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Triploidy Seeds Development in Watermelon *Citrullus lanatus* (Thunb) Matsum. & Nakai

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ABSTRACT

Watermelon, *Citrullus lanatus*, is a member of the family Cucurbitaceae; it originated from Africa and extensively grown in the tropical regions. In Nigeria, seedless watermelon is preferred by consumers; however, the cytogenetic and breeding factors are less understood leading due to limited genotypes. This study investigated the breeding and cytogenetic factors influencing the production of triploid seeds in watermelon. Diploid seeds were purchased from Technisem Agritropic Limited, Nigeria. After three weeks, the resulting seedlings were induced using colchicine concentrations. The successfully induced tetraploids were hybridised with the diploid parents, while chromosomal study was conducted using Orcein. Data were collected and subjected to one-way ANOVA at $P<0.05$. The colchicine treatment revealed 0.2% as the LD50. The hybridisation results showed successful formation of triploid seeds. Chromosomal analysis showed the chromosomal numbers: (i) diploid variety has 2n=22, (ii) tetraploid variety has 4n= 44 and the triploid has 3n=33. The triploid seeds and the established protocols are inevitable for the sustainable production of seedless watermelon.

Keywords: Hybridisation, seedless watermelon, tetraploidy, triploidy

INTRODUCTION

Watermelon, *Citrullus lanatus*, is a member of the family Cucurbitaceae; it originated from Africa and widely grown in the tropical and sub-tropical regions. Watermelon is an important herbaceous crop often grown for its sweet and juicy fruit; the crop is grown in warm climates the world over (Dube et al., 2021).

Seedless watermelons are triploid fruits due to their ploidy level. Seedless watermelon was first unravelled at Kyoto University Japan by Kihara in 1951 (Padhan & Kumar, 2022). Under natural condition

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watermelons exist as diploid plants with chromosomal number 22. The genus *Citrullus* is known to include the following species- *Citrullus lanatus, C. ecirrhosus, C. colocynths* and *C. rehmii* (Achigan-Dako et al., 2021).

Polyploidy in organisms is said to occur when there are multiple sets of chromosomes in the somatic cells other than the usual diploid numbers (Venkatesh, 2021). The cytogenetic and breeding factors for the development of the seedless watermelon are less understood; additionally, there are limited genotypes of the cultivars in Nigeria, hence the seeds of seedless watermelon are not available. The aim of the study is to investigate the breeding and cytogenetic factors influencing the production of triploid seeds in watermelon *Citrullus lanatus* (Thunb) Matsum. & Nakai.

MATERIALS AND METHODS

Source of Materials

Kaolack variety of watermelon was used for the research. Seeds were purchased from the company Technisem Agritropic limited Nigeria, Jos, Plateau State, Nigeria.

Tetraploidy Induction via Colchicine

Seedlings were induced by dropping method using cotton wool placed on the apical shoot to keep it moist. 0.2% Colchicine solution was used to induce tetraploidy on the seedlings (Wehner, 2022).

Cytological Studies

Cytological studies were conducted to affirm chromosomal number and stomata concentration. Chromosome number count using flower bud: flower bud was collected in the morning (7.00-9.00 am) and evening (4.00-6.30 pm). The sample was kept in fixative (3 Ethanol: 1 Acetic Acid) for 24 h to arrest cells and maintain them at the current stage of cell division. The flower bud was pressed for the pollen mother cell to emerge and then subjected to cytological procedures (Tong & Wang, 2024). Stomata size: Nail varnish was used to make an imprint on the lower surface of the leaf. The coated portion was carefully removed and placed on a slide containing a drop of water. The set up was covered with a cover slip and observed under light microscope. The stomata sizes were measured on Canva Software and calculated accordingly. Stomata Size= (SLXSWXK) Where SL=Length of stomata, SW= width of Stomata and k=0.78524.

Morphological Parameter Evaluations and Hybridisation

The morphological parameters (tendril length, stem diameter, number of leaves and leaf area) evaluations and hybridisation experiment were carried out in this section. Reciprocal

pollination was conducted manually with hand through cap and recap method between diploid and tetraploid plants to effect hybridisation between the diploid and the tetraploid plants (Popelka et al., 2019). The collected data were analysed using IBM SPSS version 22.0, and analysis of variance (ANOVA) at $P \leq 0.05$ level of significance.

RESULTS AND DISCUSSION

Treatment of Seedlings for Tetraploid Induction

The treatment of watermelon shoots with 0.2% colchicine concentrations successfully induced tetraploidy. Section 3.4 showed results on some cytological parameters were used to confirm tetraploidy induction. Similarly, Handayani et al. (2018) also reported that the induction of tetraploidy using 0.2% colchicine solution was effective.

Cytological Studies

Cytological study on stomata sizes showed result ranges of 47.11 µ to 54.97 µ in the tetraploids and 15.70 µ to 28.27 µ in the diploid. This showed that the tetraploids have stomata larger in size compared to that of diploid. This result agrees with the report from Handayani et al. (2018) who reported significant differences in stomata sizes between diploids and tetraploids in Watermelon. The chromosomal number showed the tetraploid plants have n=22, while the diploids have n=11 in the meiotic cells. The doubling number of chromosomes in this result confirmed the tetraploidy induction in the plant.

Morphological Parameter Evaluations and Hybridisation

The morphological parameters showed that the tendril length, number of leaves and leaf area increased significantly. There was no significant increase in stem girth. Tetraploid lines showed high growth rate over the diploids, in consonant with findings by Cui et al. (2017). Hybridisation conducted between the tetraploid and the diploid lines showed fruits formation. The tetraploids have larger size of flowers than the diploid. Triploid seeds were successfully developed: 3n=33, n=11. Triploid seeds were obtained from the resulting fruits of the crosses. In similar research, pointed Gourd revealed triploid seeds as reported by Hassan et al. (2020).

CONCLUSION

The study succeeded in the development of triploid seeds from crosses between tetraploids and diploids. The development of triploid seeds of watermelon has added to the gene bank of watermelon in Nigeria. With the triploid seeds, the production of seedless watermelon becomes seamless.

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