Journal of Multidisciplinary Engineering Science and Technology (JMEST)
ISSN: 2458-9403
Vol. 4 Issue 4, April - 2017

Effects Of Colchicine Treatments On Chromosome Doubling In Three Diploid Cotton Species

Nada S. Mustafa[1,2], Fang Liu [1], Magwanga Richard Odongo[1,3], Xingxing Wang[1], Xiaoyan Cai [1], Zhenmei Zhang [1], Kunbo Wang [1]*.

1. *Institute of Cotton Research, Chinese Academy of Agricultural Science (ICR, CAAS) /State Key Laboratory of Cotton Biology, Anyang* 455000, *China*
2. *Biotechnology and Biosafety Research Center, Agricultural Research Corporation (ARC), Sudan.*
3. *Jaramogi Oginga odinga of Science ant Technonogy. P.o Box 240-40606, Bondo.Kenya*

***Abstract*-- Colchicine has been used to induce chromosome doubling in plant species. Diploid cotton species have inherited desirable genes that are resistant to biotic and abiotic stresses and also possesses useful fiber quality traits, such as higher fiber length and strength. To transfer these desirable traits to the most extensively cultivated tetraploid cotton species through hybridization, it is a prerequisite to double the chromosome number of the diploid species. In this study we used *Gossypium herbaceum* L., *Gossypium arboreum* L. and *Gossypium raimondii* L applied two experiments: seedling, and embryo culture treatments. The results revealed that colchicine treatment was more effective in seedling treatment when the length of hypocotyls ranged from 5 to 8 mm. Further, we observed the optimum treated in the three species as the concentration of colchicine 0.5% and duration of treatment 8 & 16 hrs in seedling experiment while in embryo culture experiment the concentration of colchicines 2000ppm duration 24 hrs treatment its response. Among the experiments employed to induce polyploidy in the diploid cotton species, seedling experiment was the most effective and consistent as compared to embryo culture experiment. Though embryo culture demands less experimental space and little amount of colchicine, however the high risk of contamination and high sensitivity of embryo to colchicine make it less preferable. Colchicine treated plants are susceptible to environmental stimuli induced by high temperature and should be kept away from direct sunlight, the optimum dose of colchicine and the incubation time depends on the species and the environmental conditions.**

Keywords—Colchicine;Chromosome doubling; Diploid Cotton; Hybridization.

1. INTRODUCTION

Cotton plant, Gossypium species L, is also known as the “the white gold” due to the main product obtained from it, the soft fibrous also known the lint. Cotton plant has a wide distribution, from tropical to subtropical regions, thus has a global distribution and thus cotton has impressive diversification in relations to morphology, physiology and its growing locality. The diversification of cotton has led to a significant and remarkable evolution at the plant chromosomal level on chromosomal structure and size (Wendel and Grover 2015).

The genus, Gossypium has 45 diploid species with a total of 26 chromosomes, and also has 5 allotetraploid species, which developed through hybridization mechanism between the two diploid species, the AA and the DD diploid species, the allotetraploid forms has 52 chromosomes. The chromosomal size, pairing behavior and fertility in interspecific hybrids has enhanced the clustering of closely related species into genome groups, currently, there are 8 diploid genomic groups, marked by single alphabetical letters symbol from A to G and K, and a single tetraploid form AD. (Percival et al., 1999).

The allotetraploid group, AD sub genomes believed to have emerged 2 million years ago (Senchina et al., 2003). The allotetraploid sub genome has both AA and DD, thus marked as AADD, it has 2 mainly produced forms, namely G. hirsutum and *G. barbadense*. Due to narrow genetic diversity of the upland cotton, the ploidy manipulation has been considered as the most important tool in the improvement of genetic diversity of cotton, this has also attracted attention in sugarcane too. The wild cotton species, has significant and agronomic important traits, the rich diversity of desirable traits or genes in wild species needs to be mined and utilized. 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 among others, which would result in greater attraction of vegetative parts than fruiting parts (Mehetre 1993).

The genome manipulation is believed to be a sure way to enhance genetic diversity within the narrow genetic diversity of the upland cotton. The mechanism of chromosomal doubling will improve and enhance the genetic diversity. This attempts will lead to development of 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 among others, which would result in greater attraction of vegetative parts than fruiting parts (Mehetre 1993).

Crop plants are represented by a variety of ploidy levels including 2x, 3x, 4x, 6x and aneuploid and higher order polyploids. *G. arboreum* and *G. herbaceum* occur naturally in Africa and Asia (Wendel and Cronn, 2003). *G. arboreum* have been suggested to have many favorable traits like drought tolerance, resistant to diseases like root rot and insect pests like bollworm (Liu et al., 2006). *G. herbaceum,* commonly known as ''levant cotton'', is usually grown in rain-fed areas and found to be tolerant to salinity, drought and wind.*G. raimondii* characteristic by high quality in fiber (Jena et al., 2012). Cotton plants are able to cross-pollinate with a number of wild related species and exchange chromosome segments through homologous recombination. AD genome created from A genome and D genome. Induction of polyploidy in intergenetic 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).

Colchicine (C22H25O6N), is a hormonal plant product extracted from the seeds and bulbs of the plant *Colchicum autumnale* L., it is anti-mitotic agents, it is used to induce polyploids (Stanys et al., 2004). Colchicine act by binding to the dimmers, preventing the formation of microtubules, and consequently, spindle fibers during cell division (Petersen et al., 2003). Colchicine effect stops cell division at the early anaphase stage. The chromosomes have been duplicated but no mitosis occurred and restriction of cell wall formation at this stage results in the polyploidy cells. the polyploidy cells are generally larger than their diploid cells and do develop into thicker tissues, resulting in large-sized plant organs (Vainola, 2000).

Colchicine more effectively in seed treatment compare with seedling and steam cutting treatments in cotton (K.P.M. Dhamayanthi and Vinita Gotmare,2010)

Therefore, in the light of the above facts, this research work focuses on doubling of the chromosome, to enhance the bulking of important traits, through normal hybridization, which can be later transferred to the already cultivated upland cotton, *G. hirsutum* and *G.barbadense* . Three different diploid cotton species was used in this research, *Gossypium arbreum*, *Gossypium herbaceum* and *Gossypium raimondii.* With different colchicines concentrations with time variation. Analysis was further done both at seedling and embryo culture levels.

2. Materials and Methods

Gossypium herbaceum, Gossypium arboretum and *Gossypium raimondii* were collected from the Cotton Research Institute- Chinese Academy of Agricultural Sciences (2015-2016)

2.1 Seedling experiment:

Uniform seeds were selected for each treatment. The seeds were then sterilized with 70% ethanol for one minute and washed three times, for 3 minutes in distilled sterilized water to remove ethanol. The seeds were germinated in petri dishes with wet paper at 28^{o}C to ensure uniform germination, after 24 hours upon attaining uniform germination, treatment was initiated, using the following colchicines' concentration ; 0, 0.05, 0.5, 0.8 and 1% , in each colchicines concentrations, the seeds were exposed to different times interval ranging from 2, 4, 8 and 16 hours. Then rinsed well with distilled water and kept in petri dishes with wet paper at 27^{o}C to enhance vigorous plant growth. The seedlings were later transplanted after attaining a length of 3 to 4 cm in pots containing 1:1 vermiculite: peat-moss mixture, and kept it in 29^{o}C away from direct light. The plants were kept healthy and in a good condition for a period of three months and later transplanted to the field for the evaluation of phenotypic trait.

2.2 Embryo culture experiment:

In this experiment the seeds were sterilized with 2% hydrogen peroxide (H_2O_2) for a period of 24 hours at 29^{o}C, then washed in deionizer distilled water, three times to ensure complete removal of hydrogen peroxide. Seeds were then germinated in MS medium (macro elements, PH 6.5), then after 24 hours, put in the in cochicine concentrations of 1000, 2000 and 3000 ppm mixed with MS medium for 24 and 48 hours, after that washed it with MS liquid medium three times, then transfer to MS without colchicine and kept it at 29^{o}C away from direct light.

2.3 Chromosome counting:

The root tips of controls and the treated were pre fixed in 25 ppm cycloheximide for 25 min at 25^{o}C. Washed the tips with distilled water 2-3 times and were fixed in freshly prepared absolute ethyl alcohol-glacial aceticacid (3:1) for 24 hrs at 4^{o}C. Washed the tips again with distilled water 2-3

times. Putted the root tips in tubes contain (2% cellulose & 1% pectinase) for 40 min at 37^{o}C. Finally, washed the root tips with distilled water 2-3 times. Then make the slits root tips with 60% acetic acid. Twenty- five cells from each sample were screened and detected by the number of cells was determined by microscope.

3. Result and Analysis:

3.1 Seedling experiment:

In seedling experiment, the response to colchicines treatment was significant, when the root lengths attained growth of 5-8 mm length. When the lengths were less than the above stated, all seedlings died upon treating with colchicines, while the seedlings longer than 5-8mm, upon treating with the colchicines, grew just like the controlled ones, as illustrated in figure 1. Seeds which were treated with 0.5 % colchicines had a better response as compared to those treated with higher concentrations or concentrations lower than 0.5% among the wild cotton species. Based on the same concentration of 0.5%, the three cultivars showed varied response in terms of the total number of tetraploid cells, G. hirsutum achieved maximum numbers of tetraploid cells of 87.3% in time span of 16 hours, G. arberium achieved the highest of 69.3% both at 8 and 16 hours while G.raimondii attained a maximum of 82.0% after 8 hours. lower concentrations such as 0.05% have shown lower percentage of ploidy while higher concentrations above 0.5%, exhibit normal growth similar to control, unique observation was made at 1% concentration, a part of exhibiting low ploidy some of the plants also died as shown in(Table 1).
The result of Analysis of variance for this experiment is shown in (Table 2), indicate that the effect of colchicines treatment, colchicines concentration, genotypes, treatment duration and the interaction between its all are significant for viable seeds and seeds with tetraploid cells.

3.2 Embryo culture experiment:

In tissue culture treatment, Seeds treated with 2000 ppm colchicine at 24 hrs was found to be highly effective on the wild cotton types as opposed to concentrations above 2000ppm. However, the maximum ploidy cells were lower as compared to the seedling experiments, the maximum ploidy cells were, 34.8, 32.8, and 30.6% for *G.herbaceum* , *G.arbreum* and *G.raimondii* respectively.
The lower concentrations of 1000ppm, resulted into lower percentage of ploidy, while the least ploidy was realized at 3000ppm,as illustrated in table 3. Long exposure to colchicine
The result of ANOVA for tissue culture experiment (Table 1) is shown the effect of colchicine, concentration, genotypes and treatments duration and the interaction between its all are significant except TXG which has no significant effect.

3.3 Chromosome counting

The root tips of controls and the treated plants prepared for count the chromosome number through following steps as mentioned in materials and methods section. Finally, washed the root tips with distilled water 2-3 times. Then make the slides of root tips with 60% acetic acid.

Twenty- five cells from each sample were screened and number of chromosome was determined by the microscope.

Table 1: ANOVA results for Colchicines effects on polyploidy in seedling and tissue culture treatment.

Sources of variation	Degree of freedom	Mean of squares		Degree of freedom	Mean of squares	
		Seedling treatment			Tissue culture treatment	
		VC%	STC%		G%	TC%
Genotypes	2	90.99**	82.16*	2	107.47*	4.51**
Concentration	3	567.10*	120.97**	2	2576.46**	360.43
Time	3	166.85**	78.62**	1	281.66*	20.65*
CXT	9	12.14**	231.49**	2	21.67**	11.25**
CXG	6	7.51**	55.11***	4	42.46**	5.64**
TXG	6	11.99**	57.72*	2	3.47ns	11.25**
Error	114	0.64	3.82	40	1.83	0.19

Table 2: Colchicines effect on polyploidy in seedling treatment.

Verirty	TIME	0.05%		0.50%		0.80%		1%	
		VC%	STC%	VC%	STC%	VC%	STC%	VC%	STC%
G.H	2	100.0	20.0	96.7	34.3	93.3	38.7	80.0	51.3
	4	100.0	43.3	96.7	24.3	76.7	61.3	73.3	35.3
	8	100.0	66.7	86.7	76.7	70.0	47.0	66.7	44.3
	16	96.7	80.0	83.3	**87.3**	53.3	31.3	56.7	31.7
G.A	2	100.0	16.7	96.7	24.3	86.7	69.3	76.7	58.3
	4	96.7	34.3	93.3	47.0	73.3	68.3	73.3	35.3
	8	96.7	47.7	86.7	**69.3**	70.0	51.3	70.0	37.3
	16	96.7	47.7	86.7	**69.3**	70.0	51.3	70.0	37.3
G.D	2	96.7	27.7	90.0	34.3	80.0	57.0	66.7	58.3
	4	86.7	38.7	93.3	38.7	80.0	55.7	70.0	28.7
	8	80.0	37.3	80.0	**82.0**	66.7	44.3	60.0	27.3
	16	83.3	31.7	73.3	68.0	60.0	29.0	50.0	39.3

VS: Viable seeds after colchicine treatment (%), STC: seeds with tetraploid cells (%)

Table 3: Clochicine effect on polyploidy in tissue culture treatment.

Genpeot	time	Doses ppm					
		1000		2000		3000	
		G%	TC%	G%	TC%	G%	TC%
G.H	24	86	25.4	80	32.4	12	6.6
	48	58	33.8	70	**34.8**	0	0
G.A	24	84	23.8	84	30.6	6	10
	48	64	23.8	76	**34.8**	0	0
G.R	24	50	25.6	84	**32.8**	4	10
	48	38	20.6	52	22.6	2	0

G: germination seed %, TC: tetraploid cells %

All dose dependent increase in germination was noticed in all wild cotton species *Gossypium herbaceum*,*Gossypium arbreum* and *Gossipium raimondii* in seedlings and tissue culture treatments.higher colchicines concentrations above 0.5%, not only decreases the germination rate but also leads to whole plant death. Similarly, lower concentrations below 0.5% have no significant effect. increasing concentration of colchicine more than 0.5 % 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 of colchicines increase the germination was decrease. As shown in figure 2. This results in agreement to findings obtained by Yu Chen, Colchicines is effective in lower concentration, their results showed that, four putative hybrids were successfully chromosome doubled by treatment with 0.1% colchicine concentration for a period of 24 hours(Chen et al., 2015).

All treated seedlings had high growth vigor, the roots of the treated seedlings, were enlarged as compared to the controlled ones. Observation made on the microscope of the young tender leaves, revealed that, the shape and number of the cells were the same as in the control,similar results also seen in leaves, the only exception, is the size of the cells, the treated cells were bigger as compared to the cells obtained from the controlled plants, as illustrated in figure 3 and 4 for roots and leaves respectively.

Fig 1: show the optimum stage of seedlings in colchicine：

Fig 2: Show the effect of clochicine on seed germination

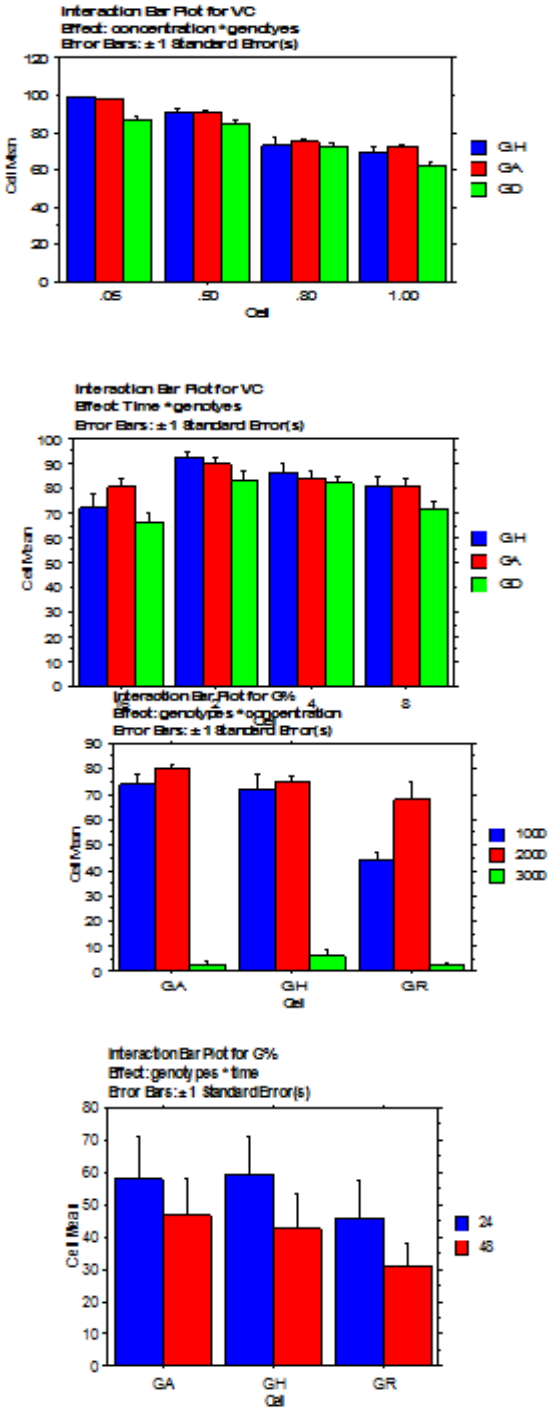
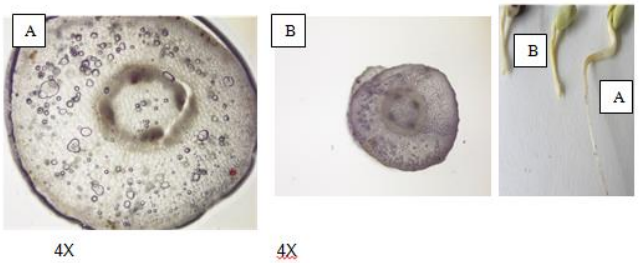
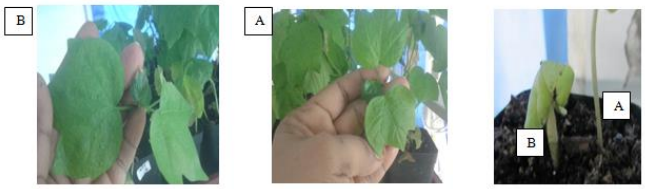


Fig 3: The different between inside the root (treated A and Control B)



4X 4X

Fig 4: different between leaf size (treated A and Control B)



4.Discussion Wild cotton species harbors many beneficial agronomic traits, these wild cotton species includes, *G. arboreum,* one of the two diploid cultivated cotton species, is known to have many favorable genes, these genes are known to genes confer the following beneficial traits , which includes, resistance to pests, Luo et al.2012, and tolerance to drought, Zhang et al., 2010.These traits are much important for the improvement of *G. hirsutum*, the main cultivated upland cotton, which its improvemt, has been a challenge due narrow genetic base.

To transfer favorable genes from *G. arboreum* into *G. hirsutum* ,a prerequisite is overcoming the cross incompatibility that exists between the above two species to obtain interspecific hybrids. For many years, numerous attempts have been made to produce these interspecific hybrids, including ovule and embryo culture studies Sacks E., J. (2008). However, it is still quite difficult

The declined growth rate of plant due to increased concentration of colchicines, as shown in this research work, also agrees to earlier findings as per K.P.M Dhamaynthi and Vinita Gotmare (2010), Similar results were also reported by Alishah Omaran and Bagherieh Najjar Mohammad (2008) in cotton species viz., *G. arboreum* L and *G. herbaceum*, L. The colchicine treatment optimum was more effective when the cells are still actively dividing at the early anaphase. The dose of colchicine and the duration of treatment control of the polyploidisation in cells (K.P.M Dhamaynthi and Vinita Gotmare (2010).

The response of tetraploid cells depend on the optimum treatment condition. Survival reveals that the growth rate of seedling decreased with i same in Beta vulgaris with Petersen et al.,(2003).

5. Conclusion:

The study reveals that the optimum colchicine treatment leads to a reasonable induction of ncreasing 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) tetraploid cells.

The seedling treatment with 0.5% colchicines for 8 hrs has best response while in embryo culture treatment 2000ppm colchicine during 24 hrs, so the optimum treatment conditions lead to reasonable induction of tetraploid cells. The optimum stage to get polyploid cells is (5-8mm) and the percentage of survival plants is high in low concentration of colchicines. Seedling treatment is more effective but in tissue culture treatment less

experimental space and little amount of colchicines, the optimum dose of colchicine and the incubation time depends on the species and the environmental conditions.

References:

Alishah Omran and Bagherich-Najjar Mohammad B. (2008).Polyploidization effect in two diploid cotton (*G. herbaceumL* and *G. arboretum* L) species by colchicine treatments. African J. Biotech : 7:102-108.

Hansen A L, Gertz A, Joersbo M, Anderson S B (1995).Short duration colchicines treatment for in vitro chromosome doubling during ovule culture of Beta vaigaris L. Plant Breeding : 114:515-519.

Jena, S.N., Srivastava, A., Rai, K.M., Ranjan, A., Singh, S.K., Nisar, T., Srivastava, M., Bag, S.K., Mantri, S., Asif, M.H.(2012), Development and characterization of genomic and expressed SSRs for levant cotton (*Gossypium herbaceum L*.). Theor Appl Genet, 124:565-576.

K.P.M Dhamaynthi and Vinita Gotmare (2010). Induction of polyploidy in two diploid wild cotton (G. armourianum and G.aridum). Electronic Journal of Plant Breeding,1(4): 966- 972.

Liu, D., Guo, X., Lin, Z., Nie, Y., Zhang, X.(2006), Genetic diversity of Asian cotton (*Gossypium arboreum* L.) in China evaluated by microsatellite analysis. Genetic Resources and Crop Evolution, 53:1145-1152.

Mehetre, S.S, 1993. Distant hybridization in cotton breeding-intergeneric hybridization (An overview). J.Cotton Res & Dev. 7 (2) :179-192.

Olsen R T, Ranney T G, Vilonia Z. 2006. Reproductive behaviour of induced allotetraploid x Chitalpa and in vitro embryo culture of polyploidy progeny. J. Am. Soc. Hort. Sci. 131 (6):716-724.

Petersen KK, Hagberg P, Kristiansen K (2003). Colchicine and oryzalin mediated chromosome doubling in different genotypes of Miscanthus sinensis. Plant Cell Tissue Organ Cult., 73: 137-146.

Percival AE, Wendel JF, Stewart JM. Taxonomy and germplasm resources. In: Smith CW, Cothren JT (eds) Cotton origin, history, technology, and production. John Wiley & Sons Inc., New York 1999, pp. 33-64.

Stanys V, Staniene G Siksnianas T (2004). In vitro induction of ploidy in Ribes. Acta Universitatis Latviensis, Biology, 676: 235-237.

Senchina D S, Alvarez L, Cronn R C, Lin B, Rong J. 2003. Rate variation among nuclear genes and the age of polyploidy plants in Gossypium. Mol. Biol. Evol : 20: 633-643.

Vainola A (2000). Polyploidization and early screening of Rhododendron hybrids. Euphytica 112: 239-244.

Wendel, J. F. and C. E. Grover, (2015). Taxonomy and evolution of the cotton genus. The age of polyploidy plants in Gossypium. Mol. Biol. Evol : 20: 633-643.

Wendel, J.F., Cronn, R.C. (2003), Polyploidy and the evolutionary history of cotton. Advances in agronomy, 78:139-186.