Investigations into the Cyto-morphogenetic Effects of Colchicine on Varieties of *Sorghum vulgare*

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INTRODUCTION

The action of colchicine on mitosis was perhaps first described by Pernice in 1889 (Eigsti & Dustin, 1955), but a full realization of the possible use of colchicine as an agent for the induction of polyploidy in plants did not gain impetus until about 1937 (Eigsti, 1938). Since 1937, many and various types of polyploid plants of agricultural importance have been derived as a result of colchicine treatment. Colchicine has been used extensively in the production of autotetraploids and amphidiploids and to a lesser degree in obtaining aneuploids in various species of plants (Eigsti & Dustin, 1955).

Franzke and Ross (1952) reported colchicine induced homozygous diploid variants in *Sorghum*. From colchicine treatment of an experimental variety which had bred true for 8 successive generations previous to treatment, they obtained diploid variants which differed from their untreated full sibs in possessing a number of ancestral traits for which in some cases they bred true immediately. Their first explanation was: "such variant plants could originate through reductional grouping of the somatic chromosomes so that a concentration of chromosomes containing gene blocks originating from one of the ancestors of the polyploid species might occur in one cell. This cell, by virtue of its inherent and perhaps environmental competitive advantage, could form a new growing point and produce a plant with a genotype entirely different from that of the original zygote."

After a study of the progeny of the variants along with a study of the progeny of the controls through four succeeding generations, Ross, Franzke and Schuh (1954) proposed a slightly different explanation: ". . . as a result of colchicine treatment, somatic reduction of the chromosome number has taken place with concurrent chromatin rearrangement

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and subsequent restoration to the diploid number. A cell so formed would perhaps, by virtue of chance genotypic or positional advantage, take over the growing point and form homozygous tissue of a different genotype than the original embryo."

Harpstead, Ross and Franzke (1954) after conducting cytogenetic examinations of the colchicine induced variants, the untreated plants and crosses between the two, did not find cytological evidence to support the above hypotheses and hence they concluded: ". . . that the changes in the chromatin must be of the nature of multiple point mutations perhaps involving minute structural changes which are not detectable through the observation of pairing relationships."

Foster, Ross and Franzke (1955) conducted a genetic study aimed at determining whether the genes controlling the various morphological characters were linked, or if they were scattered throughout several chromosomes. Their studies did not indicate any cases of linkage among the characters involved and hence they concluded that: ". . . the mutagenic effect of colchicine is not limited to one locus but must affect mutations at a large number of loci probably on different chromosomes."

Atkinson, Ross and Franzke (1956) conducted a comparative investigation of the differential reaction of two varieties of sorghum (Experimental #3 and Norghum) to colchicine treatment. Of 43 treated Experimental #3 plants which were grown to maturity, 18 were distinct morphological variants for 1 or more characters and all were diploids. Of 54 treated Norghum plants grown to maturity, no distinct morphological mutants were observed. Three of thirty treated Norghum plants examined were found to be tetraploids.

While these workers in South Dakota have achieved similar results in several experiments (i. e. the induction of homozygous diploid variants by colchicine treatment), their results have not been duplicated elsewhere and sufficient experimental evidence to determine the true explanation of the phenomenon has not been obtained. It is for this reason that the present investigation has been undertaken.

Methods and Materials

This investigation was conducted along two lines: First, an attempt to duplicate the results of Franzke and Ross and second, to obtain basic information concerning the cyto-morphological effects of the treatment on the shoot apices of treated seedlings.

Three varieties of sorghum were used in these investigations: Experimental #3 (which Franzke and Ross (1952) used in their original experiment), Redlan, and Resistant Wheatland. The seedlings were treated in the manner described by these authors, and then some were grown to maturity. Studies of sections through the shoot apices of treated and untreated seedlings at time-lapsed intervals after treatment are still in progress and are providing some basic information. To describe the cytological effects of the treatment, the terminology used by Elgsti and Dustin (1955) was adopted.

Results

Elgsti and Dustin (1955) indicated that the most outstanding single effect of colchicine in cells undergoing mitosis is to arrest the division at metaphase. The spindle fibers are destroyed, the chromosomes "divide" as usual but are held together at the centromere in the form of colchicine pairs (c-pairs). Instead of formation of the normal equatorial plate metaphase, various type of c-metaphase configurations may be observed.

The "star" metaphase (Fig. 1) is the result of destruction of the spindle fibers without concurrent destruction of the tractile fibers.

The "exploded" metaphase (Fig. 2) is the result of complete destruction of both tractile and spindle fibers, but without the effect being so great as to cause "clumping."

The "Ball" metaphase has been observed in the colchicine treated sorghum seedlings more frequently than any other type. It results from complete destruction of both the spindle fibers and the tractile fibers and by a subsequent "clumping" of the chromosomes. It indicates a strong colchicine effect. It may be recognized by the chromosomes being clumped into a tight ball and by the absence of any nuclear membrane or spindle mechanism (Fig. 3).

The occurrence of these c-metaphase types in the shoot apex (Fig. 4) indicates that the treatment might produce some genetic effects.

In October, 1956, Resistant Wheatland sorghum seedlings were treated with colchicine and were planted, along with untreated controls in the greenhouse. Treated and untreated plants were grown to maturity and were bagged to insure self pollination. Seeds from these plants were planted in the field to obtain F_1 progeny. Two of the progenies of treated plants exhibited characters by which they differed from the untreated controls. All the plants in one of these progenies failed to exsert the head properly (i. e. the peduncle was shortened) while the plants in the progeny of another treated plant were all extremely late maturing. Aceto-carmine smears of sporocytes revealed that these plants are all diploids. Genetic and cytogenetic examination of these plants is still in progress.

DISCUSSION

The explanation of the mechanism by which colchicine treatment has resulted in the production of diploid variants should take into consideration the following information:

1. The phenomenon is more readily detected in a true breeding variety of wide genetic background than in others tested.
2. The variant characters are ancestral traits in most instances.
3. No cases of linkage among the variant characters have been established.
4. The concentration of the colchicine used in the treatment is stronger than the optimum for induction of polyploidy.
5. In crosses between treated variants and untreated controls, perfect pachytene pairing is in evidence.

From the above information, the proposal of multiple spot mutations seems less likely than other proposed explanations.

On the other hand, the fact that a plant of wide genetic background reacts more readily than others suggests the possibility of colchicine induced "holohomozygousity" as an explanation. A plant like the Experimental #3 variety which has Milo, Sudan grass and Black Amber in its ancestry, while true breeding for its major morphological characters, must be heterozygous for a vast number of individual genes. If you suddenly made such a plant homozygous for all of its genes (in any given combination), one might expect a greater chance of a variant resulting than if a similar condition arose in a plant of less varied ancestry.

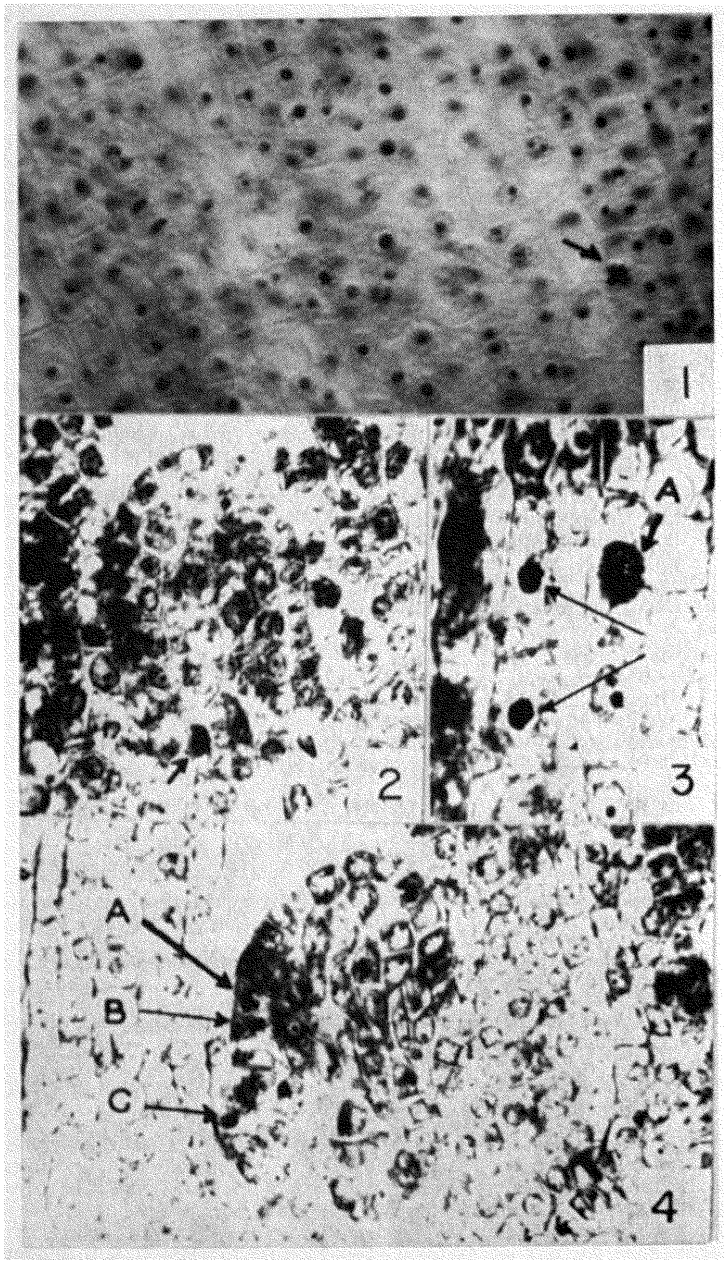

FIGURE 1. C-metaphase of the "star" type in young leaf of *Sorghum vulgare* var. Resistant Wheatland. Longitudinal section of the

shoot 22 hours after treatment with 0.5% colchicine in lanolin. (645X).

FIGURE 2. C-metaphase of the "exploded" type in the corpus of the shoot apex of *Sorghum vulgare* var. Redlan; 4 days after treatment with colchicine. (645X).

FIGURE 3. C-metaphases of the "ball" type (B) and colchicine prophase-metaphase type (A) in longitudinal section of mesocotyl of Experimental #3 variety sorghum; 24 hours after treatment with colchicine. (645X).

FIGURE 4. C-metaphases of the "star" type (A) and of the "ball" type (B) in the tunica of the shoot apex. This is on the same section as FIGURE 3. The "ball" metaphase (C) is in a leaf primordium. (645X).

Eigsti and Dustin (1955) describe the occurrence of multiple star chromosome groups in cells recovering from colchicine after having undergone several colchicine mitoses and having attained a large number of chromosomes. Cell walls are then formed between the groups. The net result is the subdivision of a large cell with many chromosomes into several cells of lower chromosome number. These authors do not attribute any genetic significance to this phenomenon. It is not clear whether the chromosomes in an individual star group are in the form of c-pairs. If these multiple stars occur in the shoot apex of colchicine treated sorghum, and if a given star group consisted of say 10 c-pairs, each pair representing a different one of each of the 10 homologues in sorghum, then the resulting cell would be homozygous for all genes and would be diploid provided the c-pairs separated as the cell reverted to the interphase. Such a cell might then undergo normal mitosis and produce cells which could replace polyploid cells and cells with non-viable gene combinations.

If such a mechanism exists in colchicine treated sorghum, it is possible that the completion of this investigation will provide evidence of its existence.

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