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Short Communication

Obtaining Synthetic Polyploid Sources through Hybridization of Diploid Species of *Gossypium* L. and Their Cytological Analysis

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Abstract

The wild diploid species *Gossypium anomalum* is considered a promising genetic resource for improving the genetic traits of cultivated cotton varieties due to its natural adaptation to abiotic (e.g., drought, high temperatures) and biotic (e.g., insects, diseases) stress conditions. Due to the challenges in hybridizing cultivated tetraploid and wild diploid species, the genome of the F1 generation, obtained from the hybridization of wild species *G. herbaceum* subsp. *pseudoarboreum* (A1 genome) and *Gossypium anomalum* (B1 genome), was amplified through synthetic polyploidy to restore crossbreeding fertility. Cytogenetic analysis of F1C hybrids revealed unbalanced tetrads and abnormal dyads during sporogenesis. These anomalies, resulting from genetic differences between the species, negatively impacted the fertility of the hybrids. Additionally, disturbances in chromosome distribution during microsporogenesis were observed, leading to reduced viability of pollen grains in hybrid plants. Based on these findings, it was concluded that further breeding efforts are necessary to enhance the genetic stability and restore the fertility of these hybrids.

Introduction

The wild diploid species *Gossypium anomalum* is a valuable genetic resource for improving the resilience of cultivated cotton varieties to various stress conditions. However, hybridization between cultivated tetraploid and wild diploid species is often challenging. To address this, synthetic polyploidy was employed to amplify the genome of F1 hybrids derived from *G. herbaceum** subsp. *pseudoarboreum* (A1 genome) and *G. anomalum* (B1 genome). This study focuses on the cytogenetic analysis of these hybrids to understand the anomalies in sporogenesis and their impact on fertility [1].

Materials and methods

The research was conducted from 2022 to 2025 at the Laboratory of Experimental Polyploidy and Phylogeny of Cotton, Institute of Genetics and Plant Experimental Biology, Uzbekistan Academy of Sciences, in Tashkent (41.369°N, 69.404°E, 480 m above sea level) [2,3]. The study involved *G. anomalum*, *G. herbaceum* subsp. *pseudoarboreum*, subsp. *frutescens*, and their polyploid hybrids: 27D F_1C (*G. herbaceum* subsp. *Pseudoarboreum* × *G. anomalum*), 28D F_1C (*G. herbaceum* subsp. *pseudoarboreum* × *G. anomalum*), 26D F_1C (*G. herbaceum* subsp. *pseudoarboreum* × *G. anomalum*), and 31D F_1C (*G. herbaceum* subsp. *frutescens* × *G. anomalum)*.

Plants were grown in Wagner pots under controlled conditions with a 16:8-hour photoperiod. Seeds were mechanically scarified and germinated in Petri dishes at 30 °C - 32 °C [4,5]. Seedlings were initially planted in a 1:1:1 mixture of compost, soil, and sand, and later transferred to Wagner pots. Polyploidy was induced by treating root tips with 0.2% colchicine solution for 24 hours in the dark. Hybridization was performed using standard methods. Cytological studies involved fixing collected anthers in Carnoy's fixative (99.5% acetic acid and 96.0% ethanol) at 24 °C, staining with acetocarmine, and examining under a Leica DM750 microscope.

Results and discussions

In the parental forms (*G. herbaceum* subsp *pseudoarboreum* and *G. anomalum*), balanced tetrads were predominantly

observed during sporogenesis, with monads, dyads, and triads occurring less frequently, which is considered normal (Figure 1, Table 1).

In the 27D F_1C hybrid (*G. herbaceum* subsp. *pseudoarboreum* × *G. anomalum*), dyads (45%) and normal tetrads (55%) were observed. In the 31D F_1C hybrid (*G. herbaceum* subsp. *frutescens* × *G. anomalum*), micro-nucleated dyads and tetrads (13%) and balanced tetrads (77%) were noted. These observations indicate unbalanced chromosome distribution and the formation of aneuploid chromosome sets (Figure 2).

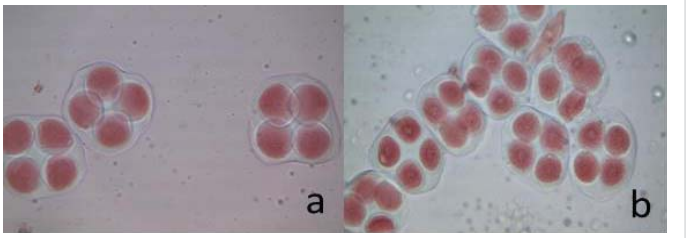

Figure 1: a) G. *herbaceum* subsp. *pseudoarboreum* b) *Gossypium anomalum* appearance of tetrads.

Table 1: Meiotic Index and Micro-nucleated Tetrads in Studied Samples.

No	Studied Samples	Total Sporads	Meiotic Index (%)	Micro-nucleated Tetrads (%)
1	*G.anomalum*	838	99,76 ± 0,16	-
2	*G. herbaceum* subsp. *pseudoarboreum*	709	98,59 ± 0,44	-
	G.herbaceum subsp. *frutescens*	803	96,66 ± 0,13	-
4	28D F_1C *G.herbaceum* subsp.*pseudoarboreum* × *G.anomalum*	324	91,74 ± 1,52	0,80 ± 0,32
5	27D F_1C *G.herbaceum* subsp.*pseudoarboreum* × *G.anomalum*	164	45,50 ± 3,85	0,60 ± 0,60
6	26D F_1C *G.herbaceum* subsp.*pseudoarboreum* × *G.anomalum*	258	85,71 ± 2,17	1,53 ± 0,76
7	31D F_1C *G.herbaceum* subsp.*frutescens* × *G.anomalum*	788	91,27 ± 1,00	0,25 ± 0,17

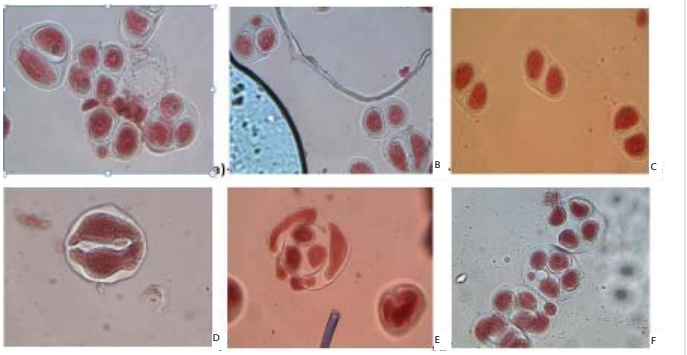

Figure 2: a), b), c), f) 27D F1C (G. *herbaceum* subsp. *pseudoarboreum* × *G. anomalum*); d), e) 31D F1C (G. *herbaceum* subsp. *frutescens* × *G. anomalum*).

Disturbances in meiosis led to the formation of polyads (1% - 2%) and unbalanced tetrads (5% - 15%). Despite the presence of unbalanced tetrads in the 31D F_1C hybrid, pollen grains were relatively well-formed [6,7] and the crossbreeding efficiency was higher.

Conclusion

Synthetic polyploidy was successfully used to amplify the genome of F1 hybrids derived from *G. herbaceum* subsp. *pseudoarboreum* × *G. anomalum* and *G. herbaceum* subsp. *frutescens* × *G. anomalum*. However, anomalies such as unbalanced tetrads and abnormal dyads were observed during sporogenesis. Further breeding research is recommended to enhance the genetic stability and restore the fertility of these hybrids.

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