Induction of tetraploids in watermelon (*Citrullus lanatus* Thunb.) through chromosome doubling

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ABSTRACT

Polyploidy is an important evolution process for the creation of new species. Up to 70% of angiosperms are reported that as polyploids. Tetraploids development in watermelon is the first most step in the seedless fruit development. In this study, the colchicine was used to generate the tetraploid watermelons. The chloroplast counting methodology was adopted to segregate tetraploids. Among the 63 plants treated, one plant showed 11- 14 chloroplasts in the guard cells. Chimeral stomatal guard cells pertaining to the number of chloroplasts also identified in this investigation.

Key words: watermelon, tetraploidy, colchicine, chloroplast, screening

Watermelon (*Citrullus lanatus* Thunb.) is available in two types in the commercial market i.e seeded and seedless. Seedless watermelons are highly preferable by the consumers than the seeded fruits and also provides premium price for the growers. Seedless fruits are sweeter than the fruits from diploid (2n =2x=22) and seeded cultivars. Triploid (2n=3x=33) nature of chromosomal content expresses the seedless form in the watermelon. The crosses between tetraploid (female) and diploid (male) will produce the triploid seedless watermelons progenies (Kihara, 1958). Compton *et al.*, 1996 reported that spontaneous chromosome doubling through tissue culture technique. Seedless watermelons were produced through using the growth hormones (Yamamuro, 1978). Development of polyploid plants with adequate fertility and viability is the challenging task for a breeder. If a tetraploid developed with perfect stability, it can be used to improve further, or it can be used directly for seedless watermelon production. The objective of the present investigation was to analyze the impact of colchicine application on the growing shoot tip at seedling stage for ploidy modification. Counting the number of chloroplasts in the stomatal guard cells was used to investigate the ploidy modification in the treated plants. Though flow cytometry is the most authenticated method to confirm the ploidy level, chloroplast counting methodology can be the best option while the screening population is large (Jaskani *et al.*, 2007).

MATERIALS AND METHODS

The seeds of Arka Manik variety were used for the colchicine treatment. One gm of colchicine (ASC2569, Avra Synthesis Pvt. Ltd.) was added into 100 ml of 2% DMSO solution to prepare 1% colchicine. A total of 63 plants were treated with 1% colchicine to induce chromosome doubling preferably tetraploids. A drop of colchicine was placed on the tip of the shoot of the seedling (Fig. 1) by using filler. This was done three times a day (at 9am, 1pm and 5pm) with three hours interval for two days. The treated plants were maintained with proper watering up to 10 leaves production. The matured leaves were used for screening the ploidy level by employing chloroplast counting in guard cells of the stomata. The lower epidermal layer of the leaf was peeled out, placed on the glass slide, and then the 1% of iodine and potassium iodide solution was added on the epidermal layer cells. After five minutes, the cells were observed under light microscope with the magnification of 400x.

Figure 1. Treating watermelon seedlings with 1% colchicine

RESULTS AND DISCUSSION

There is a need to generate the precise protocols to obtain stable performing tetraploids in watermelon. The global demand for seedless watermelons is increasing. However, limited efforts have been taken to generate watermelon coupled with stable performance and adequate seed producing capacity. Application of aqueous colchicine solution on the tip of the growing shoot induce the tetraploids in watermelon (Jaskani *et al.*, 2007). Besides, the development of efficient pollenizer also an inevitable to pollinate tetraploids efficiently. In this study, six plants out of 63 plants showed elevated chloroplast number in the stomatal guard cells. Distinguishing the different ploidy level in the treated plants according to the morphological characters such as stomatal counting could be useful approach to identify better tetraploids. Usually the guard cells of the watermelons consisting 4-7 chloroplasts (Fig 2).

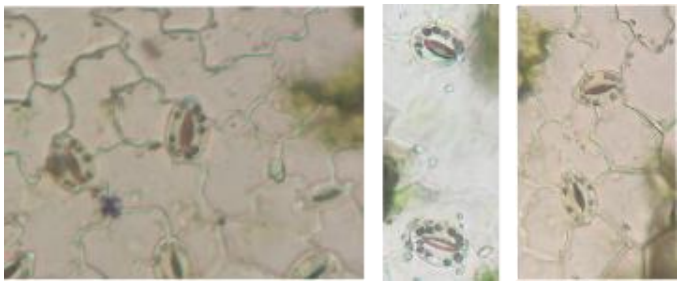

Figure 2. Chloroplast number in guard cells of the non-treated plants

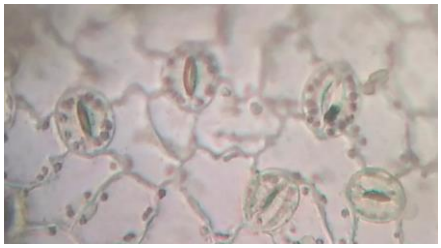

Figure 3. Chimeral guard cells of stomata

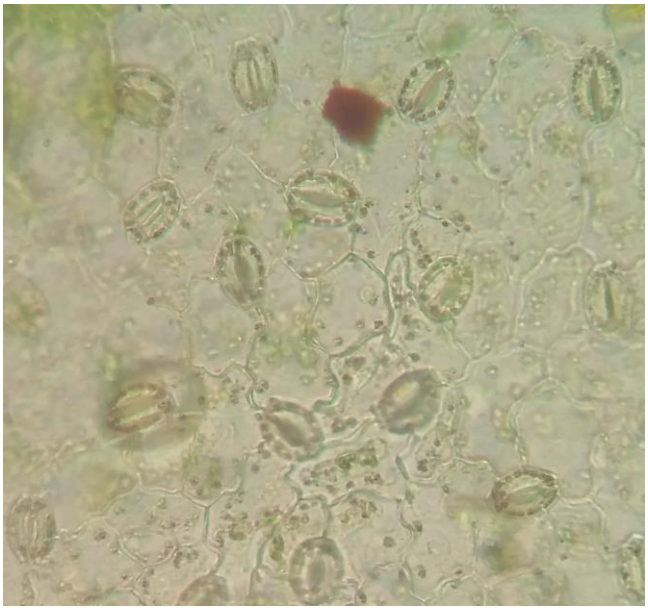

Figure 4. Expected modification in the chloroplast number in the guard cells

Among the six, five plants showed chimeral stomatal guard cells (Fig 3). The chimeral guard cells were contained the chloroplast numbers of 5-7,

8-12 and 12-14 in a same leaf. According to Jaskani *et al.*, 2007, application of colchicine solution on the shoot tip will produce less than 5% chimerasin watermelons. One plant showed elevated chloroplast number almost uniformly in all guard cells of the epidermal cells (Fig 4). The guard cells contained 11-14 chloroplast in the guard cells. Sari *et al.*,1999, reported that a total of 6–7 chloroplasts were found but in diploids it was about 11–12. Chloroplast scoring method was sucesfully used in Brussel sprout (Dore´, 1986), carrot (Rode and Dumas de Vaulx, 1987), sugarbeet (Brown *et al.*, 1991) and pepper (Abak *et al.*, 1998).

CONCLUSION

Chromosome counting and flow cytometry analysis helps to predict the ploidy level in watermelon. Chloroplast counting in guard cells also provides high throughput to handle large number of populations. Thus, it reduces the burden of large samples to be analyzed through flow cytometry for final confirmation. In addition, this will help breeder to achieve the breeding objective from large number of samples.

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