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The chromosome numbers of *Panax vietnamensis* Ha et Grushv

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ABSTRACT

The somatic chromosome number of Panax vietnamensis Ha et Grushv. was determined to be 2n = 24, based on the hypotonic shock method by potassium chloride solution. In this study, we investigated the effect of potassium chloride and colchicine solutions on chromosome dispersion of Panax vietnamensis at different concentrations. The treatment using 0.2% KCl solution for 45 minutes combined with 0.05% colchicine solution for 2 hours subsequently resulted in proper hypotonia. The result showed that chromosomes were evenly dispersed. The hypotonic shock method is deemed to be effective in equally distributing chromosomes. The result can be applied in cell genetic studies and selective breeding programs for Panax vietnamensis.

1. INTRODUCTION

Chromosomal analysis is one of the prerequisite requirements of genetic studies and breed selection, especially for highly medicinal and economic plants, to preserve precious genetic capital and for elite breeding. Ginseng has long been known as a medicine used to strengthen vitality, improve health, prevent diseases, and prolong life. *Panax vietnamensis* Ha et Grushv. (Ngoc Linh ginseng) is introduced to be an endemic species of ginseng. Although it has only been commonly used in the medical community since 1973, it can be said that Ngoc Linh ginseng is one of the most important findings in the field of medicine. Scientists have found that Ngoc Linh ginseng has the highest content of dammarane saponins (about 12-15%) and the highest amount of triterpene saponins compared to other species of the genus Panax in the world (Ha & Grushvisky, 1985).

There is currently little information on the genes or cell genetics of Ngoc Linh ginseng. This lack of data has limited the understanding of ginseng's chromosomal set and its phylogenetic relationship with other species in the genus Panax. In a report, Yi et al. (2004) mentioned that x = 12 was generally accepted as the basic chromosome number in the family Araliaceae, but this finding was not entirely certain. While there were many theories to refute, some studies have indicated that x = 6, 9 existed in many species of ginseng. Regardless of whether the difference in the number of chromosomes reported is due to a specific internal transformation, it is necessary to determine the number of chromosomes of Ngoc Linh ginseng to contribute to making the correct judgments about the chromosome of the genus Panax, which serves as a basic premise for further studies of cell genetics and the development of new breeding programs.

Determining chromosome number and karyotyping involved the following steps: Using the cell that is dividing, treating hypotonic shock, stopping cell mitosis from metaphase, preparing a microscope slide, chromosome imaging, and karyotyping (Vo, 2012). In plants, the examination of the chromosomal set to determine karyotype is usually carried out in the metaphase of mitosis in the shoot apical meristem, the root apical meristem, and the main root of the germinated seed. However, the count of chromosomes often encounters significant obstacles in species with a large number of chromosomes or chromosomes of large size. Therefore, the treatment of hypotonicity causes chromosomes to disperse in the cell, treatment to shorten the length of chromosomes, or treatment to stop cell division in mid-phase is necessary. Therefore, this study's objective was to optimize the hypotonic shock method and apply this method to determine the number of chromosomes in Ngoc Linh ginseng cells.

2. MATERIALS AND METHOD

2.1. Materials:

The chromosome microscope slides were made from the root apical meristem of Ngoc Linh ginseng (*Panax vietnamensis* Ha et Grushv.). Roots were collected from the Incubation and Support Center for Technology and Science Enterprises – Ministry of Science and Technology.

2.2. Methods:

Determine the optimal time of cell division: The roots were fixed with Carnoy's solution (3 ethanol: 1 acid acetic) at different times in the morning from 7:30 to 9 hours over a 24-hour period. After fixation, the root apex was stained with 1% acetocarmine solution to determine the time when the cells of the meristem were in vigorous mitosis. The optimal period was when the percentage of cells in the middle phase reached the highest.

The hypotonic shock methods: The temporary chromosome microscope slides were performed by hypotonic shock method according to the following procedure:

1) Treatment of roots causing hypotonic cells with potassium chloride (KCl) at different concentrations of, 0.1%, 0.2%, 0.3% and 0.4% for 45 minutes. The root sample was then washed with distilled water. Add colchicine solution at concentrations of 0.04% and 0.05%, and treat for 2 hours.

2) Fix with Carnoy's solution for 24 hours. Samples after fixation were washed several times with distilled water and stored in 70% alcohol at 4-5°C.

3) Hydrolysis of the roots with 2.5N HCl solution for 5 min.

4) Staining root tips in aceto-carmine solution 1% for 30 min at room temperature.

5) Add a few drops of 45% acetic acid solution and cover the lamelle. Gently press the slide to spread the cells evenly.

6) Observe the slide under the microscope with a 100x.

The chromosome numbers were counted in 30 different metaphase cells.

2.3. Data processing methods:

The data was collected and processed using Excel software.

3. RESULTS AND DISCUSSION

3.1. The optimal time of mitosis stage to observe chromosomes

Determining the optimal mitosis time of the species is very important for making a microscope slide to observe chromosome numbers and morphology. Chromosomes are best observed during the metaphase and anaphase of mitosis when the chromosomes are in double-stranded form and maximal constriction. In plants, each species has a different time of division and depends on the type of meristem, so it is necessary to determine the time of the division of each species to perform the chromosome sample for the best results. (Fukui & Iijima, 1991).

The experiment to determine the optimal mitosis time of Ngoc Linh ginseng was investigated between 7:30 a.m. and 9:00 a.m. on the root apical meristem sample. Roots were collected every 15 minutes. 10 to 20 roots were collected at each collection interval. Root samples were fixed with ethanol/acetic acid solution (3:1), and mitosis was observed by staining the root tips in 1% aceto-carmine solution. The results showed that Ngoc Linh ginseng had the optimal time of cell division at between 8:30 a.m. and 9:00 a.m. (Figure 1), the time for dividing cells' density with many different mitosis phases. This result is also consistent with previous studies by other authors, wherethe largest number of cells at the metaphase stage in plants was

achieved in the time frame of 8-9 a.m. and 8-10 a.m., depending on the species and seasons (Vo, 2012).

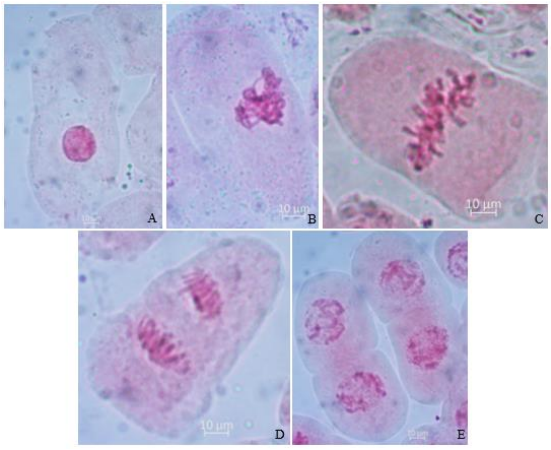

Figure 1. Ngoc Linh ginseng cells in mitosis stages

A-Interphase; B-Prophase; C-Metaphase; D-Anaphase; E-Telophase. (100x)

3.2. Effect of hypotonic shock solution concentration on the disperse of Ngoc Linh ginseng chromosomes

Cell hypotonic treatment is an important stage in producing high-quality chromosome slides. If a living cell is placed in a hypotonic environment, the osmotic pressure will cause water molecules to move into the cell, which can cause the cell to swell and burst. For plant cells or other species with strong cell walls, the cell can retain its shape in a hypotonic environment. Hypotonic shock solution with appropriate concentration and treatment time helps the cells swell sufficiently, aidingthe mid-phase chromosomes in mid-phase to disperse, not stack. Therefore, in this experiment, we treated the cells with hypotonicity with potassium chloride (KCl) solution at concentrations of 0.1%, 0.2%, 0.3%, and 0.4% for 45 min before fixation. The results showed that chromosomes were evenly dispersed, morphologically clear, and most abundant in the treatment with 0.2% KCl solution (Figure 2).

Table 1. Effect of KCl solution concentration on chromosome dispersion

KCl Conc. (%)	0	0.1	0.2	0.3	0.4
Cell shape	Broken a lot	Swell, but broken	**Swell properly**	Not swell properly	Swell properly
Chromosome dispersion	Lost, not concentrated in clusters	Not dispersed	**Evenly distributed**	Lying on top of each other	Not dispersed

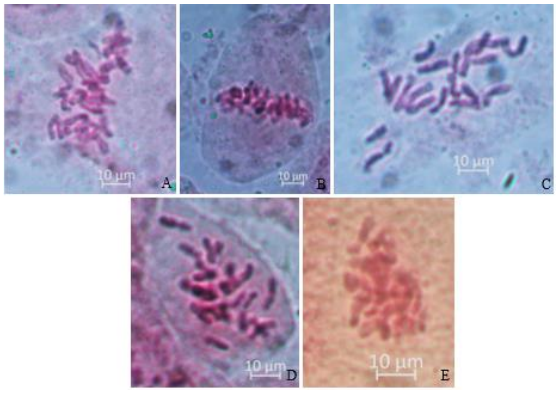

Figure 2. Effect of hypotonic shock solution concentration on the dispersion of Ngoc Linh ginseng chromosomes

A- Carnoy; B- KCl 0.1%; C- KCl 0.2%; D- KCl 0.3%; E-KCl 0.4%. (100x)

Hypotonic cell with KCl at 0.1%, 0.3%, and 0.4% was not effective. The cells were swollen, but not enough for the chromosomes to spread, so it was difficult to observe the morphology and count the number of chromosomes. Treatment of hypotonic Ngoc Linh ginseng cells with KCl 0.2% gave the best results. Here, most of the cells were swollen, and the chromosomes were evenly dispersed in the cells (Figure 2). The morphology and the number of chromosomes could be seen. The fixation solution did not cause hypotonicity of the cells, but the chromosomes were dispersed. Although the number could be observed and counted, the results were not highly accurate because most of the cells were broken, causing the loss of chromosomes.

The duration of hypotonic shock is also a factor affecting chromosome uniformity. Sufficient duration of the hypotonic shock phase should be ensured so that the cells swell sufficiently. If hypotonic shock is excessive, cells will rupture prematurely, and chromosomes will be lost. In contrast, insufficient hypotonic shock will result in overlapping chromosomes, making it difficult to count accurately. In this experiment, we treated Ngoc Linh ginseng cells with hypotonicity for 45 minutes based on the research results of Vo (2012). The treatment results showed that most of the cells treated with hypotonicity for 45 minutes were properly swollen, not broken, and the chromosomes were evenly dispersed.

Making a temporary slide is a quick and relatively easy method to visualize the chromosomes, but the time to maintain the quality of the slide is not long. Adding a few drops of 45% acetic acid solution before laminar pressing can increase the color contrast of the chromosomes, although the chromosomes may become discolored sooner. Therefore, it is necessary to devise methods of temporary preservation of specimens or implement fixed specimens of Ngoc Linh ginseng chromosomes to keep specimens longer with good quality.

3.3. Effect of concentration of colchicine solution on the dispersion of Ngoc Linh ginseng chromosomes

Research by Levan (1938) suggested that colchicine also randomly disperses chromosomes in the metaphase. *The research was treated with colchicine solution at concentrations of 0.04% and 0.05% for 2 hours. The results showed that at the concentration of 0.05%, most of the cells had chromosomal dispersion, but not in an even manner. Cells treated with 0.04% colchicine have not yet resulted in 100% of chromosomes dispersed (Figure 3). The chromosomes still aligned along the equatorial plane. Although the chromosomes in the two treatments were not completely dispersed, they still showed the influence of colchicine solution concentration. Therefore, the combination of hypotonic shock therapy and colchicine will give the best temporary chromosome slides.*

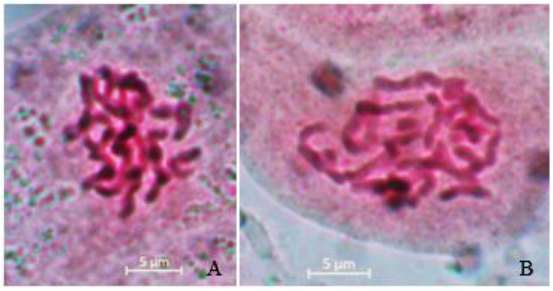

Figure 3. Effect of colchicine concentration on the dispersion of Ngoc Linh ginseng chromosomes

A- Colchicine 0.04%; B- Colchicine 0.05%. (100x)

3.4. The chromosome numbers of Ngoc Linh ginseng.

The number of chromosomes of Ngoc Linh ginseng counted on 30 cells was 2n = 22.05±1.76 in diploid (Figure 4). The known chromosomes for species in the genus Panax are mostly basal counts of x = 12 (Yi et al., 2004). In addition, phylogenetic analysis with the reported chloroplast genomes showed that four Panax species were grouped in the same genus and that *P. vietnamensis* was more closely related to *P. notoginseng* than *P. ginseng* and *P. quinquefolius* (Manzanilla et al., 2018; Nguyen et al., 2017). Another study by Zhu et al. (2003) and Phan et al. (2014) showed that two types of ginseng Lai Chau (*P. vietnamensis* var. fuscidiscus) and Ngoc Linh ginseng (*P. vietnamensis* Ha Grushv. var. vietnamensis) of Vietnamese ginseng (*P. vietnamensis* Ha et Grushv.) have close relatives. On the other hand, the diploid chromosome set in *P. vietnamensis* var. fuscidiscus and *P. notoginseng* are identified as 2n = 24 (Zhu et al., 2003; Zhang et al., 2017). Therefore, with the above data and the experimental results, we believe that the chromosome number of Ngoc Linh ginseng in diploid is 2n = 24.

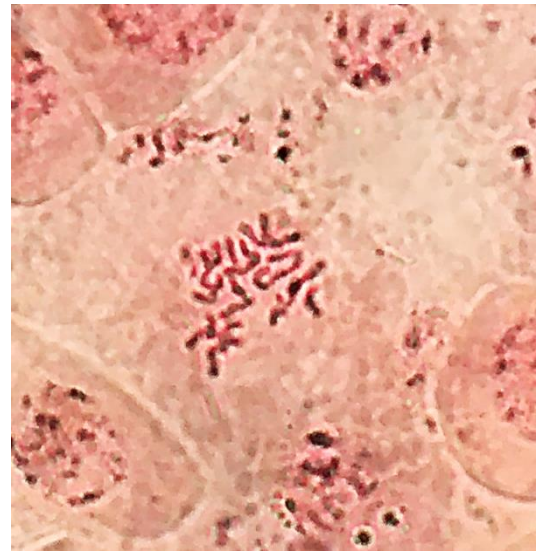

Figure 4. The chromosome number of Ngoc Linh ginseng

4. CONCLUSION

Finding the optimal cell division time, root pretreatment, and root immobilization determined that the chromosome number of Ngoc Linh ginseng was 2n = 24. However, the karyotype of Ngoc Linh ginseng has not been undertaken yet. This may cause difficulties for later cell genetic studies and breeding programs. Therefore, it is necessary to measure the length, describe the shape, and determine the centromere position of Ngoc Linh ginseng chromosomes to arrange the chromosome order and make the karyotype.

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