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INDUCTION AND IDENTIFICATION OF AUTOTETRAPLOIDS IN PEARL MILLET (*Pennisetum glaucum* L) FOR ITS UTILIZATION IN PEARL MILLET NAPIER GRASS BREEDING

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ABSTRACT

The present investigation was carried out to induce chromosomal doubling in pearl millet variety CO 8. Twenty five seeds for each treatment were exposed to colchicine @ of 0.00, 0.05, 0.06, 0.07, 0.08, 0.09 and 1.0 per cent for different durations of 6, 8, 12 and 24 hrs, and the highest germination and survival per cent was recorded in 0.05% for 6 hrs treatment with a range of 27.0 to 56.0 and, 1.5 to 19.4 per cent, respectively. Preliminary screening was based on stomatal measurements and maximum mean stomatal size was (51.7 ± 4.6) in the mutant with 0.07% for 8 hrs duration treatment. Two major variants were identified and the meiotic chromosome number of one of the variant (Pl. No. 36) was 24, against its control with 14 chromosomes. Bivalents and laggards were observed. The pollen fertility and seed set of this mutant were 53% and 5%, respectively. The values of the typical polyploidy traits of this mutant were higher than the control. The other variant was found to have bristled panicle (Pl. No. 36). Therefore, colchicines can be efficiently utilized for chromosomal doubling, however a very large population has to be maintained to isolate desirable mutants.

INTRODUCTION

India has the largest livestock population in the world and is the leader in cattle (16%) and buffalo (5.5%) population. The livestock sector contributes nearly 32% of the agricultural output, which contributes 22% of the total Indian GDP. Sustainable livestock production is highly dependent on the availability of quality feed and forage resources (Negawo *et al*., 2017). Of the several forages available, pearl millet is promising crop for green fodder supply. It can be used as sole fodder crop and also can be used in combination with Napier grass for development of Pearl Millet Napier (PMN) hybrids with improved fodder quality and yield.

Pearl millet (Pennisetum glaucumL.) is the most important drought tolerant cereal crop (Chouhan *et al*., 2015) grown primarily for food grain and fodder. It is an annual diploid (2n = 14) species with AA genome. Earlier, autotetraploidy in pearl millet was induced by Jauhar 1981, but so far, its utilization in pearl millet improvement has not been benefitted because of its poor seed set (Dujardin and Hanna, 1988). However, induced autopolyploidy in pearl millet plays an important bridging role in enhancing the crossability with other Pennisetum species and in facilitating gene flow (Hanna and Dujardin, 1985).

Induction of polyploidy in crops is a significant method for the production of new sources of germplasm that are applicable for plant genetic breeding (Tang *et al*., 2010). Besides, polyploidy can enhance quality parameters and also improves resistibility to biotic and abiotic stresses (Ahloowalia, 1967). Colchicine is widely used and found to have a significant effect on polyploid induction in plants, because of its effectiveness in arresting cell division at the anaphase stage (Kermani *et al*., 2003). It is obtained from *Colchicum autumnale* of angiosperm which binds specifically to tublins to prevent polymerization of microtubules and induces polyploidy (Ramachandran, 2013).

Limited works in tetraploid induction of pearl millet have been reported (Jauhar, 1981). In the present study, attempts were made for doubling the pearl millet genome by using colchicine. The induced plants were studied for various morphological and cytological traits (stomatal measurements and pollen fertility) and were further confirmed by meiotic chromosomal counts. The aim of this present study was to generate tetraploid pearl millet line for its utilization in crosses with tetraploid napier grass for further crop improvement studies in pearl millet napier hybrids.

MATERIALS AND METHODS

The pearl millet variety CO 8 was used in the current investigation. The study was conducted at the Department of Forage Crops, Centre for Plant Breeding and Genetics, TNAU, Coimbatore.

The experiment was carried out with diploid seeds (2n = 14), which were surface sterilized by immersing in sodium hypochlorite for 15 min, followed by thorough rinsing with deionised water (Liu *et al*., 2007). The sterilized seeds were pre- soaked for 3 hours in distilled water and carefully blotted

with a paper towel (to remove excess water) and immediately placed in a petridish containing germination paper soaked in different concentrations (seven) of colchicine for four durations. A total of 28 treatments including control were formulated to identify the effects of colchicine treatment. After ten days of treatment, the germination percentage and after 30 days of transplantation the survival percentage for each concentration was recorded and the data were subjected to statistical analysis.

To observe the phenotypic effects of colchicine, data were collected on plant height, number of tillers per plant, flag leaf length, flag leaf width, internodal distance, panicle length, seed set percentage. Besides, data on pollen fertility percentage (kumar *et al.*, 2013) and stomatal size was recorded. The stomatal measurements were carried following the procedure conducted by Quesenberry *et al.*, 2010 in Bahia grass. Chromosomal counts were made on pollen mother cells. The panicles from the control and variant plants were fixed in Carnoy's fluid (glacial acetic acid and alcohol 1:3) at appropriate stage between 8: 45 am during the bright sunshine hours. The material was left in the fixative for one day, rinsed thoroughly in 75% alcohol and transferred to 70% alcohol and stored in refrigerator for further use (vidya *et al.*, 2003). The course of meiosis was studied in temporary smears of pollen mother cells using 1% acetocarmine stain.

RESULTS AND DISCUSSION

Effect of colchicine on germination and survival per cent on pearl millet

Among the different sets of treatments, the concentration, duration and interaction of concentration vs duration showed significant differences for germination and survival percentage. It indicates the potentiality of the alkaloid in inducing the variability in pearl millet (Table 1). The germination and survival per cent got affected as the concentration increases in the treatments (Table 2 and graph 1). Among the different treatments, the highest germination and survival per cent was recorded in 0.05% concentration (Table 2). Pertaining to the durations, highest germination percent (50.33) was obtained at 6 hrs duration and the least (33.33) was recorded at 24 hrs duration treatment. Similarly, the highest survival per cent (16.66) was noticed at 6 hrs duration and least per cent (4) at 12 hrs duration treatment (Table 2).

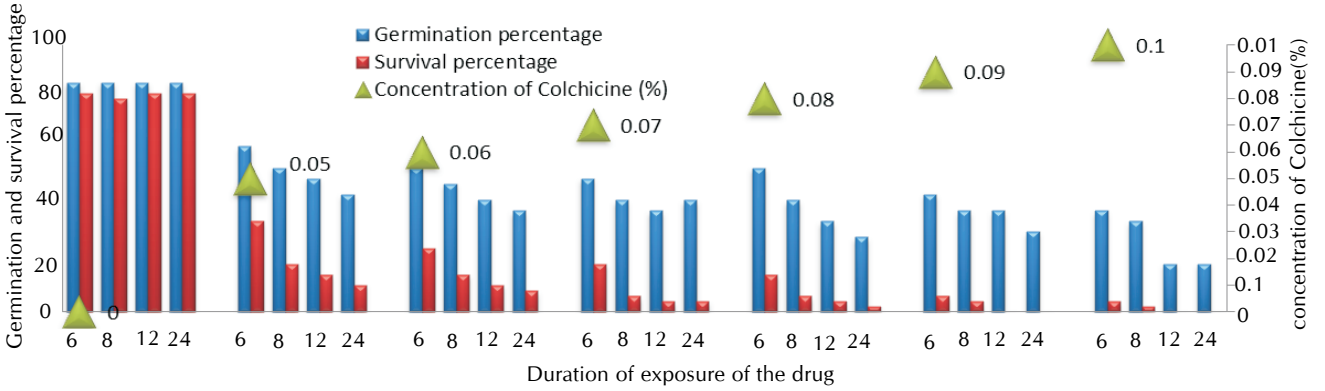
In combination of the duration and concentration, highest germination per cent (62.0) was recorded at the treatment, 0.05% colchicine for 6 hrs, followed by 0.05% for 8 hrs, 0.06 % for 6 hrs and 0.08% for 6 hrs with 54.0 per cent germination. In contrast, the least germination percent (18.0) was recorded at 0.10% colchicine for 12 hrs and 24 hrs treatment. Regarding the survival per cent, the highest value (34.0%) was obtained at the combination of 0.05% for 6 hrs. However, the treatments, 0.09 and 0.10% for 12 hrs and 24 hrs resulted in complete mortality of the plants. Therefore, of all the treatments, 0.05% for 6 hrs treatment was found to be rewarding for obtaining high germination percent (62.0%) and survival percent (34.0%).

Morphometric variations in colchiploids

The mean performance and their range for various traits *viz.*, plant height, number of tillers per plant, flag leaf length, flag leaf width, internodal length, panicle length, pollen fertility per cent, seed set per cent and stomatal measurements of the survived colchiploids were recorded and presented in Table 3. The highest mean value for plant height (149.3 ± 7.7) was recorded at 0.08% concentration for 8hrs duration treatment, but the range was low (141.6 to 157.0). However, higher range (119.5to182.0) for plant height was obtained at 0.08% for 6 hrs duration treatment. For number of tillers and inter nodal length, highest mean value (10.0 ± 1.8 and 18.5 ± 0.5, respectively) was recorded at 0.09% for 6 hrs treatment and their range was highest at 0.05% for 12 hrs treatment (3.0 to 12.0) and 0.08% for 6 hrs treatment (16.2 to 21.0), respectively. The treatment, 0.07% for 6 hrs recorded the highest mean

Table 1:Analysis of variance for germination, survival percentage and dosage effect

	Treatments (df =27)	Concentration (df= 6)	Duration (df= 3)	Concentration × Duration (df= 18)	Error (df= 28)
Germination per cent	276.98 NS	1090.05 **	218.83 **	15.66 *	5.81
Survival per cent	788.49 NS	3296.29 **	399.53 **	17.40 *	7.59

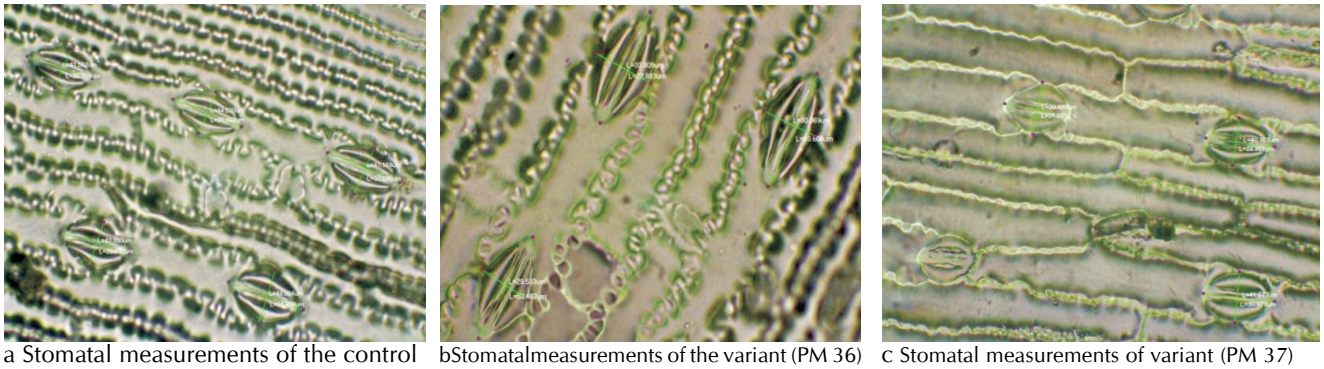


Graph 1: Summary of germination and survival per cent obtained by colchicine treatment at various concentrations and durations in pearl millet

Table 2: Effects of colchicine concentration and duration on the germination and survival percentage of treated bajra lines

Germination percentage						Survival percentage				
Concentration of colchicine	6hrs	8hrs	12hrs	24hrs	Mean of concentration	6hrs	8hrs	12hrs	24hrs	Mean of concentration
Control C0	86^{a} (68.8)	86^{a} (68.8)	86^{a} (68.8)	86^{a} (68.8)	86 (68.8)	80^{a} (64.9)	84^{a} (63.4)	80^{a} (64.9)	84^{a} (63.4)	82^{a} (64.4)
0.05%	62^{b} (51.9)	54^{bc} (47.2)	50^{cd} (45)	44de (41.5)	52 (46.5)	34^{b} (35.6)	18^{cd} (25)	14de (21.9)	10^{ef} (18.3)	19.4 (25.2)
0.06%	54^{bc} (47.2)	48^{cd} (43.8)	42^{defg} (40.3)	38^{efg} (38.0)	45.5 (42.3)	24^{c} (29.3)	14def (21.9)	10^{ef} (18.3)	08^{efg} (16.4)	14.3 (21)
0.07%	50^{cd} (45)	42^{defg} (40.3)	38^{efg} (38)	42^{defg} (40.3)	43 (40.9)	18^{cd} (25)	06^{fg} (13.9)	04^{gh} (11.5)	04^{gh} (11.5)	8.2 (15.5)
0.08%	54^{bc} (47.2)	42^{defg} (40.3)	34^{fgh} (35.6)	28^{h} (31.9)	39.5 (38.8)	14de (21.9)	06^{fg} (13.9)	04^{gh} (11.5)	02^{hi} (6)	6.5 (13.3)
0.09%	44de (41.5)	38^{cd} (43.8)	38^{efg} (38)	30^{h} (33.2)	37.5 (39.1)	06^{fg} (13.9)	04^{gh} (11.5)	0^{i} (0.5)	0^{i} (0.5)	2 (6.6)
0.10%	38^{efg} (38)	34^{gh} (35.6)	18^{i} (25)	18^{i} (25)	27 (30.9)	04^{gh} (11.5)	02^{hi} (6)	0^{i} (0.5)	0^{i} (0.5)	1.5 (4.6)
Mean of duration	50.3 (45.1)	43 (41.9)	36.6 (37.0)	33.3 (35.0)		16.6 (22.9)	8.3 (14.5)	5.3 (10.7)	4 (8.9)	
For comparing means of		SEd		CD @ 5%		SEd		CD @ 5%		
Concentration		1.2		2.46		1.37		2.82		
Duration		0.91		1.86		1.04		2.13		
Concentration x Duration		2.41		4.93		2.75		5.64		

Values in the parenthesis are arcsine transformed;The letter followed by the parameters indicates significance.



a Stomatal measurements of the control bStomatalmeasurements of the variant (PM 36) c Stomatal measurements of variant (PM 37)

Figure 1: Adaxial stomatal measurements of the control and variants in pearl millet

(70.7 ± 5.2) and range values (57.9 to 79.7) for flag leaf length. Highest mean panicle length (27.0 ± 4.0) was observed at 0.08% for 8 hrs treatments, while the range was highest (19.0 to 28.3) at 0.07% for 8 hrs duration treatment.

Status of pollen fertility and seed set among the variants

Pollen fertility per cent, seed set per cent and stomatal size are major traits that helps for polyploidy screening. In general the autoployploids were recorded to be having lower pollen fertility and seed set percentage and larger stomatal size when compared to its diploid counterparts (Table 3). The highest mean value for pollen fertility per cent (93.4 ± 2.8) and seed set per cent (80.5 ± 0.5) was recorded at 0.09 % for 6 hrs treatment. The lowest mean value for pollen fertility per cent (49.1 ± 3.0) and seed set per cent (34.5 ± 2.0) was recorded at 0.08 % for 8hrs treatment. The range for these traits was higher at 0.08% for 8 hrs treatment (16.1 to 82.2) and 0.07% for 8 hrs (55 to 79%), respectively

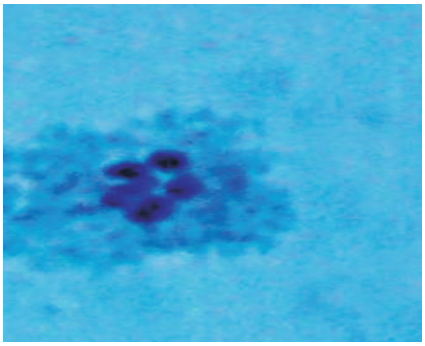
Stomatal variation in the colchiploids

To distinguish the induced polyploidy plants from their diploid counter parts, indirect technique of stomatal size measurement was considered as an alternative to the classical method of chromosome counting which is time demanding. Though exact ploidy level cannot be determined, using this technique, variants can be screened in a short time by examining the epidermal tissues without the requirements of specific equipment and high expenditure. In general, a correlation between stomatal length and plant ploidy has been established in a wide variety of angiosperms (Beaulieu *et al*., 2008), which was thought to be based on the enlargement of cells with increasing ploidy level. In the current study, preliminary screening of the variants was done based on the adaxial stomatal size measurements. Highest mean value for stomatal length (51.7 ± 4.6) was obtained at 0.07% for 8 hrs duration (Fig.1b) (Table 3) and highest mean value for width (30.4 ± 1.8) was obtained at 0.07% for 6 hrs duration treatment, against the control with the length and width of 45.0 ± 0.7 and 28.1 ± 0.4, respectively. In contrast, some of the variants have recorded lesser stomata measurements than control (Fig. 1c); however they are insignificant. The mean range of the stomata

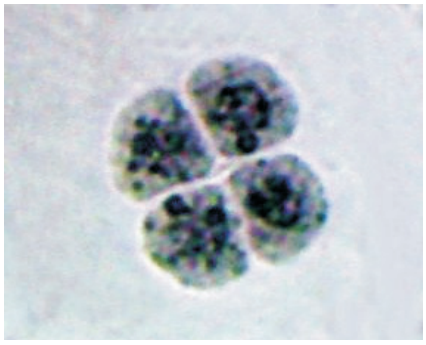
Table 3: Mean performance and range of colchiploids for various morphological, reproductive and stomatal traits.

S. No.	Conc. Of col chicine %control	Time (hrs)	Plant height (cm) Mean ± SE	Range	No. of tillers/ plant Mean ± SE	Range	Flag leaf length (cm) Mean ± SE	Range	Flag leaf width (cm) Mean ± SE	Range	Internodal length (cm) Mean ± SE	Range
			143.7 ± 1.3	142.4 - 145.1	8.5 ± 0.5	8.0 - 9.0	71.5 ± 0.5	71.0 - 72.3	4.1 ± 0.1	4.1 - 4.2	16.5 ± 05	16.2 – 17.0
1	0.05	6	97.3 ± 4.6	86.0 - 127.6	6.5 ± 0.4	4.0 - 8.0	64.5 ± 3.1	49.6 - 78.3	3.4 ± 0.2	2.0 - 4.5	15.5 ± 0.4	13.4 – 17.0
		8	106.3 ± 9.5	90.0 - 132.6	3.2 ± 0.7	2.0 - 5.0	70.0 ± 1.6	66.0 - 73.2	3.7 ± 0.1	3.2 – 4.0	13.2 ± 1.0	12.0 - 16.5
		12	115.5 ± 14.6	95.5 - 144.0	6.6 ± 2.7	3.0 - 12.0	67.7 ± 4.9	57.9 - 73.0	3.9 ± 0.1	3.6 - 4.2	15.6 ± 2.1	12.0 - 19.4
		24	112.6 ± 8.0	104.6 - 120.7	7.5 ± 0.5	7.0 - 8.0	70.4 ± 3.0	67.4 - 73.4	4.4 ± 0.1	4.3 - 4.5	17.5 ± 0.5	17.0 – 18.0
2	0.06	6	134.2 ± 5.7	112.0 - 153.4	7.8 ± 0.6	6.0 - 10.0	70.4 ± 5.6	49.5 - 89.5	4.1 ± 0.2	3.0 - 4.8	17.2 ± 0.7	14.6 - 19.8
		8	128.3 ± 4.3	102.6 - 152.0	9.6 ± 0.3	9.0 - 10.0	56.1 ± 3.4	52.4 – 63.0	4.4 ± 0.2	4.0 - 4.8	14.3 ± 0.7	13.3 - 15.7
		12	137.3 ± 8.3	129.0 - 145.6	5.0 ± 0.0	5.0 - 5.0	69.7 ± 9.3	60.4 – 79.0	3.6 ± 0.4	3.2 – 4.0	16.9 ± 0.5	16.4 - 17.4
		24	134.7 ± 14.3	120.4 - 149.0	9.0 ± 1.0	8.0 - 10.0	62.2 ± 2.6	59.6 - 64.9	3.9 ± 0.1	3.8 - 4.1	17.0 ± 1.0	16.0 – 18.0
3	0.07	6	138.8 ± 11.8	105.7 - 160.4	4.5 ± 0.6	3.0 - 6.0	70.7 ± 5.2	57.9 - 79.7	3.8 ± 0.2	3.2 - 4.4	18.3 ± 0.5	17.4 - 19.8
		8	147.8 ± 26.8	121.0 - 174.6	9.0 ± 3.0	6.0 - 12.0	68.8 ± 6.3	62.5 - 75.2	3.6 ± 0.6	3.0 - 4.2	18.1 ± 0.0	18.1 - 18.2
4	0.08	6	144.1± 19.2	119.5 - 182.0	9.3 ± 1.4	7.0 - 12.0	68.8 ± 6.6	60.8 - 82.0	3.6 ± 0.1	3.5 - 3.8	18.4 ± 1.4	16.2 – 21.0
		8	149.3 ± 7.7	141.6 - 157.0	6.5 ± 1.5	5.0 - 8.0	61.7 ± 10.9	50.8 - 72.6	3.2 ± 0.2	3.0- 3.5	17.7 ± 1.4	16.3 - 19.1
5	0.09	6	139.4 ± 10.8	128.6 - 150.2	10.0 ± 10.8	9.0 - 11.0	69.5 ± 8.9	60.6 - 78.4	3.9 ± 0.5	3.4 - 4.4	18.5 ± 0.5	18.0 – 19.0
S. No.	Conc.	Time	Panicle length (cm)		Pollen fertility percentage		Seed set percentage		Adaxial Stomatal measurements (μm) Length		width	
			Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
	control		24.6 ± 0.5	24.0 - 25.1	98.5 ± 0.5	98.0 - 99.0	85.5 ± 0.5	85 - 86	45.0 ± 0.7	44.1 - 45.9	28.1 ± 0.4	28.1 – 29.2
1	0.05	6	20.3 ± 1.0	14.8 - 24.6	80.9 ± 2.9	70.0 - 92.6	71.8 ± 2.1	65 - 85	43.6 ± 2.1	33.6 - 53.2	29.2 ± 0.7	26.2 - 32.2
		8	13.0 ± 1.9	8.4 - 16.9	77.7 ± 5.7	64.9 - 90.4	70.0 ± 5.4	56 - 81	41.3 ± 3.8	31.8 - 50.2	28.4 ± 2.1	25.2 - 33.6
		12	19.8 ± 0.4	18.9 - 20.5	79.8 ± 3.3	75.9 - 86.4	71.0 ± 4.1	65 - 79	40.8 ± 2.7	37.5 - 46.3	29.3 ± 0.4	27.2 - 32.3
		24	15.5 ± 1.3	14.2 - 16.9	91.1 ± 4.2	86.9 - 95.4	78.0 ± 7.0	71 - 85	40.6 ± 1.7	38.9 - 42.3	26.3 ± 2.0	24.2 - 28.4
2	0.06	6	22.7 ± 1.0	19.9 - 26.4	89.6 ± 1.7	83.4 - 95.4	73.5 ± 1.95	69 - 80	42.5 ± 2.4	34.8 - 48.1	27.1 ± 1.2	23.9 - 31.7
		8	20.2 ± 0.9	19.0 - 22.1	82.5 ± 1.0	80.5 - 83.5	71.6 ± 1.4	69 - 74	45.3 ± 3.6	39.8 - 52.2	26.4 ± 1.7	23.4 - 29.5
		12	20.2 ± 2.3	17.9 - 22.5	91.1 ± 1.6	89.5 - 92.8	78.0 ± 1.0	77 - 79	41.7 ± 4.7	37.0 - 46.4	25.9 ± 2.3	23.6 - 28.3
		24	22.4 ± 1.9	20.5 - 24.3	90.0 ± 6.2	83.8 - 96.3	69.5 ± 4.5	65 - 74	44.6 ± 2.7	41.9 - 47.4	27.8 ± 1.6	26.1 - 29.5
3	0.07	6	18.8 ± 0.6	17.2 - 20.3	87.4 ± 4.4	75.9 - 97.3	75.7 ± 5.2	62 - 86	46.4 ± 2.4	41.4 - 52.7	30.4 ± 1.8	27.1 - 35.0
		8	23.6 ± 4.6	19.0 - 28.3	73.8 ± 0.6	53.2 - 94.4	42.0 ± 3.0	55 - 79	51.7 ± 4.6	47.0 - 56.3	27.7 ± 0.3	27.4 - 28.0
4	0.08	6	21.8 ± 0.9	20.0 - 23.3	82.1 ± 4.7	72.8 - 88.1	69.3 ± 3.8	62 - 75	44.1 ± 1.0	42.1 - 45.7	27.4 ± 1.2	25.5 - 29.8
		8	27.0 ± 4.0	23.0 - 31.0	49.1 ± 3.0	16.1 - 82.2	34.5 ± 2.0	54 - 69	47.0 ± 0.2	46.8 - 47.2	28.1 ± 2.4	25.7 - 30.6
5	0.09	6	22.3 ± 2.7	19.6 – 25.0	93.4 ± 2.8	90.5 - 96.3	80.5 ± 0.5	80 - 81	44.3 ± 2.4	41.9 - 46.7	27.1 ± 1.0	26.1 - 28.2

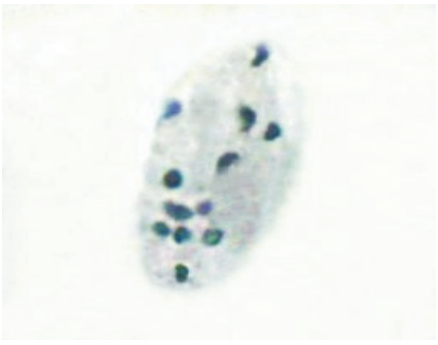
Note: Data was depicted only for the treatments showing survival per cent >4.0.



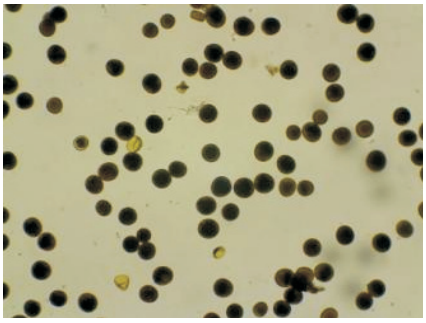
a. Control (7 II);



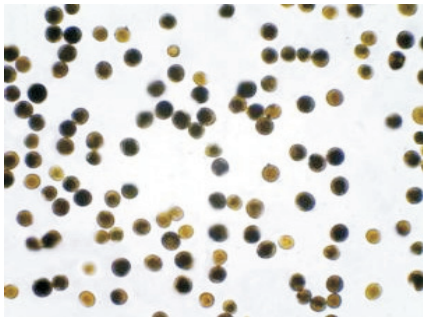
b. Control (Tetrad)



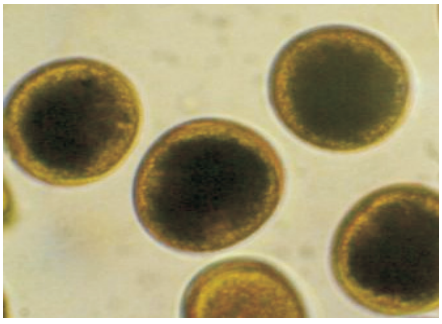
c.Variant (12 II)



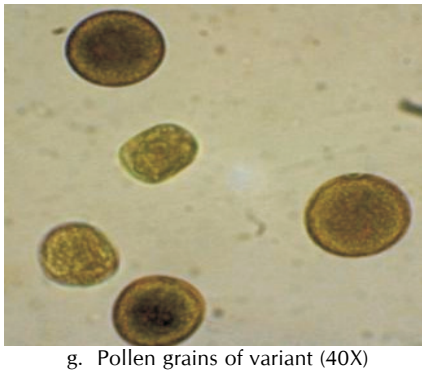
d. Pollen fertility of control



e. Pollen fertility of variant

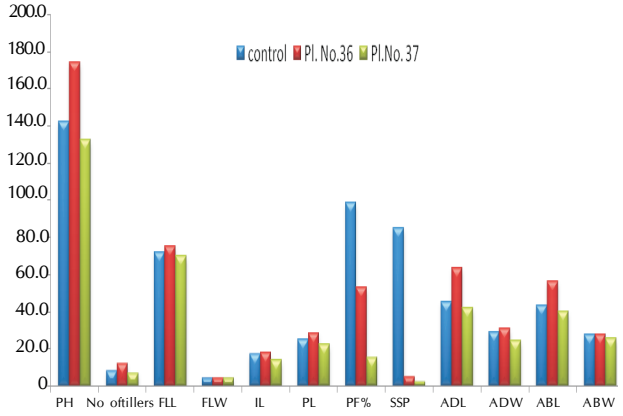


f. Pollen grains of control (40X)



g. Pollen grains of variant (40X)

Figure 2: Cytological and pollen fertility variations observed in the control and aneuploidy (PM 36) of pearl millet



Graph 2: PH = plant height; FLL = Flag Leaf Length; FLW = Flag Leaf Width; IL = Internodal Length; PL = Panicle Length; PF = Pollen Fertility; SSP = Seed Set Percentage; ADL and ADW = Adaxial stomatal Lengthand Width and ABL and ABW = Abaxial Stomatal Length and Width.

length was highest at 0.05% treatment for 8 hrs (31.8 – 50.2) and width at 0.06% treatment for 6hrs (23.9 – 31.7). The ploidy level of the variant with higher mean values of stomata length and width was further confirmed by meiotic studies.

Cytological behavior of the varaiant

The variant was obtained at colchicine treatment concentration of 0.07 per cent for 8 hrs and recorded to be aneuploid, with increased chromosomal number (24 chromosomes). These 24 chromosomes were observed as bivalents (12) (Fig. 2c). Univalents ranging from 1 to 4 and laggards were rarely observed. Pollen grains of various sizes were seen and fertility of 53% was observed (Fig.2e and 2g), while the control recorded 98.0% fertility and pollen of almost similar size (Fig. 2d and 2f). The seed set percentage of this variant was 5%. Similar such results were reported by Sun *et al*. (1994) in sorghum. When compared to the control, the variant has recorded higher mean values for plant height, number of tillers per plant, flag leaf length and width, internodal distance, panicle length and stomatal measurements (Graph 2), which

Figure 3: Bristle panicle mutant

are typical characters of induced polyploids. Another mutant with bristled panicle (Fig. 3) was observed at 0.07% for 12 hrs duration. When compared to the control it has recorded, lower values for all the traits studied, except for flag leaf width (Graph 2).

Besides, utilization of diploid pearl millet as fodder crop, tetraploid pearl millet can be developed and utilized for hybridization with tertaploid napier, that may generate tetraploid fertile hybrid. Therefore, attempts were made to double the chromosomes of pearl millet. In the current experiment, as the concentration and duration of the colchicine treatment was increased, the germination and survival per cent decreased (Graph 1). Similar such findings were also reported by Liu *et al*.(2007) in Platanus acerifolia, Zeinab *et al*.(2012) in Berseem, Lam *et al*.(2014) in *Acacia crassicarpa* for germination percentage. Pertaining to survival per cent, Kadota and Niimi (2002) in Japanese pea cultivar and by Timbo *et al*. (2014) in *B. ruziziensis* reported similar findings.

The variant has recorded higher plant height than the control and the results were in accordance with the findings of Gonzalez and Hanna (1984) in pearl millet napier hybrids and Dujardin *et al*. (1989) in induced Tift 23 R pearl millet inbred line. In contrast, reduced plant height was recorded by Sourour *et al*. (2014) in barley. The induced polyploid recorded enhanced panicle length and these findings were similar to the results of Watanabe and Yamazaki (1973) in rice and Dujardin *et al*. (1989) in pearl millet. In contrast, shorter panicles in induced polyploids were observed by Siddiqi and Marwat (1983) in wheat and Vanitha *et al*. (2013) in sorghum.

The variant recorded reduction in pollen fertility and similar documentations in induced polyploids when compared to their control was done by Jb *et al*. (1968) in Brassica, Kaushal *et al*. (2015) in Pennisetum orientale and Wang *et al*. (2017) in buck wheat, while Dibyendu (2010) in grass pea and Pereira *et al*. (2014) in Lolium multiflorum reported increased pollen fertility. For seed set per cent, decreased seed set in induced autopolyploids was also documented by Hanna *et al*. (1976) in pearl millet, Siddiqi and Marwat (1983) in wheat and Sun and Gh (1994) in sorghum, which were similar to the current findings.

Therefore, based on the results obtained, the current investigation reveals that colchicine is an efficient antimitotic agent that generates variants and it could be further utilized for crop improvement programme. Eigsti and Dustin (1957) reported that morphological variations can be induced by colchicine which may be a direct action of the drug by affecting the rate of enzymatic reaction rather than nuclear change. It is also certain that the reaction to the chemical depends on the concentration and duration of the treatment, which was evident from the wide range of results obtained while treating with the same concentration for different durations. It also reveals the importance of stomatal measurements in ploidy determination. Therefore in conclusion, 0.05% and 0.06% were found to be effective in generating variants, while the treatment with 0.07% with 8hrs and 12hrs was found to be most effective in the generation of selective mutants. These obtained mutants will be further screened for its cytological stability and seed set percentage and the stable ones will be utilized for crop breeding programme to develop pearl millet napier allopolyploids.

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