. Materials and Methods

2.1. Callus Induction and Plantlet Regeneration

Leaf base of 10-day old seedlings was used as a source of explant. This explant was derived from foxtail millet seeds germinated on MS basal media [5] without the addition of plant growth regulators (PGRs). Explants were cultured in MS basal media supplemented with 2 mg/L 2,4-D, 800 mg/L casein acid hydrolysate, 1 mg/L proline, 5 mg/L AgNO₃, 3% sucrose and 2.5% gelrite (with pH 5.9) [6]. Cultures were incubated for 4 weeks in a 16/8 h light/dark photoperiod at 25 ºC.

2.2. Colchicine Treatment and Plantlets Regeneration

Colchicine solution was made by dissolving colchicine on half-strength MS basal media (½ MS). Then, callus was immersed in colchicine solution with various concentrations and exposure time (Table 1).

| Colchicine Concentration (ppm) | Exposure Time (hour) |
|-------------------------------|----------------------|
| 0                             | K0T1                 |
| 125                           | K1T1                 |
| 250                           | K2T1                 |
| 500                           | K3T1                 |

Soaked-colchicine callus was transferred into MS basal media supplemented with 2 mg/L BAP, 800 mg/L casein acid hydrolysate and 1 mg/L proline for shoot induction. The induced shoots were transferred into MS basal media without the addition of PGRs (MS0) for root induction. These cultures were incubated in a 16/8 h light/dark photoperiod at 25 ºC.

2.3. Chromosome Observation and Plantlets Acclimatization

The squashing method was used for chromosome observation. Prior to acclimatization, plantlets were placed in a room with light intensity above 1000 lux for 7 days hardening. Then, plantlets were removed from the bottle, rinsed with tap water followed by soaking in 1.5 g/L of dithane (fungicides) and 1 g/L of bactericide for 10 minutes. Plantlets were planted into rice husk charcoal growing media.

2.4. Field Experiment

7-day old plantlets in rice husk charcoal growing media were transferred into 30 cm diameter polybags. The polybags consisted of three different growing media such as soil, rice husk charcoal, and manure in a ratio of 1:1:1. Polybags were placed in a place full of sun. Then, plants fertilized with 10 g NPK fertilizer in a ratio of 15:15:15.

3. Results and Discussion

The effects of colchicine on the growth of explants were assessed three weeks after treatment (Figure 1). Colchicine treatments resulted in a lower survival rate than control, although it has various effects among of colchicine concentrations. The lowest survival rate was produced by K2T2 treatment. Most of the treatment resulted in higher browning callus than control. Browning callus probably caused by colchicine which affected cell death by inhibiting spindle tubulin polymerization [7]. It impacted cell division retardation and caused cell death. Furthermore, Ascough et al. [8] have explained that higher colchicine concentration retarded the survival rate of Watsonia lepida N.E. Brown. Viehmannova et al. [9] also reported that colchicine has decreased the survival rate of yacon (Smallanthus sonchifolius).
Shoot formation has detected 4 weeks after treatment at first subculture. In the second subculture, only 4 combinations produced shoots. In this experiment, the difference in time exposure shows a distinctive result. The result showed that 48 hours is lethal exposure time if it was combined with colchicine because it didn’t produce shoot (Figure 2). Colchicine treatment decreased the number of shoots. It was probably the effect of colchicine which made cell death, so it impacted the retardation of callus development into the shoot. This result was in accordance with the result of Benici et al. [10] which showed that high concentration colchicine and longtime exposure did not induce shoots and caused the complete death of the explants. Furthermore, Nilanthi et al. [11] explained that callus was treated with 60 ppm colchicine reduced shoot formation in the coneflower plant and the number of shoots declined when the callus was exposure longer at colchicine solution.

In this experiment, the shoot which was produced from K2T1 become browning and died when it was transferred at the MS0. It was probably due to the phenolic compound produced when the shoots cut and transferred on MS0. Plantlets with different morphological were obtained on K3T1. Then, in this experiment, only K0T1 plantlets (control) and K3T1 plantlets were used. K3T1 Plantlets have a bigger root size and crested (Figure 3b). The plantlets were indicated a polyploid plant. According to Comai [12] Increasing the genomic content of an organism in line with the rise of cell volume, with the consequent shift in the relationship between the tridimensional and bidimensional. Some polyploid organisms show bigger of organ size. Ahmadi et al [13] reported that tetraploid plant obtained from the Egyptian henbane plant (Hyoscyamus muticus) was exposed to a 0.2 % colchicine solution (w/v) has root and leaf’s size bigger than control.
Putative polyploid was obtained on K3T1 plantlets. Based on cytological observation, K0T1 plantlets has fewer chromosome number than K3T1 plantlets (Figure 4).

The putative polyploid plant has a different morphological form to diploid plants (Table 2). The putative polyploid plant is shorter and has a fewer number of tillers than diploid plants (Figure 5). This has the same result as the other researches with different plants experiment subject. Alam et al. [14] explained that colchicine produced tetraploid potato which has a shorter plant height than diploid potato. Ajijah and Bermawi [15] also reported that the tetraploid aromatic galangal (Kampferia galanga) is shorter and produces fewer tillers than the diploid plant.
Figure 5. Diploid K0T1 Plant (a) and Putative Polyploid K3T1 Plant (b)

Table 2. The morphological comparison in diploid K0T1 and putative polyploid K3T1 plant

| Parameter                  | Plants                                      |
|---------------------------|---------------------------------------------|
| Plant Height              | Diploid K0T1 > Putative Polyploid K3T1      |
| Leaf Width                | Diploid K0T1 < Putative Polyploid K3T1      |
| Leaf Colour               | Diploid K0T1 < Putative Polyploid K3T1      |
| Panicle Length and Width  | Diploid K0T1 < Putative Polyploid K3T1      |
| Number of Seeds           | Diploid K0T1 > Putative Polyploid K3T1      |
| Seed Size                 | Diploid K0T1 < Putative Polyploid K3T1      |

Putative polyploid K3T1 plant has a wider leaf than diploid K0T1 (Figure 6). It also has a darker green color leaf than the diploid plant. The result indicates the chlorophyll content has increased in putative polyploid K3T1 plant. Sukamto [16] reported that the putative polyploid leave has a darker green color than the diploid plant on arrowroot (*Maranta arundinacea*). Mathura et al. [17] explained that the chlorophyll content in tetraploid acacia (*Acacia mearnsii*) is higher than the diploid plant which makes the leaf has a darker green color.
Figure 6. Comparison of Diploid K0T1 and Putative Polyploid K3T1 Leaves

The putative polyploid plant has a shorter and wider panicle than control and it can be seen that the panicle diameter of putative polyploid and control showed a significant difference (Figure 7a). Based on this experiment result, colchicine treatment increased the volume of the cell on the panicle organ which leads to the enlargement of panicle length and diameter. Shahid et al. [18] explained that allotetraploid rice has a longer and wider panicle than polyploid rice. The risen of organ size was also reported by Wu et al. [19], in which colchicine treatment with a concentration of 0.2 % and 0.4% produced adzuki bean (Vigna angularis) tetraploid that has longer and wider pod than the diploid plant.

Then, seed per panicle on putative polyploid K3T1 plant has fewer numbers than diploid K0T1 plant. Based on this result, K3T1 plant might probably produce a polyploid plant. Plant has not adapted with a new genomic. The cells undergo abnormal meiosis and it leads to a reduction in the number of seeds. Gupta and Yahsvir [20] reported that abnormal meiotic was found on hexaploid whorled pigeon grass (Setaria verticillata) which showed an univalent chromosome on metaphase I. It would retard gametogenesis and decrease sexual reproduction capability. Furthermore, Bonato. et al. [21] explained that abnormal meiosis also found on palisade grass (Brachiaria brizantha) and impacted in decreasing on pollen fertility and low pollination number which caused low seed formation.

Figure 7. (a) Comparison of Diploid K0T1 and Putative Polyploid K3T1 Panicles, (b) Comparison of Diploid K0T1 and Putative Polyploid K3T1 Seed Size

In addition, Putative polyploid K3T1 has a bigger seed size than diploid K0T1 (Figure 7b) and the weight of 100 seeds also heavier than control. The weight increased might be caused by the plant cell architecture changes into bigger size and volume. The polyploid plant has the ability to synthesize components, for instance, starch grains and protein. These components might be increasing the weight of seed. This result in line with Haryanti [22] experiment which reported that the green bean plants (Phaseolus vulgaris) treated by 0.20% colchicine formed bigger seed and higher protein content than
control. Nura et al. [23] also reported that sesame plants (Sesamum indicum) treated by 0.1-2 mM colchicine produced 1000 seeds bigger and heavier in all treatments than control.

4. Conclusion
Based on the present results, it can be concluded that the K3T1 (500 ppm colchicine concentration, 24 hours exposure) combination produced polyploid plant on foxtail millet (Setaria italica (L) Beauv) cv. Buru Hotong. The field experiment data showed that putative polyploid plant has smaller characteristics in the term of plant height, the number of tillers, and number of seed per panicle than the diploid plant. However, leaf width, panicle length and diameter, seed size, and weight of 100 seeds of putative polyploid plant bigger than the diploid plant.

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