Colchicine induced polyploidy in Common Ice plant

*Mesembryanthemum crystallinum* L

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Abstract. The aim of this research was to obtain optimum concentration with mutation breeding techniques by using colchicine for polyploid induction in the common ice plant *Mesembryanthemum crystallinum* L. The common ice plant *Mesembryanthemum crystallinum* L wild type seed were soaked in five levels of colchicine concentration: 0% (control), 0.01%, 0.05%, 0.10% and 0.20% for 24 hours. Seeds were germinated in petri dish using growth medium with compound of 1.15 gr ml half strength MS salts, 0.5 ml B5 vitamins, 0.2% (w/v) Sucrose 1gr, 25 mg L⁻¹ myo-inositol and Agar. After 15 days, seeds germinated and then the young plant were transferred to a fresh medium soil. The observation parameters consisted of leaf thickness, width of the leaf, and length of the leaf, calculated from 1st month until 8th month. The observational data obtained were analyzed in variance and if the treatment has significant effect, then the follow up test will be done with DMRT test at 95% confidence level. For polyploidy analysis was conducted using Flow Cytometry. The results show that colchicine can affect the vegetative growth and the number of chromosomes from *Mesembryanthemum crystallinum* L. plants. The colchicine treatment of 0.10% was the best treatment compared to other treatments in the parameters of the length, width and thickness of the leaves. Use of colchicine treatment of 0.10% changed the chromosome number of the *Mesembryanthemum crystallinum* L. plant from diploid (2n = 18) to Octaploid (8n = 108).

1. Introduction

*Mesembryanthemum crystallinum* is an annual plant native from Nambian in South Africa and is widely distributed in western Australia, southwest US, Pacific coast of Mexico and Chile [1]. This plant has a high tolerance to drought and salinity. This plant can complete its life cycle in soils containing NaCl at concentrations equivalent to seawater (about 500 mM) [2].
This plant can be utilized for consumption as a vegetable, as a substitute for soap by means of leaves being crushed first, used as herbal medicine, cosmetic raw materials [3] and planted as ornamental gardens [4]. *Mesembryanthemum crystallinum* is also used as a role model in the study of plant physiology [1]. Based on the salt accumulation ability [5], *Mesembryanthemum crystallinum* can also be used as a bioremediation plant [6]. In addition, *Mesembryanthemum crystallinum* contains high myo-inositol, pinitol, and proline, so it can be indicated that this plant has high potential as a functional food with high polyol content [7].

Mutation breeding techniques have been widely used to obtain various plant varieties. By using mutation breeding techniques, we can more quickly obtain new varieties than by using conventional breeding techniques. Through this mutation technique, one of the characteristics of a variety can be improved without changing the nature of others [8]. One of the mutation breeding techniques for the improvement of properties is the polyploidy induction technique. Polyploidized plants will have a larger size of flowers, fruit, and seeds, wider and thicker leaf size, greener leaf color, and longer vegetative age compared to diploid (normal) plants.

With the development of technology, mutation breeding techniques are now being developed using colchicine mutagen. Colchicine is a mutagen that prevents the formation of microtubules and is usually used to multiple chromosomes. The application of colchicine in vivo can be done by soaking the seeds, plant roots and sprouts or by dripping the colchicine on sprouts or seeds. Based on the description above, research will conducted on polyploidy at the common ice plant *Mesembryanthemum crystallinum* L. at various levels of concentration using colchicine.

2. Materials and method
This research was conducted in Food Production Laboratory at Kagawa University from December 2018 until September 2019. The trial was arranged based on a Randomized Block Design with three replications. Seeds of the common ice plant *Mesembryanthemum crystallinum* wild type were induced using colchicine by soaking treatment.

Seeds of the common ice plant, *Mesembryanthemum crystallinum*, were dipped into 2.5% (v/v) sodium hypochlorite for 20 minutes and then rinsed 10 times with distilled water. Seed were soaking in different concentration of Colchicine (0.01%, 0.05%, 0.10%, and 0.20%) in 24 Hours. Following soaking, seed were rinsed 5 times with distilled water. Seeds were germinated in petri dish using growth medium with compound 1.15gr ml half strength MS salts [9], 0.5 ml B5 vitamins, 0.2% (w/v) Sucrose 1gr, 25 mg L⁻¹ myo-inositol and Agar [10]. After 15 days, seeds germinated and then the young plant were transferred to a fresh medium, soil.

The observation parameters were leaf thickness, width of the leaf, and lenght of the leaf, calculated from 1st month until 8th months. The observational data obtained were analyzed in variance and if the treatment has significant effect, then the follow up test will be done with DMRT test at 95% confidence level. Polyploidy analysis was carried out using Flow Cytometry. Nuclear sampel were prepared from fresh rosette leaf (approximately 25 mm²) of treated plants *Mesembryanthemum crystallinum* L. Sample tissue were chopped altogether with a razor blade in a plastic petri dish with 0.2 ml drop of nucleiid extraction buffer (solution A of the Partec kit) and incubated for 10 minutes at room temperature and filtered through a 30 μm nylon mesh. The flow though was combined with 1 ml of DAPI solution and incubation for 1 minute at room temperature. Flourescence intensity of the nucleiid in the filtrate was measured using flow cytomtry (FCM) equipped with a mercury lamp.

3. Results and discussion

3.1. Germination and survival rate
Induction of polyploidy mutations through inhibition of spindle yarn formation activity in the process of mitosis in somatic cells is possible using colchicine compounds. Polyploidy induction mutations have been successful in several plants such as Stevia Plants [11], Taro Plants [12], and Dendrobium
Plants [13]. This method requires accuracy in the use of planting materials, concentration and soaking time with colchicine to obtain the expected results.

**Figure 1.** Germination rate of *Mesembryanthemum crystallinum* L.

Increasing the concentration of colchicine does not reduce the percentage of plant life of *Mesembryanthemum crystallinum* L. as seen in Figure 1. This is thought due to the condition of the cells in each different plant so that the sensitivity of cells is different in responding to the treatment of colchicine concentration. Colchicine is a mutagen that prevents the formation of spindle threads during cell division, because in the nucleus the colchicine binds to α and β microtubules during cell division. Colchicine causes mutations causing prevention of spindle thread formation when exposed to colchicine cells, hence causes the chromosomes in cells that have multiplied are not separated towards the poles resulting in polyploidy cells. Changes in the amount of genetic material in plant cells after the treatment of colchicine causes rearrangement in which mutated cells must be able to adjust the conditions of their natural cell biology with different chromosome numbers. Mutant cells that cannot adapt cannot grow and will be deleted during cell division in the process of plant growth.

**Figure 2.** Survival rate of *Mesembryanthemum crystallinum* L. on 8th month.
Figure 2 shows that immersion of *Mesembryanthemum cristallinum* L. in various concentrations of colchicine had an effect on the life percentage of this plant. Treatment control (0%) and soaking of 0.01% colchicine did not show any mortality up to 8 months after planting (MAP), while immersion at a concentration of 0.05%, 0.10% and 0.2% cause death since 2 MAP. Observations at 1 MAP show 100% live seed in the control and all colchicine concentrations. The results of observations at the age of 8 MAP show that the percentage of seed life decreased compared to the age of 1 MAP. Control and immersion in colchicine 0.01% produced a percentage of life of 100%, soaking colchicine 0.05% produced a life percentage of 73.33%, soaking colchicine 0.10% produced a life percentage of 66.67%, and immersion of colchicine 0.20% produced a life percentage of 25%.

3.2. Morphological characteristics

Effect of colchicine concentration treatment on length, width and thickness of *Mesembryanthemum cristallinum* L leaf are shown in Figure 3. The chromosome doubling due to colchicine is characterized by rapid individual growth after the inhibition phase. The growth speed of each individual is different because colchicine has a different sensitivity in each concentration in doubling the chromosomes. Each species has a different response to the application of colchicine. This can also due to cell age and somaclonal variation in these individuals [14]. Colchicine causes slow cell division and also causes slow formation and development of primordial leaves. Leaves are the main photosynthetic organ, thus determining the amount of assimilate produced that is needed during plant growth and development. Chloroplasts in plants develop from differentiated micro structures called proplastids. Proplastids divide during mitosis. When the seeds are treated with colchicine, mitosis in embryonic cells is followed by proplastid cleavage, even though the chromosomes that have multiplied may fail to separate from the anaphase due to damage to the formation of microtubules which make up spindle threads by colchicine, resulting in plants that have higher levels of chlorophyll and resulted in the color of the leaves of the plants being treated more concentrated than the color of the leaves of the plants on the untreated. The large amount of chlorophyll as the main pigment in photosynthesis increases the efficiency of photosynthesis so that more dry matter can be stockpiled by plants.
Larger leaf size in the plant treated with colchicine has a positive value for the growth of the plant. Larger leaves cause the photosynthetic reaction to take place more maximum. In larger leaves, the absorption of sunlight takes place more optimally than smaller leaves in the environment of maximum sunlight intensity. In addition, the carrier file which is enlarged due to the enlargement of plant cells is also very influential in the transport of better assimilation and water products so that the plants grow taller, the stem is bigger, and the flowering time is faster. Plant cells tend to be more resistant to even higher concentrations of colchicine. Liquid colchicine can diffuse rapidly through plant tissue and can be circulated through the vessel system so that it can directly affect cells during mitosis [15]. In addition, the shape in the form of liquid can also stop plant seed dormancy so that the seeds germinate. Mutations can occur in each part of the plant and plant growth phase, but more occur in the active part of cell division such as shoots, seeds, etc.

![Comparison between control plants and plants treated with Colchicine](image)

**Figure 4.** Comparison between control plants and plants treated with Colchicine (A) Control Plant; (B) Plant with Colchicine Treatment.
Each stem segment produces one leaf stem except for the first leaf in the form of a pair of leaves facing each other and each is a single leaf. Variations are shown from the results of observations, namely that there were the first leaves totalling of 2 leaves in the main stem segment, one of which was larger than the leaf before it (Figure 4). This is thought to be the result of mutations, namely in the form of khimera from the treatment of colchicine in the seed phase. However, along with plant growth and development, no leaf morphology has been found like this until the plants are 8 MAP.

3.3. Polyploidy analysis of *Mesembryanthemum cristallinum* L.

Polyploidy analysis using the flowcytometry against *Mesembryanthemum cristallinum* L. is shown in Table 1. Some examples of the results of the flowcytometry analysis are displayed in the form of histograms (Figure 5).

| Colchicine Concentration (%) | Stage Life of *Mesembryanthemum cristallinum* L. |
|-----------------------------|-----------------------------------------------|
|                             | Seedling  | Juvenile | Adult   |
| 0                           | 2n        | 2n       | 2n-4n   |
| 0.01                        | 2n        | 2n       | 2n-4n   |
| 0.05                        | 2n        | 2n-4n    | 2n-8n   |
| 0.1                         | 2n        | 2n-4n    | 2n-8n   |
| 0.2                         | 2n        | 2n-4n    | 2n-8n   |

The treatment of colchicine has an influence in inducing mutations, as shown this recent study, some individuals found to be polyploid (tetraploid), while others remain to be diploid. Based on Table 1, plants at the adult plant stage, with the treatment of 0% (control) and 0.01% colchicine produced diploid plants, while the plants treated with higher concentration of 0.05%, 0.10%, and 0.20% produced octoploid plants. Colchicine treatments of 0.05, 0.1 and 0.2% with 1 day immersion can induce polyploidization on *Mesembryanthemum cristallinum* L. In the plant *Acacia carpa*, colchicine 0.2% for 24 hours is most efficient in producing tetraploid plants [16]. Each plant has a different response to colchicine depending on the type and organ of the treated plant. Therefore there is a diversity of results from colchicine concentration and soaking time which are used to induce polyploidy optimally.

Figure 5. Flowcytometri histogram of *Mesembryanthemum cristallinum* L. Control Plant; (B) Plant with 0.01% Colchicine Concentration; (C) Plant with 0.05% Colchicine Concentration; (D) Plant with 0.10% Colchicine Concentration; (E) Plant with 0.20% Colchicine Concentration.
Colchicine affects the growth of *Mesembryanthemum crystallinum* L. plants by influencing the preparation of microtubules in cells. Spindle as a mitotic apparatus, composed of microtubules in a dublet form. Microtubule dublets are composed of two singlet microtubules, while singlet microtubules are composed of protofilament. Protofilament is a polymer of tubulin protein dimers a and b. The work of colchicine basically is to inhibit the formation of microtubules. It will bind to tubulin dimers a and b, so protofilament is not formed. With no protofilament formed, singlet microtubules and dublet microtubules are not formed, resulting in no spindle formation. With the inhibition of cleavage spindle formation, the chromosomes that are already in a doubling state are not divided in the opposite direction, thus forming polyploid cells. In polyploid cells, cell size and cell nucleus will increase as the chromosome number increases.

In plants that have undergone polyploidization, an increase in the number of chromosomes in their cells will take place. The increase in the number of chromosomes in cells also results in increased activity of genes that function to regulate various metabolism in cells, including protein synthesis, resulting in an increase in the production of plant growth hormones which act as a driver of plant growth. Based on the results of the study it is shown that higher concentration affect the growth in the direction of increasing plant biomass.

4. Conclusions

Colchicine can affect vegetative growth and the number of chromosomes from *Mesembryanthemum crystallinum* L. plants. The colchicine treatment 0.10% is the best treatment compared to other treatments in the parameters of the length, width and thickness of the leaves. The chromosome number in the Colchicine concentration of 0.10% changes the chromosome number of the *Mesembryanthemum crystallinum* L. plant from diploid (2n = 18) to Octaploid (8n = 108).

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