Published January, 1973

The Origin of 2n and n Sectors of Chimeral Pima Cotton Plants[1]

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ABSTRACT

The tetraploid tissue of American Pima cotton *(Gossypium barbadense* L.) plants chimeral for tetraploid and haploid tissues was found to be of hybrid origin. We crossed 57-4, a doubled haploid that is semigametic, as female, with a multiple-marked stock homozygous for glandless and heterozygous for red plant color. A number of the plants from this cross were chimeral for glanded or glandless tissue, red or green tissue, and tetraploid or haploid tissue. The combinations of tissues in tri-chimeral plants were such that polyspermy appeared to be involved in the development of their tetraploid tissue. We theorized that a sperm nucleus divided in the egg cell before fertilization. The semigametic tendency of the female parent may be associated with the stimulation of divisions of the sperm nucleus.

Additional index words: **Amphidiploid, Chimeral, *Gossypium barbadense* L., Haploid, Polyspermy, Semigamy, Tetraploid.**

THE occurrence of cotton (*Gossypium barbadense* L.) plants that were chimeral for sectors of tissue of different origins has been reported (11). Some of the chimeral plants were haploid[3] and sectored for maternal and paternal tissue. These plants were considered to be the result of semigamy (11), an abnormal type of fertilization (1). Other chimeral plants were sectored for tetraploid and haploid tissue. We concluded (11) that a tetraploid sector was the result of semigamy followed by lack of cytokinesis of the first mitotic division of the egg nucleus. A restitution nucleus was formed, resulting in a female nucleus with the tetraploid number of chromosomes. It appeared that this nucleus divided to form a sector of tetraploid tissue that we considered doubled haploid. Later, progeny tests of 90 plants, representing several crosses, showed the tetraploid sectors to be hybrid in origin. The study reported herein was an investigation of the origin of the hybrid, tetraploid tissue of chimeral plants.

MATERIALS AND METHODS

The female parent in this study was 57-4, a doubled haploid that produces up to 60% haploids from single-embryo seeds (10). Cuttings were made from one plant of 57-4, rooted, and transferred to soil in 20-gallon cans.

The male parents consisted of several plants of a multiple-marked stock that were homozygous for the glandless genes gl_2 and gl_3 (6) and heterozygous for the red plant color gene R_1 (13). The heterozygous red plant color gene provided a means of identifying sperm nuclei.

Seed from crossing the above parents were planted in flats of sand. Seedlings were scored for chimeral expression as evidenced by sectors of tissue of different origins. Sectors of maternal tissue were green and glanded. Sectors of paternal tissue were red and glandless or green and glandless. Sectors of hybrid tissue were red and glanded or green and glanded. Thus, the green and glanded sectors could be maternal or hybrid tissue. The chimeral seedlings were transferred to 15-cm (6 in.) pots where their ploidy level was determined phenotypically. Plants were considered tetraploid if their anthers shed pollen, and they set bolls. Plants were considered haploid if their anthers did not shed pollen, and they had smaller plant parts (7).

RESULTS AND DISCUSSION

The characteristics and number of plants we obtained from a cross of 57-4, as female, and the heterozygous red, glandless stock are shown in Table 1. In this cross the female parent had the genotype $r_1r_1Gl_2Gl_2Gl_3Gl_3$. It produced egg nuclei that were $r_1Gl_2Gl_3$. The male parent had the genotype $R_1r_1gl_2gl_2gl_3gl_3$ and produced male gametes, sperm nuclei, that were $R_1gl_2gl_3$ and $r_1gl_2gl_3$.

Three thousand three hundred and fifty-six seedlings were scored. Of these, 1,530 were red and glanded. We assumed that these seedlings were F_1 hybrids. A random sample of the population was grown and the ploidy level of the red, glanded plants was determined. All plants were fertile, indicating tetraploidy. The red, glanded plants came from the union of an egg nucleus, $r_1Gl_2Gl_3$, and a sperm nucleus, $R_1gl_2gl_3$.

Sixteen hundred and twenty-nine seedlings were green and glanded. The majority of these were F_1 hybrids, and a few were maternal haploids. The F_1 hybrids came from the union of an egg nucleus, $r_1Gl_2Gl_3$, and a sperm nucleus, $r_1gl_2gl_3$. The maternal haploids had the genotype $r_1Gl_2Gl_3$. In a randomly selected population of green seedlings, 88 were green, glanded, and tetraploid, and one was green, glanded, and haploid.

One seedling was green and glandless. It may have been a paternal haploid, genotype $r_1gl_2gl_3$, of androgenetic origin, or it may have been a chimeral seedling whose other tissue was masked when examined.

One hundred and seventy-one seedlings were chimeral for two kinds of tissue. Four chimeral combinations were represented (Table 1). Twenty-five seedlings were chimeral for three kinds of tissue. They were sectored for green, glanded tissue; red, glanded tissue; and red, glandless tissue.

We scored, at flowering, 170 of the 196 seedlings classified chimeral (Table 2). Classification at the two stages frequently differs, since one tissue usually outgrows and masks the other. Seventeen of the 77 seedlings chimeral for green, glanded tissue and green, glandless tissue were tetraploid, green, and glanded. They were F_1 hybrids from the union of a female

Table 1. Number of nonchimeral and chimeral plants from a cross of Pima cotton 57-4 and a red, glandless stock.

Plant characteristics		Number of plants
Type	Tissue(s)	
Non-chimeral	Red, glanded	1,530
Non-chimeral	Green, glanded	1,629
Non-chimeral	Green, glandless	1
Chimeral	Green, glanded--green, glandless	94
Chimeral	Green, glanded--red, glandless	56
Chimeral	Green, Glanded -- red, glanded	17
Chimeral	Red, glanded--red, glandless	4
Chimeral	Green, glanded--red, glanded--red, glandless	25

[1] Cooperative investigations of the Agricultural Research Service, USDA, and the Arizona Agricultural Experiment Station, Tucson, Arizona Journal paper No. 1978 of the Arizona Agr. Exp. Sta. Received Aug. 16, 1972.

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[3] Chromosome number of haploid = 26 and tetraploid = 52.

Table 2. Characteristics and ploidy level classification at flowering of 170 Pima cotton plants scored as chimerals in seedling stage.

Classification at seedling stage		Classification at flowering stage — Number of plants							
No. of plants and tissue combinations		Green, glanded		Green, glandless		Red, glanded		Red, glandless	
		2n	n	2n	n	2n	n	2n	n
77	Green, glanded--green, glandless	17	41		19				
53	Green, glanded--red, glandless		41						12
16	Green, glanded--red, glanded		8			8			
3	Red, glanded--red, glandless					3			
21	Green, glanded--red, glanded--red, glandless		7			6	1		7

gamete, $r_1Gl_2Gl_3$, and a male gamete, $r_1gl_2gl_3$. Forty-one plants were haploid, green, and glanded. Their haploid tissue was from female gametes, $r_1Gl_2Gl_3$. Nineteen plants were haploid, green, and glandless. Their haploid tissue was from male gametes, $r_1gl_2gl_3$.

Forty-one of the 53 seedlings chimeral for green, glanded tissue and red, glandless tissue were haploid, green, and glanded. Their haploid tissue was from female gametes, $r_1Gl_2Gl_3$. Twelve plants were haploid, red, and glandless. This tissue was from male gametes, $R_1gl_2gl_3$.

The 16 seedlings chimeral for green, glanded tissue, and red, glanded tissue appeared as follows at flowering. Eight green, glanded plants were haploid, and eight red, glanded plants were tetraploid. The haploid, green, glanded tissue was from female gametes, $r_1Gl_2Gl_3$. The tetraploid, red, glanded tissue was hybrid. It was the result of the union of female gametes, $r_1Gl_2Gl_3$, and male gametes, $R_1gl_2gl_3$.

Three seedlings scored as chimeral for red, glanded tissue, and red, glandless tissue were, at flowering, tetraploid, red, and glanded. They were F_1 hybrids and resulted from the union of female gametes, $r_1Gl_2Gl_3$, and male gametes, $R_1gl_2gl_3$.

Seven of the 21 tri-chimeral seedlings were scored haploid, green, and glanded at flowering. This tissue was from female gametes, $r_1Gl_2Gl_3$. Seven plants were haploid, red, and glandless. Their tissue was from male gametes, $R_1gl_2gl_3$. One plant was haploid, red, and glanded. Its origin is unknown. It is a recombinant type with genotype $R_1Gl_2Gl_3$. Six plants were tetraploid, red, and glanded. Their tissue was from the union of female gametes, $r_1Gl_2Gl_3$, and male gametes, $R_1gl_2gl_3$. These tri-chimeral plants were sectored for green, glanded tissue, red, glanded tissue, and red, glandless tissue, with genotypes $r_1Gl_2Gl_3$, $R_1r_1Gl_2gl_2Gl_3gl_3$, and $R_1gl_2gl_3$, respectively. These genotypes are equivalent to the female, hybrid, and male.

The tri-chimeral plants are of interest because two female and two male gametes are involved in each plant. The presence of two female gametes, or egg nuclei, in amphidiploid cotton has been reported previously (3, 5, 8, 14). The presence of two sperm nuclei in tissues of amphidiploid cotton has not been reported previously.

The two sperm nuclei involved in the tri-chimerals may be due to polyspermy. We assume that one sperm nucleus contained in a pollen tube unites with the two fused polar nuclei to form endosperm tissue. It is unlikely that the two polar nuclei alone could develop into endosperm tissue, which is considered necessary for growth and development of embryos (2).

Polyspermy is the presence of more than two sperm nuclei in the embryo sac at the time of fertilization. It may occur as a result of the entry of several pollen tubes into the embryo sac or through repeated divisions in a pollen grain or pollen tube (9). Iyengar (4) reported the entry of more than one pollen tube into ovules of cotton. However, he reported no evidence of polyspermy based on the number of nucleoli in the endosperm and fertilized egg. He indicated that the nuclei of only one pollen tube enters the embryo sac and functions in double fertilization. The two sperm nuclei involved in these tri-chimeral plants may be from one pollen tube. The genotype of the two sperm nuclei involved in the 20 tri-chimeral plants was the same, i.e., $R_1gl_2gl_3$.

The two sperm nuclei may have come from additional mitotic divisions in the pollen grain or pollen tube, but it seems unlikely since the male parent appeared normal. The more feasible explanation is that a sperm nucleus divides in the egg cell before fertilization. The semigametic tendency of the female parent may be associated with stimulating the division of the sperm nucleus. If the latter is correct, this would be another source of polyspermy.

The results reported in this paper have a bearing on the procedures used in developing doubled haploids via semigamy (12). Employing semigamy involves the doubling of paternal, haploid tissue. In the seedling stage, paternal, haploid tissue and hybrid, tetraploid tissue are indistinguishable, but are distinguishable at flowering.

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