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

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Abstract

The present study was carried out during 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). In the present study chloroplast count and pollen grain study were undertaken as the morphological markers to carry out the experiment. The true leaf samples collected from 10 numbers of diploid(2x) var. Sugar Baby and 7 numbers of triploid (3x) var. Swarna(KAU-CL-TETRA-1×CL-5) seedlings of watermelon were taken for the study whereas Binocular compound microscope (Olympus ICON-Tr Freedom) at 100x magnification was used for counting the number of chloroplasts present in the guard cell of stomata. Around 40 numbers of stomatal guard cells were taken into consideration for counting the average chloroplast number for each sample. The pollen grain was studied after staining with red safranin and observed under the compound microscope. The results obtained from the average chloroplast count of 10 samples of diploid (2x) watermelon var. Sugar Baby was 11.07 whereas the average chloroplast count of 7 samples of triploid (3x) watermelon hybrid var. Swarna (KAU-CL-TETRA-1×CL-5) was 12.11. The pollen grain of triploid (3x) watermelon hybrid var. Swarna (KAU-CL-TETRA-1×CL-5) was found to be not retained from red safranin stain color being sterile in nature whereas the fertile pollen of diploid (2x) watermelon var. Sugar Baby retained the stain color.

Keywords: Chloroplast, polyploidy, swarna, triploid, 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 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) × 2x (Male) crosses and 3x (female) × 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 (McCuistion 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 High-tech seed testing laboratory under the Department of Vegetable Science under Kerala Agriculture University, Trissur, Kerala to differentiate between normal diploid watermelon var. Sugar Baby (2x) and triploid F1 hybrid watermelon var. Swarna(3x) (KAU-CL-TETRA-1 × CL-5) 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 was 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 watermelon seedlings true leaf samples and 7 numbers of suspected triploid (3x) watermelon seedlings var. Swarna (KAU-CL-TETRA-1 × CL-5) true 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 normal diploids(2x) and triploids (3x) watermelon. 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) F1 hybrid watermelon var. Swarna (KAU-CL-TETRA-1 × CL-5) 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) (Table 1).

Table 1: Mean Value of Chloroplast Count of no. of plant leaf samples taken into consideration

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 color as visible under the microscopic image (Figure 1) whereas the suspected triploid (3x) (Sample no.121) having sterile pollen which didn't retained the red

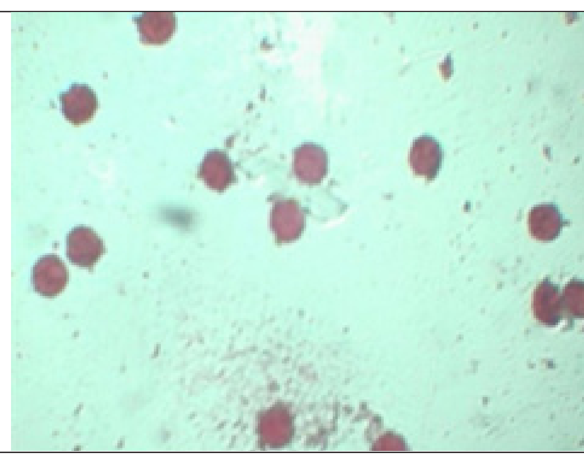

Figure 1: Microscopic image of fertile diploid (2x) watermelon pollen (Sample no.25) which retained red safranin colour

safranin stain color with appearance of dark brown color as visible from the microscopic image (Figure 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. Fruits harvested from tetraploid (4x) female parents will have either triploid (3x) hybrid or selfed tetraploid(4x) seeds. Further study to differentiate the morphological characteristics between diploid (2x) and triploid (3x) watermelon can be carried out with higher number of plant leaf and pollen samples.

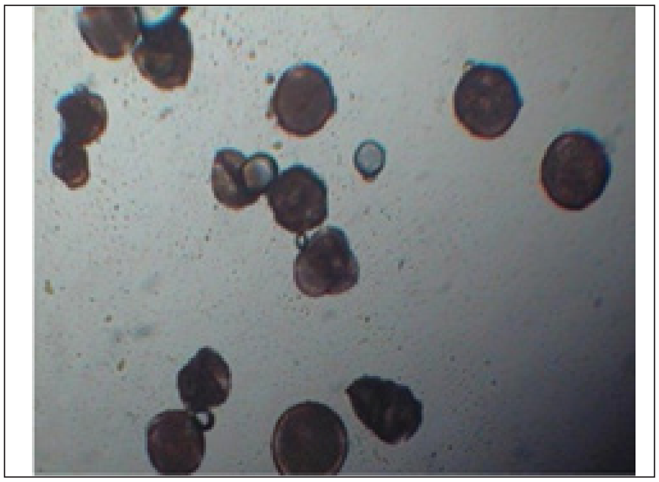

Figure 2: Microscopic image of sterile triploid (3x) watermelon pollen (Sample no.121) which didn't retained red safranin colour

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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7. References

Ahamad, I., Hussain, T., Nafees, M., Zamil, M., Ashraf, I., Akhtar, M., Iqbal, M., Rafay, M., Ruby, T., Ali, L., 2013. Morphological dissimilarity between tetraploid and diploid watermelon (*Citrullus lanatus* Thunb). World Applied Sciences Journal 21(6), 858–861

Andrus, S.P., Jeyachandran, R., 1971. *In vitro* multiple shoot regeneration from nodal explants of zehneriascabra (l.f.) sonder-an important medicinal climber. Plant Tissue Culture 14(2), 10–106

Can, S., 2012. Polyploid organisms. Science China Life Sciences 55(4), 30–311

Compton, M.E., Gray, D.J., Elmstrom, G.W., 1993. A simple protocol for micro propagating diploid and tetraploid watermelon using shoot tip explants. Plant Cell, Tissue and Organ Culture 33, 21–217

Cushman, K.E., Horgan, T.E., Snyder, R.G., Hudson, P.M., Coker, C.H., Ely, M., 2003. Evaluation of elongated and oval triploid (seedless) watermelon genotypes. Annual Report 2002 of the North Mississippi Research & Extension Center. Mississippi Agriculture & Forestry Experiment Station Information Bulletin 398, 339–345 Accessed on 08.04.22

Duval, J.R., NeSmith, D.S., 2000. Treatment with hydrogen peroxide and seed coat removal or clipping improve germination of 'Genesis' triploid watermelon. Hort Science 35, 85–86

Galbraith, D.W., 1990. Flow cytometric analysis of plant genomes. Methods in Cell Biology 33, 549–562

Grange, S., Leskovar, D.I., Pike, L.M., Cobb, B.G., 2000. Excess moisture and seed coat alteration influence germination of triploid watermelon. Hort Science 35, 1355–1356

Jaskani, M.J., Sung, W.K., Dae., H.K., 2005. Flow cytometry of DNA contents of colchicines treated watermelon as a ploidy screening method at m1 stage. Pakistan Journal of Botany 37, 685–696

Joachimiak, A., Kula, A., Sliwinska, E., Sobieszczanska, A., 2001. C-banding and nuclear DNA amount in six Bromus species. Acta Biologica Cracoviensia Series Botanica 43, 105–115

Jones, K.D., Reed, M., 2007. Analysis of ploidy level and its effects on guard cell length, pollen diameter, and fertility in hydrangea macrophylla. Hort Science 42, 483–488.

Kazi, N.A., 2015a. Polyploidy in flower crops. Scholarly Research Journal of Interdisciplinary Sciences. III/XVL, 2930–2936

Kazi, N.A., 2015b. Polyploidy in Vegetables. Journal of Global Biosciences 4(3), 1774–1779.

Kihara, H., 1951. Triploid watermelons. Proceedings of the American Society for Horticultural Science 58, 217–230

Koh, G.C., 2002. Tetraploid production of Moodeungsan watermelon. Journal of Korean Society of Horticultural Science 43, 671–676

McCuistion, F., Elmstrom, G.W., 1993. Identifying polyploids of various cucurbits by various guard cell chloroplast numbers. Proceedings of the Florida State Horticultural Society 106, 155–157

Miyashita, T., Ohashi, T., Shibata, F., Araki, H., Hoshino, Y., 2009. Plant regeneration with maintenance of the endosperm ploidy level by endosperm culture in *Lonicera caerulea* var. emphyllocalyx. Plant Cell, Tissue and Organ Culture 98, 29–301.

Padoan, D., Mossad, A., Chiancone, B., Germana, M.A., Khan, P.S.S.V., 2013. Ploidy levels in Citrus clementine affects leaf morphology, stomatal density and water content. Theoretical and Experimental Plant Physiology 25, 283–290

Parisod, C., Holderegger, R., Brochmann, C., 2010. Evolutionary consequences of auto polyploidy. New Phytologist 186, 5–17.

Researchers submit patent application "Inbred tetraploid watermelon line 4×asss4" for approval. In Acton, Q.A., 2013 (Ed) Cell nucleus structures – Advances in Research and Application, Atlanta, Scholarly Editions, 150–151

Rhodes, B., Zhang, X.P., 1999. Hybrid seed production in watermelon. Journal of New Seeds 1, 69–88.

Sari, N., Abak, K., Pitrat, M., 1999. Comparison of ploidy level screening methods in watermelon: *Citrullus lanatus* (Thunb.) Matsum. and Nakai. Scientia Horticulturae Amsterdam 82, 265–277

Sattler, M.C., Carvalho, C.R., Clarindo, W.R., 2016. The polyploidy and its key role in plant breeding. Planta 243, 281–296

Sliwinska, E., Steen, P., 1995. Flow cytometric estimation of ploidy in anisoploid sugarbeet populations. Journal of Applied Genetics 36, 111–118.

Tan, F.Q., Tu, H., Liang, W.J., Long, J.M., Wu, X.M., Zhang, H.Y., Guo, W.W., 2015. Comparative metabolic and transcriptional analysis of a doubled diploid and its diploid citrus rootstock (*C. Junos* cv. Ziyangxiangcheng) suggests its potential value for stress resistance improvement. BMC Plant Biology 15.

Tapan, K.M., 2014. Polyploid breeding. Breeding and Biotechnology of Tea and its Wild Species. Delhi, Springer India 9–34.

Tiku, A.R., Razdan, M.K., Raina, S.N., 2014. Production of triploid plants from endosperm cultures of Phloxdrummondii. Biologia Plantarum 58, 153–158.

VandePeer, Y., Mizrachi, E., Marchal, K., 2017. The evolutionary significance of polyploidy. Nature Reviews Genetics 18, 41–424.

Wang, X., Cheng, Z., Zhi, S., Xu, F., 2016. Breeding triploid plants: a review. Czech Journal of Genetics and Plant Breeding 52(2), 41–54.