

THE EFFECTS OF COLCHICINE AND THE INDUCTION OF POLYPLOIDY
IN SORGHUM (*SORGHUM VULGARE* PES.)

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Polyploidy is one of the best known **evolutionary** processes and a method of producing new types among the higher plants. The morphological and **physiological** effects of **autopolyploidy** are to increase the size of the vegetative parts of the **plant** thereby making **autopolyploids** huskier and rather more vigorous than their corresponding **diploids**. Sorghum is the most important millet grown in India and it is a **major** source of food and feed. It is essentially a crop of the **tropics** and is **generally** grown under less favourable conditions than the other **major** food grains such as rice, wheat and maize. *Sorghum alnum* (Columbus grass), *S. halepense* (Johnson grass) and *S. Vulgare* (Var. Sudanese) (Sudan grass) are perennial fodders. *S. Vulgare* is grown for its grain and straw. The grain **yield**, straw quality and yield of *S. Vulgare* are mainly dependent on the total leaf area of the plant. The present study aims at developing autotetraploids, suited for fodder purposes, by exploiting the **gigantism** of vegetative parts generally exhibited by them.

Materials and methods

Seeds (S1) and seedlings (S2) of Grain sorghum, variety V2 were treated with combinations of three concentrations of Colchicine solution, viz. 0.15% (C1) 0.25% (C2) and 0.5 (C3) percent, at three durations, viz. $2\frac{1}{2}$ h (D1), 5h (D2) and $7\frac{1}{2}$ h (D3). The controls for concentrations and durations were Co and Do respectively. The experiment was laid out in a **RBD** with four replications. The data were **analysed statistically** according to the method of Snedecor and Cochran (1967).

The seeds were soaked in distilled water for **12** hours prior to treatment. The treated seeds were washed in running water (or one hour and allowed to sprout on moist filter papers. Seeds soaked in distilled water and **allowed** to sprout on filter **paper** were used as control. Seedling treatment was done adopting the method of Dogget (1957).

Germination percentage, survival percentage of treated seeds and seedlings, early deformity, height of plant, size and number of stomata per unit area, leaf area, number of days to flower, sterility and size of **pollen**, **length** of panicle, thousand seed weight, and dry weight of plant were studied. After studying the morphological characters, plants suspected as polyploids were **subjected** to cytological examination. Plants having $n = 20$ were labelled as **tetra-**ploids and five such tetraploids were isolated.

Results and Discussion

The mean values of germination are given in Table 1. The Anova indicated that the treatment had a significant influence on the percentage of germination. Germination percentage was very low in Colchicine treated seeds and with *increasing dosage* the *germination* percentage decreased *significantly*. Similar results were obtained by Sharma and Datta (1957) in *Coriandrum sativum* and Saxena and Nanda (1960) in Phlox. This may be due to the binding action of colchicine on the protein molecules of living organisms, which finally upsets the functioning of the cell Deysson (1968).

Table 1 **Germination** and survival percentage of seeds and seedlings of *Sorghum vulgare*, treated with various concentration of colchicine (in **angles**),

Treatments	Percentage of Germination (Mean)	Percentage of survival		
		S ₁	S ₂	Mean
C ₀ D ₀	79.80	66.04	64.30	65.17
C ₁ D ₁	68.74	54.80	41.17	47.90
C ₁ D ₂	52.78	46.88	35.25	41.07
C ₁ D ₃	46.91	37.23	33.38	35.31
C ₁	56.14	46.30	36.60	41.45
C ₂ D ₁	56.80	50.79	43.03	46.91
C ₂ D ₂	50.77	44.83	33.18	39.03
C ₂ D ₃	43.07	28.94	14.83	21.84
C ₂	50.24	41.54	30.35	35.94
C ₃ D ₁	52.56	50.75	37.25	44.00
C ₃ D ₂	47.48	39.23	18.01	28.62
C ₃ D ₃	37.28	21.41	10.53	15.97
C ₃	45.77	37.13	21.93	29.53
D ₁	59.36	52.11	40.48	46.30
D ₂	50.34	43.66	28.81	36.24
D ₃	42.41	29.19	19.58	24.34
Mean	53.61	44.09	33.09	38.59

The mean values of survival of treated seeds and seedling are given in Table I. The treatment had a significant effect on survival of plants. The survival percentage of control was significantly higher than that of the treatment. Seedling treatment gave a lower survival percentage than that of the seeds. Sanders *et al.* (1976) suggested that Colchicine treated seedlings require special medium, nutrients and controlled temperature for an increased growth rate of root and shoot in the early stages of development and survival.

Table 2 Height of plants grown from seeds and seedlings of *Sorghum vulgare* treated with various concentrations of colchicine (in c. m.)

Treatments	S ₁	S ₂	Mean
C ₀ D ₀	131.07	120.89	125.98
C ₁ D ₁	112.00	107.98	109.99
C ₁ D ₂	107.33	99.73	103.56
C ₁ D ₃	96.08	102.11	99.09
d	105.14	103.29	104.21
C ₂ D ₁	105.56	104.89	105.22
C ₂ D ₂	108.75	107.33	108.06
C ₂ D ₃	99.94	104.11	102.03
C ₂	104.76	105.44	105.10
C ₃ D ₁	100.17	102.67	101.42
C ₃ D ₂	97.22	102.00	99.61
C ₃ D ₃	92.56	96.52	94.54
C ₃	96.65	100.39	98.52
D ₁	105.91	105.18	105.54
D ₂	104.44	103.04	103.74
D ₃	96.19	100.91	98.55
Mean	105.07	104.83	104.95

In the treated seeds the germination was delayed and the growth was at a slow rate. In seedling treatment the growth was arrested for 5 to 10 days, especially in higher doses. Similar results were reported by Karivanov (1969) in hybrids of maize. Between seeds and seedling treatment, maximum lethality was noted in seedling treatment which may be due to the direct

contact of the meristematic region to the action of colchicine in impairing the normal cell division. Similarly Damon (1958) reported arrested metaphase cells in the shoot spines of colchicine treated sorghum seedlings. In dividing cells, Colchicine inhibits the proteins that form the spindle of the mitotic apparatus, by binding to the protein subunits of nuclear microtubules (Deysson, 1968). Control plants showed significantly increased height (Table 2). Foster *et al.* (1961) in Sorghum and Cirstea (1970), in maize reported similar results. This can be due to the mutation effect of colchicine.

Table 3 Measurement of number, length and width of stomata of plants grown from seeds and seedlings of *Sorghum vulgare*, treated with various concentrations of Colchicine

Treatment	Number of Stomata per unit area			Length of Stomata (μ)			Width of Stomata (μ)		
	S ₁	S ₂	Mean	S ₁	S ₂	Mean	S ₁	S ₂	Mean
C ₀ D ₀	10.43	10.77	10.60	43.43	42.03	42.73	33.33	30.60	31.97
C ₁ D ₁	10.78	9.67	10.22	45.10	40.40	42.75	28.69	34.85	31.77
C ₁ D ₂	11.38	10.77	11.07	42.82	43.68	43.25	29.03	34.40	31.72
C ₁ D ₃	10.47	10.83	10.65	44.46	44.18	44.32	29.29	29.80	29.55
C ₁	10.87	10.42	10.65	44.13	42.75	43.44	29.00	33.02	31.01
C ₂ D ₁	10.60	10.82	10.71	42.67	38.12	40.40	38.68	34.59	34.64
C ₂ D ₂	10.81	a.62	11.22	38.38	41.16	40.27	31.31	32.06	31.69
C ₂ D ₃	10.30	10.00	10.15	42.92	40.16	41.54	32.21	33.83	33.02
C ₂	10.57	10.81	10.69	41.32	40.99	41.16	34.07	33.49	33.78
C ₃ D ₁	10.33	10.09	10.21	42.42	40.40	41.41	30.80	34.29	32.55
C ₃ D ₂	10.50	10.50	10.50	42.11	38.12	40.12	31.91	34.84	33.38
C ₃ D ₃	10.37	10.75	10.81	36.36	39.13	37.75	29.29	33.58	31.44
C ₃	10.57	10.45	10.51	40.30	39.22	39.76	30.77	34.24	32.47
D ₁	10.57	10.19	10.38	43.40	39.64	41.52	32.76	34.57	33.67
D ₂	10.90	10.96	10.93	41.10	41.32	41.21	30.75	33.77	32.26
D ₃	10.54	10.53	10.54	41.25	41.16	41.21	30.26	32.41	31.34
Mean	10.65	10.58	10.61	42.07	41.09	41.58	31.46	33.28	32.37

The mean number of stomata per unit area is given in Table 3. In seedling treatment C₂ D₂ S₂ treatment had more number of stomata than any

other treatment. In treatments the mean number was only 7.38 while it was 10.60 in diploids. Siddiq (1967) and Gill *et al* (1970). Reported similar observations in sorghum and bajra respectively. Mean values of the size of stomata are also given in Table 3. The length and breadth of stomata in treatments were 53.65μ and 39.88μ respectively, while in diploids these were only 42.73μ and 31.97μ . Siddiq (1967) and Gill *et al.* (1970) reported similar results in sorghum and bajra respectively. In seeds and seedling treatment the size of stomata was greater in the lower doses. This may be due to the formation of Chimeras than stable autotetraploids. Table 4 gives the mean leaf area. In both, seed and seedling treatment controls had more leaf area than any other treatment. The leaf area was more in seed treatment than in seedling treatment. The mean leaf area of treatments were 810.68 sq. cm. but in diploids

Table 4 Values of leaf area of plants grown from seeds and seedlings of *Sorghum vulgare*, treated with various concentrations of colchicine (Sq. cm)

Treatments	S ₁	S ₂	Mean
C ₀ D ₀	1593.93	1560.36	1577.15
C ₁ D ₁	896.33	1019.72	958.03
C ₁ D ₂	915.10	826.52	870.81
C ₁ D ₃	793.25	786.32	789.79
C ₁	868.22	877.52	872.87
C ₂ D ₁	916.66	763.76	840.21
C ₂ D ₂	935.15	736.80	836.00
C ₂ D ₃	663.97	797.73	730.85
C ₂	838.61	766.09	802.35
C ₃ D ₁	839.02	1018.21	928.61
C ₃ D ₂	936.22	976.58	955.90
C ₃ D ₃	1532.79	569.43	1051.11
C ₃	1101.45	854.74	978.10
D ₁	884.00	933.90	908.95
D ₂	928.50	846.63	887.57
D ₃	995.78	717.00	856.80
Mean	1001.88	905.54	953.71

it increased to 1577,15 sq. cm. This general reduction of leaf area in treated plants may be due to narrower leaves and lesser number of leaves in the treated plants than in controls. This observation is in agreement with those of Raman and Krishnaswamy (1955) in tetraploids of *Sorghum halepense*. Flowering was delayed in the treated plants. Between treatments seedling treatment took more days for flowering than the seed treatment. In treatments it took 82 days for flowering while in diploids it was 74 days. Dussean (1945) and Schertz (1962) obtained similar results in the same material. Late flowering in polyploidy can be explained as, due to the low permeability accompanied by lower respiration and transpiration rates. This upsetting of the transport of prepared food from the site of production to the growing point in polyploids also may lead to late flowering as suggested by Schwanitz (1951),

Table 5 Percentages of pollen sterility (in angles) of plants grown from seeds and seedlings of *Sorghum vulgare*, treated with various concentration of colchicine.

Treatments	S ₁	S ₂	Mean
C ₀ D ₀	21.24	21,82	21.53
C ₁ D ₁	28.92	22.84	25.88
C ₁ D ₂	28.33	28.74	28.53
C ₁ D ₃	33.44	31.11	32.27
C ₁	30.23	27.56	28.89
C ₂ D ₁	30.81	31.32	31.07
C ₂ D ₂	29.31	33,17	31.24
C ₂ D ₃	33.19	48.65	40.92
C ₂	31.10	37.72	34.41
C ₃ D ₁	32.38	29.87	31.13
C ₃ D ₂	34.56	29.48	32.02
C ₃ D ₃	35.17	50.19	42.68
C ₃	34.03	36.51	35.27
D ₁	30.70	28.01	29.36
D ₂	30.73	30.46	30.60
D ₃	30.93	43.32	38.62
Mean	30.73	32.72	31.73

The present study indicated that colchicine treatment increased pollen sterility both in seed and seedling treatments significantly (Table 5) with a mean sterility of 41.46% for treated plants 13.5% for controls. Siddiq (1967) obtained totally sterile dwarf plants among colchicine treated sorghum seedlings. The present study shows that, though there were multivalents, the anaphase disjunction was regular in most cases. Since pollen sterility was a constant feature in all treatments, it suggests that there are certain other factors also involved besides multivalent formation in inducing pollen sterility. Size of pollen grain did not range significantly. In the various treatments the mean size of pollen grain was 60.60 μ while in control it was only 48.00 μ . Pollen grain size is directly proportional to chromosome number (as suggested by Gill *et al* 1970), The length of panicle in treated plants was less than that of control. In diploids the mean length was 27.61 cm. but in treated plants it was reduced to 20.42 cm. Such a reduction is in accordance with the reports of Sankaram (1964), Siddiq (1967) and Majisu (1971) in *S. vulgare*. The reduced length may be due to the shortened nature of treated plants. Treatments did not bring any significant change in thousand gram weight. But in treatments the weight was slightly higher than that of diploids. Majisu (1971) reported large sized grains in sorghum tetraploids. So the increased weight may be due to enlarged size of tetraploids. There was a significant reduction in the dry weight of treated plants. The mean dry weight of tetraploids was 20.25 g. while in the diploids it was 25.84 g. The reduction in dry weight is due to reduction in leaf size and reduced height of plant. Tetraploids were detected in treatments $C_1D_1S_2$, $C_2D_1S_2$ and $C_3D_3S_2$. They had dark green leaves and all were shorter than diploids. Eventhough the thousand grain weight was more, the total yield was reduced in tetraploids. The gigantism often exhibited by polyploids was noted in the size of stomata and size of pollen grain of the polyploids obtained in the present study.

Summary

Sorghum vulgare Var. V2, was treated with (3 concentrations) colchicine (in 3 durations) to get autopolyploids. The treatment reduced the germination percentage significantly and the reduction increased with higher concentrations and longer duration. The survival percentage of treated seeds and seedlings was also less. The isolated polyploids were dwarf in nature with shorter panicles compared to the diptoids and had larger sized stomata and lesser leaf area. The flowering was delayed in the pollen mother cells and showed several kinds of chromosomal abnormalities. Pollen sterility was higher in treatments, and as concentrations and durations increased pollen sterility also increased.

സംഗ്രഹം

സ്വബഹുദ്വൈയസൂകര കിട്ടാൻവേണ്ടി മൂന്നു ഗോഡതയിലും മൂന്നു കാലാവധിയിലും സോർഗം വംശേർ ഇനം V_2 കിളിർപ്പ് ലായനിയിൽ മുക്കിവെക്കുകയുണ്ടായി. ഈ ഉപചാരംകൊണ്ട് വിത്തിന്റെ കിളിർപ്പ് ശതമാനം പ്രകടമാംവിധം കുറഞ്ഞതായി കണ്ടു. ഗോഡതയും ഉപചാരകാലാവധിയും കൂടുന്നതനുസരിച്ച് കിളിർപ്പ് കുറയുന്നതായിട്ടാണ് കണ്ടത്. ഉപചരിച്ച വിത്തിലും തൈകളിലും അതിജീവനവും കുറഞ്ഞതായിക്കണ്ടു. വേർതിരിച്ചെടുത്ത ബഹുദ്വൈയസൂകര ദ്വിദ്വൈയസൂകളെ അപേക്ഷിച്ച് കുറിയവയും ചെറിയ പൂങ്കലുകളോടു കൂടിയവയും ആയിരുന്നു. കൂടാതെ അവയുടെ സ്റ്റോമാറ്റ വലുതും പത്രപാളി ചെറുതും ആയിരുന്നു. ബഹുദ്വൈയസൂകളിൽ പൂവിടീൽ താമസിച്ചാണ് നടന്നത്. അവയിലെ പരാഗമാതൃകോശങ്ങളിൽ പലവിധ അപസാമാന്യ രീതികളും ദൃശ്യമായിരുന്നു. ഉപചാരങ്ങളിൽ പരാഗവന്ധ്യത കൂടുതലായി കണ്ടു. ഗോഡതയും കാലാവധിയും കൂടുന്നതനുസരിച്ച് പരാഗവന്ധ്യതയും കൂടിക്കണ്ടു.

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