

**Research Article****Induction of polyploidy in two diploid wild cotton (*G. armourianum* and *G. aridum*) species by colchicine treatment**

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**Abstract**

In cotton, wild species generally have in built in resistance of desirable genes for biotic and abiotic stresses and possessing useful fibre qualities such as high length and strength. Among the antimetabolic agent, colchicine is widely used to induce doubling of the chromosomes in plant species. Attempt has been made to increase the ploidy level in two wild cotton species viz. (*G. armourianum*, L and *G. aridum*) to make crosses with tetraploid *G. hirsutum*, L cotton. A dose dependent increase in ploidy level was observed. Significant difference was noticed in induction of polyploidy and plant growth rate that depends on dose of colchicine, duration of the treatments and type of plant material have been observed in the study. Increase in the concentration of colchicine or duration of the treatment showed negative effects on seed viability in both the species under study. The colchicine treatment is more effective when the length of hypocotyls is between 4 - 8 mm. The root meristem of *G. armourianum*, L treated with 0.7% colchicine exhibited more tetraploid cells in 20 hrs treatment. The increase in the concentration of colchicine with increase in duration leads chromosomal abnormalities in both the wild species. Among the three methods employed in inducing polyploidy in wild diploid cotton, seed treatment was more efficient and reliable as compared to other seedling and cuttings treatments.

**Key words:** Colchicine, *G. armourianum*, *G. hirsutum*, wild species

**Introduction**

Ploidy manipulation has been considered as a valuable tool in genetic improvement of several plant species including commercial crops as Cotton and Sugarcane. The wealth of desirable genes present in wild species deserves special attention and needs systematic exploration and utilization. Such attempts will lead to develop varieties having built-in inherent resistance to major pests and diseases by modification of structural characters of the plants, such as flared or deciduous bracts, tight and straight calyx, coarse and pubescence, thick and stiff boll rind etc, which would result in greater attraction of vegetative parts than fruiting parts for oviposition (Mehetre 1993). Crop plants are represented by a variety of ploidy levels including 2x, 3x, 4x, 6x and aneuploid and higher order polyploids.

Cotton plants able to cross-pollinate with a number of wild related species and exchange chromosome segments through homoeologous recombination. The polyploidisation between an A genome diploid and a D genome diploid created the AD allotetraploids group of cotton in more than 2 million years ago (Senchina *et al.*, 2003). Induction of polyploidy in interspecific or intergeneric hybrids results in duplication of the two genomes present in a hybrid and formation of an allopolyploid. This allows for continued introgression of desired genes in to the cultivated gene pools (Olsen *et al* 2006).

In wide hybridization of cotton, incompatibility plays as a bottleneck in introgression of genes from wild species to cultivars. The major reason may be the ploidy level differences prevailing in cotton species. *Gossypium aridum* (D<sub>4</sub>) and *G. armourianum* (D<sub>2-1</sub>) are wild American cotton species belonging to D genome, which are the source for drought, sucking pest resistance and fibre strength. Leaves of *G. armourianum* emit a strong odour of lemon oil,

which is a character known to contribute resistance to sucking pests. Imparting resistance to pests from wild species to cultivated types has not been rewarded to the desirable extent mainly because of different causes like genetic and chromosomal (Thombre and Mehetre 1982 and Mehetre 1993) factors. Earlier studies indicated the scope of utilization of the *Gossypium armourianum* x *G. hirsutum* and *G. aridum* x *G. hirsutum* crosses for the improvement in respect of fibre strength and sucking pest resistance. A widely used chemical colchicine has been known for its mutagenic action that capable of changing the ploidy level in any plant species. Colchicine can effectively arrest the cell division at an early anaphase and the chromosomes have been duplicated resulted in polyploid cells (Alishah Omaran and Bagherieh-Najjar Mohammad, 2008).

Therefore, in the light of the above facts, attempts have been made to explore the possibilities of doubling the chromosome of the wild diploid *Gossypium armourianum* and *G. aridum* species thereby making crosses with tetraploid *G. hirsutum*. Analysis was done on phenotypic and cytological effects of various doses and duration of colchicine treated with seeds and seedlings and cuttings.

## Materials and Methods

### Seed treatment

Cotton seeds were sown in the petriplates lined with wet paper towels incubated at 26°C for germination. After 24 hrs, seeds were washed thoroughly and immersed in aqueous solution of colchicine with the concentration of 0.5, 0.7, 0.9, 1, and 1.5 percent for 4, 8, 12, 16, 20 and 24 hrs. After completion of their incubation, the seeds were rinsed thoroughly and washed with distilled water and kept in petridishes lined with wet paper towels and incubated in 27°C. After getting 4-8 cm emergence of hypocotyls region, the root tips were excised and the ploidy level was determined by squash method suggested by Singh (1993).

### Seedling treatment

Seeds were grown in earthen pots under normal condition (14 hrs light period at 29°C with 60 % humidity). Various concentrations of aqueous colchicine solution (0.6, 0.7, 0.9, 1 and 1.5%) were applied for 24 hrs at four hrs interval on the terminal meristamatic region of the young plants in four-leaved stage. The cotton plug method was followed. Cotton plantlets were kept at room temperature to avoid water evaporation. Plants were screened for the

survival percentage. The morphological abnormalities were recorded. The root tip squash preparation was done as described by Singh (1993).

### Stem cutting treatments

Fifteen cuttings of 10 cm of each wild species were collected for the study. The cuttings were immersed in aqueous solution of colchicine with the concentration of 0.5, 0.7, 0.9, 1, and 1.5 percent for 4, 8, 12, 16, 20 and 24 hrs. One set of cuttings of each wild species were planted with out any treatment in earthen pots as control. The cuttings were washed thoroughly, planted in earthen pots, and kept in a controlled condition.

### Chromosome counting

The root tips of controls and the treated were pre fixed in 2 mM 8-hydroxyquinoline for 5-6 hrs at 18°C washed with distilled water and were fixed in freshly prepared absolute ethyl alcohol-glacial acetic acid (3:1) for 24 hrs at 4°C and finally stored in 70% ethanol at 4°C. Hydrolysis was done in I N H Cl for 10-15 min at 60°C (45-60 min at room temperature) and stained with acetoorecin (Singh, 1993). Twenty-five cells from each sample were screened and the number of cells was determined by squash method and microscopic visualization. Suitable photomicrographs were taken for determining the chromosome numbers.

## Results and Discussion

A dose dependent increase in germination was noticed in wild cotton species viz. *Gossypium armourianum* and *Gossypium aridum* in seeds, seedlings and stem cutting treatments. The increasing concentration of colchicine more than 1% leads to decrease the rate of germination. Similarly lower concentration does not have much effect. Similar trend was observed in case of the period of application (duration) of colchicine.

### Seed treatments

In seed treatment, the response of the seed germination was better in 4-8 mm long hypostyle region. Seeds treated with 0.9 % colchicine was found highly effective than other higher concentrations of colchicine in both the wild cotton species. However, the maximum of 83.5% was obtained in *G. aridum* while it was 71.4% in *G. armourianum*. The lower concentrations such as 0.5, 0.7 doses have not shown any ploidy enhancement. The lower concentrations such as 0.5, 0.7 doses have

not shown any ploidy enhancement. The analysis of variance (ANOVA) for this experiment was shown in table-1. The results indicated that the effects of colchicines dose (D) ( $P \geq 0.01$ ), genotype (species) (G), D x treatment duration (T as well as D x T x G were all significant ( $P \geq 0.05$ ). However, the effects of replication, D x G and D x T were not significant. The combining effects of colchicine with different duration G x D, D x T ( $P \geq 0.01$ ) and the effects of G x D x T ( $P \geq 0.05$ ) on tetraploid cells were not statistically significant.

#### **Seedling (apical meristem) treatments**

In seedling treatments (apical meristem), higher concentrations viz. 1.2, 1.4, 1.6 percentages injured the apical meristem of both the species. Lower concentration (0.5 %, 0.7 %) doesn't show any change in their phenotype. However, success was obtained only in 0.9 % + 20 hrs in *G. aridum* and 1.0% with 16 hrs in *G. armourianum*, while the higher concentration of 1.2 % colchicine + 12 hrs expressed phenotypic changes at the beginning but later the plant type appeared normally. The combined effects of 0.9 % colchicines (0.9 + 16 hrs, 0.9 + 20 hrs, 0.9 + 24 hrs) and 1% colchicines concentration (1.0 + 16 hrs, 1.0 + 18 hrs, 1.0 + 20 hrs) exhibited polyploidisation to some extent. Maximum polyploidisation occurred in 0.9 +20 hrs treatment in *G. aridum*, while in *G. armourianum* maximum was observed in 1.0 % + 16 hrs treatment. The higher colchicine concentration + higher duration expressed chromosomal abnormalities and mixoploidy.

Among the two wild species studied, *G. armourianum* responded better in 1% colchicine concentration with 16 hrs duration than *G. aridum* thereby revealing that *G. aridum* is more sensitive to colchicine than *G. armourianum*. Data reveals that growth rate of the apical meristem declined when the concentration of colchicine increases. The survival percentage of seedlings were 33% in 0.9 + 20 hrs in *G. aridum* while in *G. armourianum* the survival percentage is 24 %. Lower concentrations does not have any effects and few plants survived in the combination of 0.5 % with 24 hrs duration and showed morphological variation in newly formed leaves. However, in course of time growth has completely stopped.

#### **Stem cuttings treatments**

In stem cutting treatments, there was slow growth at the beginning but in later stages, there was no response. In total two plants in *G. armourianum* and a single plant in *G. aridum* exhibited tetraploids as it

was confirmed through stomatal studies and enhanced abnormal plant growth. However, from the studies, it was observed that among the materials studied (seeds, seedlings and stem cuttings) for the polyploid induction, seed treatment (root tips) was found better in inducing polyploid plants than seedling treatment (apical meristem) and stem cutting treatments which was confirmed through root mitosis and stomatal studies of the epidermal peelings.

In all the cases, the polyploid plants showed enhanced leaves and overall plant structure. The results indicated that increasing the dose of colchicine declined the growth rate of plants suggesting that apical meristem zone are very sensitive to mutagenic agents like colchicine. Similar results were reported by Alishah Omeran and Bagherieh Najjar Mohammad (2008) in cotton species viz., *G. arboreum* L and *G. herbaceum*, L. The optimum effect of colchicine treatment can be achieved when the cells are actively dividing. The rate of polyploidisation in a cell depends on both the dose of colchicine and the duration of treatment.

The optimum treatment conditions lead to reasonable induction of tetraploid cells. The data on plant growth, survival reveals that the growth rate of seedling decreased with increasing concentrations of colchicine suggesting that active cell division of the apical meristem is highly affected by the increasing dosage of colchicine. Hansen *et al.*, (1995) support the findings in *Beta vulgaris* and Petersen *et al.*,(2003) in *Miscanthus sinensis*. The seedling survival was 16-34 % in *G. armourianum* while the survival percentage was 16-22 in *G. aridum*.

Even lower doses less duration like 6 hrs, 8 hrs does not have any positive effect in seedling treatment. However, in *G. armourianum* 11 days old seedling treated with 0.9 % colchicine with 12 hrs duration exhibited thickening of the first formed dark coloured leaves. The leaf colour has been changed from pale green to dark green. Thao *et al* (2003) reported similar findings in *Alocassia* using colchicine and Oryzalin mutagens. De Jesus- Gonzalez and Weathers (2005) suggested that increasing doses of colchicine with moderate duration (period) generally induce polyploidy in *Artemisia annua* which enhance the alkaloid content of Artemisinin.

The study reveals that the optimum treatment leads to a reasonable induction of tetraploid cells. the colchicine treatments with medium doses of 0.8 for

10 hrs or 12 hrs are the optimum dose for the induction of polyploid in wild cotton species. The doses above 0.8 and duration above 12 hrs are highly affected the growth rate thereby indicating that increasing the dose of colchicine would lead to a decline growth rate in plants. It suggest that the apical meristem of the seedling are highly sensitive than the seeds to the mutagenic agent colchicine. Data suggest that for polyploidy induction, 16hrs incubation of the *G. armourianum* and *G. aridum* seeds with 0.8 and 0.9 % colchicine respectively gives the best results. The optimum dose of colchicine and the incubation time depends on the species and the environmental conditions.

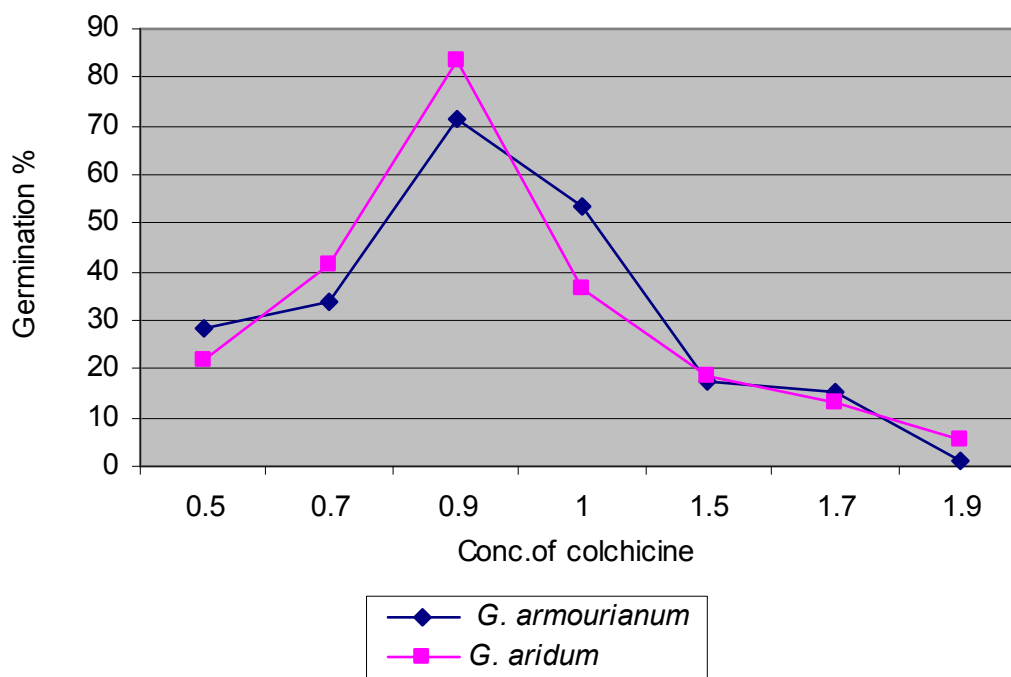
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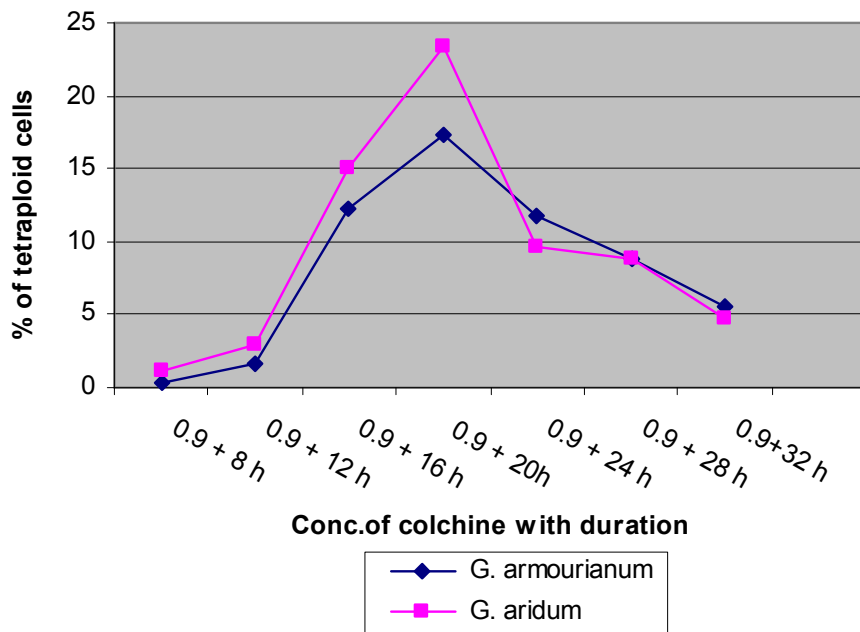
**Table -1. ANOVA results for colchicine effects on polyploidy in seeds and stem cuttings**

Source of variation	Degree of freedom (df)	Means of squares Seed treatment		Degree of freedom (df)	Means of squares Stem cutting treatment	
		STC	NVS		SCN	SCT
Replication (R)	3	7.08 <sup>ns</sup>	12.45	3	0.77**	42.08 <sup>ns</sup>
Genotype (G)	1	13.01 <sup>ns</sup>	33.80	1	0.48*	72.71
Dose (D)	3	132.05	48.92	3	1.17**	79.23
Time (T)	3	51.14	39.38	1	0.53*	91.51
G x D	3	70.21	22.45	3	0.23*	35.7 <sup>ns</sup> 1
G x T	3	31.51 <sup>ns</sup>	19.58	1	0.41 <sup>ns</sup>	59.3 0 <sup>ns</sup>
D x T	9	42.24	36.19	3	0.36*	31.32
G x D x T	9	29.16	29.45	3	0.58**	19.11
Error	93	14.08	12.15	45	0.103	11.31

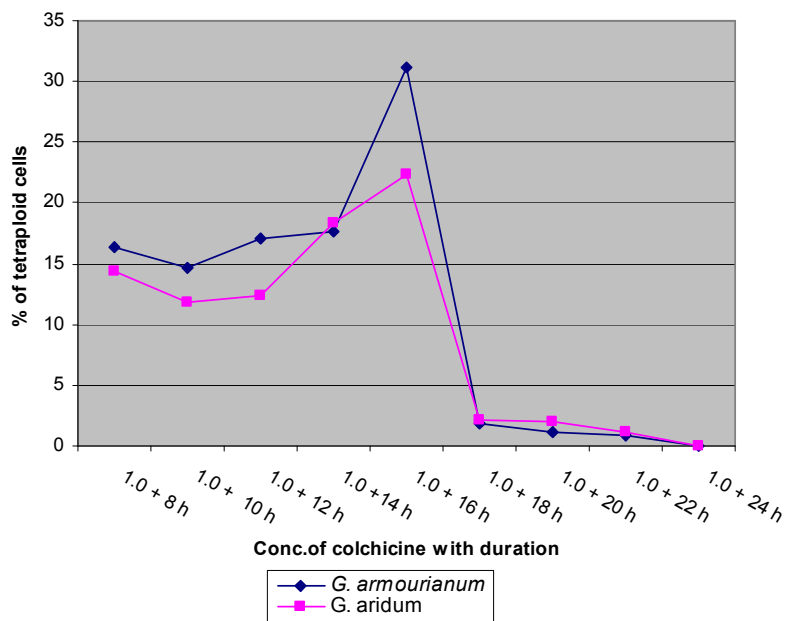
STC – Number of seeds with tetraploid cells; NVS- Number of viable seedlings after treatments; SCN- Stem cuttings with normal growth; SCT- - Stem cuttings with tetraploid cells

**Fig.1. Effect of colchicine on seed germination in *G. armourianum* and *G. aridum***


**Fig.2. Effect of colchicine in an effective concentration of 0.9% in seedling treatment**



**Fig.3. Effect of colchicine in an effective concentration of 1.0% with different duration in seedling treatment**



**Fig.4. Effect of colchicine on survival percentage of tetraploid seedlings in *G. armourianum* and *G. aridum***

