

A Comparative Study for Various Morphological Characters between Diploid and Triploid Watermelon

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Abstract

Breeding activities to generate seedless cultivars of fruits and vegetables have been initiated around the world due to its increased market demand and consumer acceptance. Seedless fruits and vegetables are becoming increasingly popular across the world due to their higher flesh content, enhanced sweetness and ease of consumption thanks to the lack of seeds. Polyploidy breeding is a very important aspect in horticultural crops to create genetic and morphological variation among horticultural crops. Seedless watermelons are produced through polyploidy breeding by doubling the chromosome number and crossing between normal diploid(2x) with colchicine induced tetraploids (4x).The present study was carried out in the year (May-July)2015 at the High-tech seed testing laboratory under the Department of Vegetable Science, Kerala Agriculture University, Trissur, India to differentiate between the normal diploid(2x) watermelon (var. Sugar Baby) with the yellow seedless triploid (3x) watermelon hybrid var. Swarna (KAU-CL-TETRA-1 × CL-5). The true leaf samples collected from 10 numbers of diploid (var. Sugar Baby) and 7 numbers of triploid (var. Swarna) seedlings of watermelon were taken for the study. In the present study chloroplast count and pollen grain study were undertaken as the morphological markers to carry out the experiment. The results obtained from the average chloroplast count of 10 samples of diploid (2x) sugar baby was 11.07 whereas the triploid (3x) Swarna hybrid was 12.11. The pollen grain of triploid (3x) was found to be not retained from red safranin stain colour being sterile in nature whereas the fertile pollen of diploid (2x) watermelon retained the stain colour.

Keywords: Triploid, Swarna, Chloroplast, Polyploidy, Watermelon

1. Introduction

Breeding various fruits and vegetables for seedlessness is a horticulturally important objective of the 21st century. The application of polyploidy breeding in production of seedless fruits and vegetables has resulted in significant economic and societal gains (Can, 2012). Increased cell size and gene expression, changes in physiological properties and higher ecological tolerance to a larger range of environmental challenges are all the benefits and superior performance of polyploid plants over their related diploid counterparts (Parisod et al., 2010, Tan et al., 2015, Van de Peer et al., 2017). Triploid plants like citrus, banana and watermelon are important horticulture crops that produce seedless fruit (Wang et al., 2016). The first triploid watermelons were developed during the 1950's at Kyoto University in Japan (Kihara, 1951). Because of its high-quality flesh that is almost free from seeds, the triploid (3x) watermelon fetches premium rates (Acton, 2013). Triploid(3x) plants have desirable characters which includes increase in vigour and broad, thick, dark green leaves, larger numbers of flowers or fruit which result in higher yield or higher harvest index (Wang et al., 2016) as a result of the energy that would normally be allocated for seed development is rather used for flowers or other organs (Miyashita et al., 2009, Tiku

et al., 2014). The watermelon var. Vertigo ($2n=3x=33$) can be taken as an excellent example that has produced the highest fruit yield ($41000 \text{ lb acre}^{-1}$) (Cushman et al., 2003). Production of triploid ($3x$) led to increase in the size of somatic cells and guard cells (Jones and Reed, 2007) and increases chloroplast numbers which further enhances the rate of photosynthesis (Padoan et al., 2013, Tapan, 2014). Tetraploid ($4x$) breeding lines are developed by chromosome doubling which are used as female parent in crossing with the normal diploid ($2x$) as male parent to produce triploid ($3x$) F1 hybrids (Andrus et al., 1971). Different concentrations of colchicine were used to double the chromosomes (Kazi, 2015a) whereas *in vivo* colchicine treatment results in a mixed population of diploid, tetraploid and aneuploids, as well as sectorial and periclinal chimaeras (Compton et al., 1993). Selfing tetraploid ($4x$) plants produced fewer fruits and seeds than $4x$ (female) \times $2x$ (Male) crosses and $3x$ (female) \times $2x$ (Male) crosses which gave seedless fruits (Kazi, 2015b). Identification of true triploids ($3x$) at the seedling phase can be done through various morphological, cytological and molecular studies. The use of flow cytometry is an established method to identify nuclear DNA content in the case of watermelon (Galbraith, 1990) and can also be used for plants growing in the field and in the greenhouse (Joachimiak et al., 2001, Sliwinska and Steen, 1995). It is estimated that the pollen size of tetraploid ($4x$) is 1.44X larger than the normal diploid ($2x$) (Rhodes et al., 1999). The number of chloroplasts in watermelon leaf guard cells is positively correlated with plant ploidy level (Jaskani et al., 2005, Ahmad et al., 2013) which can be used as a rapid and effective method to differentiate between the normal diploid and triploid hybrid watermelon (McCustion et al., 1993, Sari et al., 1999). Although there are many advantages of polyploid plants a major drawback associated it includes difficulties in seed germination which can be successfully improved by various mechanical practices such as scarification, seed nicking and seed coat removal in case of triploid ($3x$) watermelon (Duval and NeSmith, 2000, Grange et al., 2000). Another major drawback associated with it includes its lower commercial adaptability due to slower growth rate leading to delay in flowering and reduction in fertility compare to relative diploid ($2x$) due to meiotic irregularities (Sattler et al., 2016).

2. Materials and Methods

The present comparative study was done in the year (May–July)2015 at the Department of Vegetable Science under Kerala Agriculture University, Trissur, Kerala between var. Swarna (triploid($3x$)F1hybrid) and var. Sugar Baby ($2x$) (normal diploid) by examining various morphological characters. The present comparative study includes the following methods: 1. Chloroplast count of guard cells of leaf samples 2. Study of pollen grains of different samples.

2.1. Chloroplast count

Chloroplast count per guard cell of fully expanded leaves is used as a method to identify between diploid($2x$) and tetraploid($4x$) watermelon plants. The true leaves sample of different plants were taken and the leaf was peeled out and a peel was taken on a slide to count the number of chloroplasts in stomatal guard cell using a Binocular compound microscope (Olympus ICON-Tr Freedom) at 100x magnification. For each leaf sample, around 40 numbers of stomatal guard cells were taken into consideration and the number of chloroplasts in each stomatal guard cell is counted. The average chloroplast number of 40 different stomatal guard cells were counted for each sample. For the chloroplast count 10 numbers of different normal diploid ($2x$) (var. Sugar Baby) plant leaf samples whereas 7 numbers of suspected triploid ($3x$) (var. Swarna) plant leaf samples were taken into study.

2.2. Pollen study

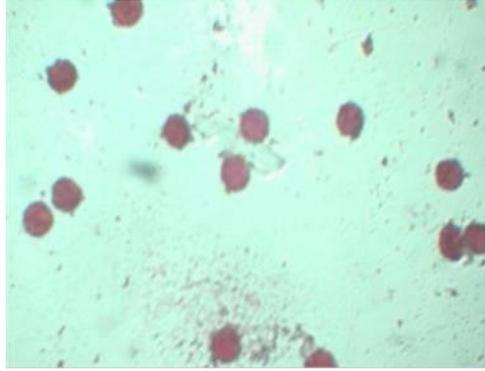
Pollen grain from both the suspected triploid (3x) line and the normal diploid (2x) plant is collected from the field. It was then subjected to staining with red safranin and viewed under a compound microscope. Those pollen grain samples collected from suspected triploid (3x) line will be sterile in nature which can be confirmed by not retaining the stain colour whereas diploid (2x) pollen grain will retain the stain colour being fertile in nature.

3. Results and Discussion

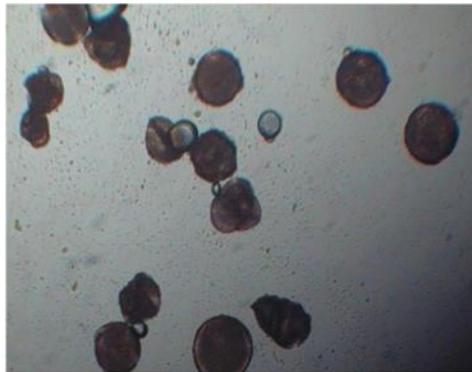
The objective of the study was to count the average chloroplast number which was suggested as a technique to differentiate between the diploids (2x) and triploids (3x). If we can identify true triploids (3x) in the seedling phase, F1 hybrid seed production of triploid (3x) hybrid can be carried out in the open field by interplanting tetraploid (4x) female parent and diploid (2x) male parent. Fruits harvested from tetraploid (4x) female parents will be either triploid hybrid seeds or selfed tetraploid seeds. The mean value of observed 10 samples of chloroplast count of normal diploid (2x) watermelon (var. sugar baby) was found to be 11.07 whereas the mean value of observed 7 samples of chloroplast count of triploid (3x) was found to be 12.11. Present investigation with the result from the chloroplast count did not give a convincing result. However, there are some samples that cross over this limit in each ploidy level. Mixoploid nature of leaf tissue may be the cause of this cross over (Koh, 2002).

Serial No:	Ploidy Level	No. of different plant leaf samples taken into consideration	Chloroplast Count (Mean Value)
1	Diploid (2x)	10	11.07
2	Triploid (3x)	7	12.11

Pollen grain sterility was confirmed at the flowering stage of the triploid hybrid. Pollen grain was collected from suspected triploid (3x) and a diploid (2x) watermelon plant and was subjected to staining with safranin. The normal diploid (2x) (Sample no.25) having fertile pollen which retained the red safranin stain colour as visible under the microscopic image (Fig.1) whereas the suspected triploid (3x) (Sample no.121) having sterile pollen which didn't retained the red safranin stain colour with appearance of dark brown colour as visible from the microscopic image (Fig.2) studied under the microscope. By using various morphological markers comparative studies between diploid (2x) and triploid (3x) watermelon can be helpful for us in the identification of true triploids (3x) at seedling phase which in terms will be beneficial for us in F1 hybrid seed production in open field condition by interplanting tetraploid (4x) female parent and diploid (2x) male parent. Further study to differentiate the morphological characteristics between diploid (2x) and triploid (3x) watermelon can be carried out with higher number of plant samples.



(Fig. 1) Microscopic image of fertile diploid (2x) watermelon pollen (Sample no.25) which retained red safranin colour



(Fig. 2) Microscopic image of sterile triploid(3x) watermelon pollen (Sample no.121) which didn't retained red safranin colour

4. Conclusion

According to the findings, triploid (3x) watermelon differs from the normal diploid (2x) watermelon by having a larger number of chloroplasts in the stomatal guard cells of true leaf. The pollen grain study was conducted to further prove triploid (3x) plant's pollen grain sterility, which was confirmed by staining the pollens of both triploid (3x) and diploid (2x) watermelon with red safranin and examining them under a compound microscope.

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6. Conflict of Interest

The authors have declared no conflict of interests exists.

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