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Colchicine-induced chromosome doubling in *Pennisetum* interspecific hybrids and its effect on plant morphology

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Abstract

We report the production of hexaploid plants of interspecific hybrids of *Pennisetum*, with the ultimate aim to improve the biomass yield, drought tolerance and multicut behaviour of this genus. Chromosome doubling was achieved with the application of colchicine at three different concentrations (0.05, 0.1 and 0.2%) for two time durations (12 and 24 hours). The root slips and stem cuttings of interspecific hybrids were used for treatment and the root slips were found to be more efficient. The preliminary screening to select the putative hexaploid plant was done based on stomatal frequency and morphology. Plants containing significantly lower stomatal frequency and larger stomata size were selected for further analysis by chromosome counting. This experiment confirmed that 0.1% concentration of colchicine treatment to root slips for 24 hours was more effective to induce the amphiploids in *Pennisetum.*

Key words: Interspecific hybrids, *Pennisetum*, amphiploids, stomatal morphology, chromosome doubling

Introduction

Plant breeding is a perpetual task to develop high yielding varieties which are well adapted to the target environment. *Pennisetum* is one of the important genera of the Poaceae family (Brunkun 1977). The most economically important cultivated species are pearl millet [*Pennisetum glaucum* (L.) R. Br.] and Napier grass [*Pennisetum purpureum* (K.) Schum]. Pearl millet is a diploid with 2n = 2x = 14 (genomes AA) and Napier grass is an allotetraploid species with 2n = 4x = 28 (genomes A'A'BB). The species are closely related and have a good capacity for genetic combination, producing interspecific hybrids with 2n = 3x = 21 (genomes A'AB). This type of hybridization is performed in an attempt to combine the characteristics of Napier grass such as high biomass, perennial nature, aggressiveness and high dry matter yield (Diz 1994, Jauhar and Hanna 1998) with well adapted variety, pearl millet having characteristics like high vigour, tolerance to diseases, forage quality etc. The resulting Interspecific hybrids have higher palatability to cattle than Napier grass. However, the sterility of these hybrids (Burton 1944, Hanna 1981) due to meiotic irregularities has been considered as a major constraint for breeding programme.

The difficulty of sterility can be overcome by doubling the chromosome number of a hybrid so that each chromosome get homologous chromosome and make perfect pairing of chromosomes. Such organisms or hybrids in which the chromosome number is doubled are called amphiploids. The chromosomal duplication has been done in buds and shootings by Barbosa and coworkers (2007). The use of antimitotics for chromosome duplication represents an important tool to produce hexaploid hybrids with 2n = 6x = 42 with genomes A'A'AABB coupled with restored fertility. Common antimitotic agents used for chromosome doubling are colchicine, trifluralin and oryzalin (Yu et al. 2009, Dhooghe et al. 2011) but colchicine is the most commonly used chromosome doubling agent (Dermen and Henry 1944, Griesbach 1981, Ramulu et al. 1991, Tuyl et al. 1992, Ishizaka and Uematsu 1994, Wu et al. 2011, Segraves and Anneberg 2016). For the induction of polyploidy in plants, an alkaloid that is Colchicine is widely used for chromosomal doubling (Pasakinskiene 2000, Petersen et al. 2002, Nimura et

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al. 2006, Segraves and Anneberg 2016). Kadota and Niimi in 2002 used this *in vitro* treatment of explants with colchicine in many cases.

Stomata are found in the epidermis of aerial parts of flowering plants. Stomata is organised with a pair of guard cells flanking a microscopic pore.Vandenhout et al. (1995) and Quesenberry et al. (2010) have successfully used stomatal frequency and size as the indirect measure of altered ploidy in banana and bahiagrass, respectively.

Materials and methods

Plant material

The four interspecific hybrids (FBC 16 × M 30086, PIB 394 × M 30086, PIB 394 × K 52440, PIB 339 × K 59347 and two check varieties (PBN 233 and PBN 346) were taken.

Chromosome doubling

The root and stem cuttings of interspecific hybrids and two check varieties that is PBN 233 (2n=3x=21) and PBN 346 (2n=3x=21) were treated with the three different concentrations of colchicine i.e. 0.05%, 0.1% and 0.2% at two different durations i.e. 12 hours and 24 hours in a pot containing colchicine solution prepared in water.After treatment, the plants were transplanted in the field. The F_1 plants were evaluated morphologically and cytologically for confirmation of hybridity.

Characterization of Amphiploids

Stomata analysis

A thin coating of natural nail polish or quick fix was applied to approximately one cm^2 strips from the central portion of the lower epidermis of leaves. As the quick fix or nail polish dries, the imprints of the stomata were drawn away slowly with nails or forcep and mounted on the glass slide. This method is commonly known as Fevicol sticker method (Nayeem and Dalvi 1989). The number of stomata was determined using light microscope at 40X magnification (Solangi 2001). For measurements of stomata, micrometry technique was used. The ocular micrometer was first calibrated using stage micrometer. Then the length and width of stomatal aperture was measured with the help of ocular micrometer. CD and CV was calculated using CPCS1 (Computer package by Cheema and Singh 1). The plants showing significant results for stomata frequency and size were analysed by Scanning Electron Microscopy (SEM).

Cytogenetic study

For meiotic studies, young flower buds were collected and fixed in carnoy's fixative consisting of absolute alcohol, glacial acetic acid and chloroform (1:1:1) for 24 hours. Later, they were washed under running water to remove all the traces of the fixative and stored in 70% ethanol at low temperature. Freshly prepared one percent acetocarmine stain was used for staining chromosomes by usual squash method. For different stages of microsporogenesis, minimum of 10 well spread and stained pollen mother cells were observed.

Results and discussion

Stomata analysis was made to determine whether it could be an effective and convenient way to select the putative plants. There is a wide variation in stomata frequency and measurements among the interspecific hybrids and the hybrids obtained after treatment of colchicine. Therefore the stomata frequency and measurements were analysed as a screening approach for altered ploidy (Singh and Sethi 1995; Tavan et al. 2015; Nalawade and Gurav 2017; Manzoor et al. 2019). It was concluded that the stomatal density is more on the abaxial surface than on the adaxial surface in all the interspecific hybrids and check varieties. From the four interspecific hybrids and two check varieties, only one interspecific hybrid and one check variety was found to be significant at $P \leq 0.05$ for stomata density and size.

From Table 1 and Fig. 1 among four interspecific hybrids and two check varieties, only one interspecific hybrid and one check variety was found to be significant at $P \leq 0.05$ for stomata density and size. In this study, the average density of stomata of PIB 394 × k 52440 (control) was 132.40 ± 1.30 and 89.81 ± 7.72 for abaxial and adaxial surfaces respectively and the hybrid following 0.05% for 24 hours was found to have 99.62 ± 5.95 and 65.92 ± 9.53 for abaxial and adaxial surfaces respectively (Table 2, Fig. 3). The same hybrid was having average polar and equatorial length 26.23 ± 1.37 μm and 16.25 ± 1.79 μm on the abaxial surface, and 27.11 ± 1.22 μm and 16.71 ± 1.22 μm on the adaxial surface respectively. While the colchicine treated plant following 0.05% colchicine treatment for 24 hr has average polar and equatorial length 36.12 ± 3.35 μm and 27.02 ± 2.12 μm on the abaxial surface, and 36.51 ± 2.84 μm and 18.37 ± 1.59 μm on the adaxial surface.

Table 1. Effect of colchicine at different concentrations (0, 0.05%, 0.1% and 0.2%) for two different durations (12 hour and 24 hour) on stomatal frequency (mm^2) of new interspecific crosses and check varieties

S.No.	PMN hybrid	Colchicine concentration	Stomatal frequency (mm^2)			
			Leaf surface			
			Abaxial		Adaxial	
			Duration of treatment			
			12 hr (mean ± SE)	24 hr (mean ± SE)	12 hr (mean ± SE)	24 hr (mean ± SE)
1.	PIB 394 × K 52440	0	132.4 ± 1.30	132.4 ± 1.30	89.81 ± 4.72	89.81 ± 4.72
		0.05%	85.92 ± 1.31	**99.62 ± 5.95**	62.96 ± 7.49	**65.92 ± 9.53**
		0.1%	94.44 ± 5.23	128.08 ± 1.65	80.86 ± 7.25	91.35 ± 4.93
		0.2%	94.81 ± 3.65	128.14 ± 3.94	65.18 ± 8.18	94.07 ± 7.51
	CD (p=0.05)		**NS**	**8.53**	**NS**	**15.34**
2.	PBN 233	0	190.74 ± 4.72	190.74 ± 4.72	110.18 ± 2.61	110.18 ± 2.61
		0.05%	129.62 ± 5.01	184.56 ± 5.26	101.85 ± 2.50	104.31 ± 3.85
		0.1%	105.55 ± 5.09	**101.47 ± 5.26**	103.55 ± 5.45	**54.44 ± 10.9**
		0.2%	143.56 ± 8.68	179.62 ± 9.65	111.11 ± 9.27	102.59 ± 8.08
	CD (p=0.05)		**NS**	**21.90**	**NS**	**15.27**

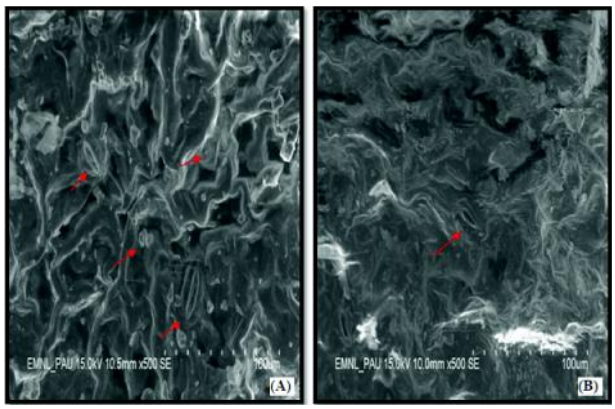

Fig. 1. Scanning electron microscope showing stomata frequency of interspecific hybrid PIB 394 × K 52440 (A): control (having high stomata frequency), (B): treated with 0.05% colchicine concentration for 24 hrs (having low stomata frequency)

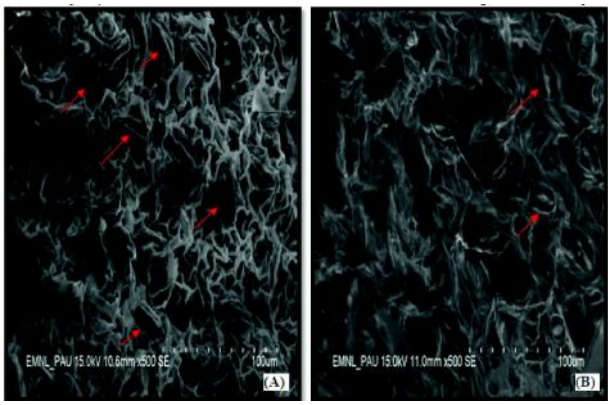

Fig. 2. Scanning electron microscope showing stomata frequency of check variety PBN 233 (A): control (having high stomata frequency), (B): treated with 0.1% colchicine concentration for 24 hrs (having low stomata frequency)

Therefore it can be concluded that there is 24.75 and 28.52% reduction in stomatal frequency on abaxial surface and adaxial surface respectively, and also, there is 9.64 μm and 10.77 μm increase in polar and equatorial length on abaxial surface and 9.40 μm and 1.66 μm increase in polar and equatorial length on the adaxial surface. Our results were coordinated by Mo et al. (2020).

In the check variety PBN 233, the control plant has 190.74 ± 4.72 and 110.18 ± 2.61 stomata/mm^2 on the abaxial and adaxial surfaces respectively while the hybrid following 0.1% colchicine treatment to the root slips for 24 hours duration was having 101.47 ± 5.26 and 54.44 ± 10.9 stomata/mm^2 on the abaxial and adaxial surfaces (Table 1, Fig. 2). The same hybrid was found to have mean polar and equatorial length 12.83 ± 1.95 μm and 10.49 ± 1.28 μm on the abaxial surface, and 10.75 ± 0.75 μm and 9.95 ± 0.65 μm on the adaxial surface respectively. Whereas the hybrid following 0.1% colchicine treatment for 24 hours has mean polar and equatorial length 28.69 ± 1.25 μm and 15.6 ± 0.06 μm on the abaxial surface, and 26.73 ± 1.19 μm and 15.25 ± 0.00 μm on the adaxial surface respectively (Table 2, Fig. 4).

So, we observed there were 46.80 and 50.58%

Table 2. Effect of colchicine at different concentrations (0, 0.05%, 0.1% and 0.2%) for two different durations (12 hour and 24 hour) on stomatal size (mm) of new interspecific crosses and check varieties

S.No.	PMN Hybrid	Colchicine concentration	Polar length (µm)				Equatorial length (µm)			
			Abaxial		Adaxial		Abaxial		Adaxial	
			12 hr	24 hr	12 hr	24 hr	12hr	24 hr	12hr	24 hr
1.	FBC 16 × M 30086	0	25.73 ± 0.72	25.73 ± 0.72	23.36 ± 2.51	23.36 ± 2.51	17.71 ± 0.21	17.71 ± 0.21	16.86 ± 0.01	16.86 ± 0.01
		0.05%	26.00 ± 0.04	28.48 ± 0.18	23.42 ± 2.63	24.72 ± 1.60	17.96 ± 0.78	18.09 ± 0.78	16.91 ± 0.94	17.31 ± 0.94
		0.1%	25.75 ± 0.70	26.25 ± 2.83	24.45 ± 1.13	22.55 ± 1.83	18.05 ± 0.84	18.63 ± 0.67	14.96 ± 0.40	17.82 ± 1.78
		0.2%	26.86 ± 2.00	24.52 ± 1.22	24.52 ± 1.35	25.26 ± 0.57	17.75 ± 0.42	17.29 ± 1.91	17.68 ± 2.68	18.91 ± 0.94
	CD (p=0.05)		NS	NS	NS	NS	NS	NS	NS	NS
2.	PIB 394 × M 30086	0	23.09 ± 0.75	23.09 ± 0.75	22.90 ± 0.48	22.90 ± 0.48	13.45 ± 3.11	13.45 ± 3.11	13.75 ± 3.53	13.75 ± 3.53
		0.05%	24.45 ± 1.13	24.91 ± 0.94	24.45 ± 1.69	23.25 ± 1.41	12.55 ± 1.55	14.11 ± 2.17	12.75 ± 3.53	13.55 ± 3.25
		0.1%	23.45 ± 3.11	24.41 ± 0.23	23.55 ± 3.25	22.35 ± 2.61	12.38 ± 3.01	13.49 ± 2.78	12.76 ± 3.55	11.87 ± 0.88
		0.2%	23.4 ± 3.11	24.2 ± 2.26	23.65 ± 1.20	22.85 ± 1.90	11.05 ± 0.77	13.05 ± 2.05	13.5 ± 1.41	11 ± 0.70
	CD (p=0.05)		NS	NS	NS	NS	NS	NS	NS	NS
3.	PIB 394 × K 52440	0	27.11 ± 1.22	27.11 ± 1.22	26.23 ± 1.37	26.23 ± 1.37	16.71 ± 1.22	16.71 ± 1.22	16.25 ± 1.79	16.25 ± 1.79
		0.05%	25.11 ± 0.84	36.51 ± 3.35	24.44 ± 1.12	36.12 ± 2.84	16.75 ± 0.35	27.02 ± 2.12	16.55 ± 1.27	18.37 ± 1.59
		0.1%	27.75 ± 1.06	27.85 ± 0.56	25.05 ± 0.28	25.42 ± 0.31	15.87 ± 0.88	15.71 ± 1.60	15.68 ± 0.25	15.38 ± 0.51
		0.2%	27.87 ± 0.88	27.00 ± 0.62	26.91 ± 1.88	25.54 ± 1.35	16.5 ± 0.00	16.3 ± 0.70	15.39 ± 0.71	16.24 ± 0.39
	CD (p=0.05)		NS	6.72	NS	5.53	NS	4.11	NS	1.81
4.	PIB 339 × K 59347	0	27.90 ± 0.48	27.90 ± 0.48	27.12 ± 0.53	27.12 ± 0.53	16.98 ± 0.65	16.98 ± 0.65	16.35 ± 1.55	16.35 ± 1.55
		0.05%	26.55 ± 1.34	25.11 ± 0.75	26.62 ± 2.92	25.74 ± 0.14	17.05 ± 0.74	17.71 ± 2.64	15.99 ± 0.74	13.91 ± 0.94
		0.1%	26.05 ± 0.71	27.16 ± 2.15	25.61 ± 4.28	26.61 ± 2.87	17.98 ± 0.75	18.05 ± 0.84	17.10 ± 2.05	17.61 ± 1.64
		0.2%	26.45 ± 0.07	28 ± 0.70	26.7 ± 3.11	27 ± 1.97	14.5 ± 5.65	15.85 ± 0.91	16.5 ± 0.42	15.4 ± 0.70
	CD (p=0.05)		NS	NS	NS	NS	NS	NS	NS	NS
5..	PBN 233	0	12.83 ± 1.95	12.83 ± 1.95	10.75 ± 0.70	10.75 ± 0.70	10.49 ± 1.28	10.49 ± 1.28	9.95 ± 0.65	
	9.95 ± 0.65									
		0.05%	14.75 ± 0.70	13.92 ± 1.92	17.58 ± 1.56	10.95 ± 0.98	10.43 ± 0.21	9.56 ± 0.02	10.77 ± 0.74	10.77 ± 0.74
		0.1%	13.30 ± 2.90	28.69 ± 1.25	14.25 ± 2.26	26.73 ± 1.19	9.90 ± 0.48	15.6 ± 0.06	10.09 ± 0.62	15.25 ± 0.00
		0.2%	13.54 ± 1.43	13.32 ± 2.92	11.25 ± 4.24	11.40 ± 1.63	10.77 ± 3.31	10.77 ± 2.14	10.75 ± 0.71	10.53 ± 1.43
	CD (p=0.05)		NS	7.58	NS	4.28	NS	3.38	NS	2.93

*The values given in table 2 are the mean data of three plants recorded in a replicated trial

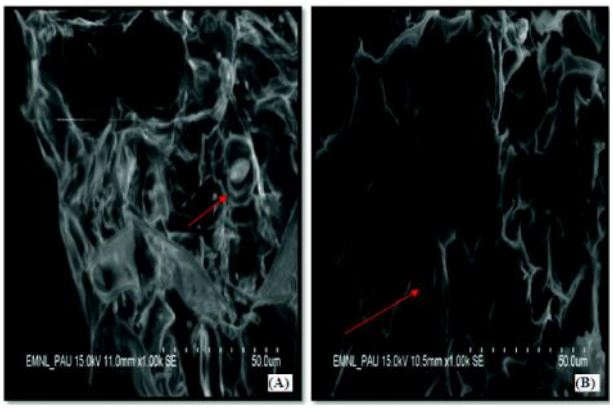

Fig. 3. Scanning electron microscope showing stomata size for interspecific hybrid PIB 394 × K 52440 (A): control (having smaller size of stomata), (B): treated with 0.05% colchicine concentration for 24 hrs (having larger size of stomata)

reduction in the stomatal frequency at abaxial surface and adaxial surfaces respectively. Also there was average 15.86 μm and 5.11 μm increase in the polar and equatorial length of abaxial surface and average 16.24 μm and 5.30 μm increase on the abaxial surface from the triploid to the hexaploid plant. The similar

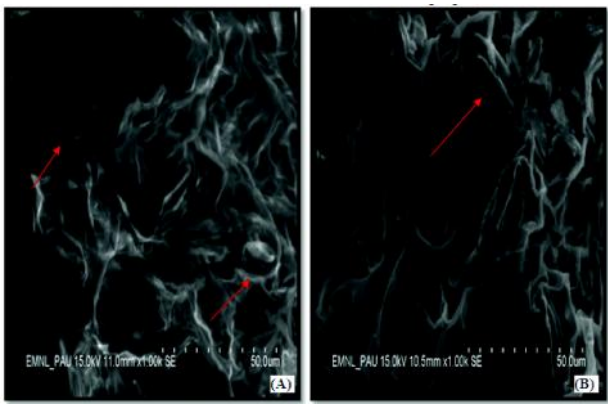

Fig. 4. Scanning electron microscope showing stomata size for check variety PBN 233 (A): control (having smaller size of stomata), (B): treated with 0.1% colchicine concentration for 24 hrs (having large stomata)

results were reported by Soetopo and Hosnia (2018) and Mo et al. (2020).

There were only two putative hybrids after stomatal morphology analysis. One of which was the interspecific hybrid (PIB 394 × K 52440) following 0.05% colchicine concentration for 24 hours to root slips and the other was the check variety PBN 233 following 0.1% colchicine concentration for 24 hours to root slips were analysed for cytological investigation.

The interspecific hybrid PIB 394 × K 52440 (Control) was having 21 chromosomes as given in [Fig 5 (A)] and the interspecific hybrid PIB 394 × K 52440 following 0.05% for 24 hours showed the mixoploids as given in [Fig. 5 (B)] which means that the colchicine 0.05% for 24 hours was ineffective to induce amphiploidy.

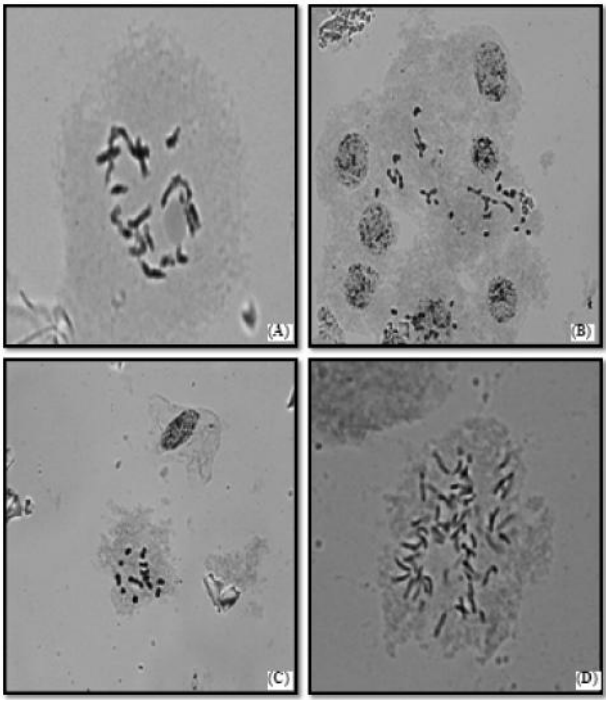

Fig. 5. Cytological investigation of putative amphiploids in PMCs at metaphase stage prepared from young flower buds (A) Interspecific hybrid PIB 394 × K 52440 [Control showing triploid (2n = 3x = 21)], (B) Interspecific hybrid PIB 394 × K 52440 (0.05% for 24 hrs showing mixoploid); (C) Hybrid PBN 233 (Control showing 21 chromosomes) (D) Hybrid PBN 233 (0.1% for 24 hrs showing more number of chromosomes)

The check variety PBN 233 treated (Control) was having 21 chromosomes [Fig. 5 (C)] and the same treated with 0.1% concentration of colchicine for 24 hours to root slips was reported to have more chromosomes when compared with the untreated (triploid) plant [Fig. 5 (D)].

Authors' contribution

Conceptualization of research (RK, YV, AK); Designing of the experiments (RK, YV); Contribution of experimental materials (RK); Execution of field/lab experiments and data collection (AK); Analysis of data and interpretation (AK, RK, YV, AK); Preparation of manuscript (AK, RK, YV).

Declaration

The authors declare no conflict of interest.

References

Aryavand A., EhdaieB., TranB. and Waines J. G. 2003. Stomatal frequency and size differentiatae ploidy levels in *Aegilops neglecta,* Gen. Res. and Crop Evol., **50**: 175-182.

Barbosa S., Davide L. C., Pereira A. V. and Abreu J. C. 2007.Chromosomal duplication of triploid hybrids of elephant grass and millet. Bragantia, **66**: 365-372.

Brunkun J. N. 1977. A systematic study of *Pennisetum* sect. *Pennisetum* (Gramineae). Am. J. Bot., **64**: 161-176.

Burton G. W. 1944. Hybrids between Napier grass and cattail millet. J. Hered., **35**: 227-232.

Chen G., Sun W. B. and Sun H. 2009.Morphological characteristics of leaf epidermis and size variation of leaf, flower and fruit in different ploidy levels in *Buddleja macrostachya* (Buddlejaceae). J. Syst. and Evo., **47**(3): 231-236.

Derman H. and Henry F. B. 1944. A general cytohistological study of colchicine polyploidy in Cranberry. Am. J. Bot., **31**: 451-463.

Dhooghe E., Laere K. and Eeckhaut T. 2011.Mitotic chromosome doubling of plant tissues in vitro.Plant Cell Tiss. Organ Cult., **104**: 359-373.

Diz D. A. 1994. Breeding procedures and seed production management in pearl millet × elephantgrass hexaploids hybrids. Ph.D. Dissertation. University of Florida, USA.

Griesbach R. J. 1981. Colchicine induced poltploidy in *Phalaenopsis* orchids. Plant Cell Tiss. Org. Cult., **1**: 103-107.

Hanna W. W. 1981. Method of reproduction in Napier grass and in the 3X and 6X alloploid hybrids with pearl millet. Crop Sci., **21**: 123-126.

Ishizaka H. and Uematsu J. 1994. Amphiploids between *Cyclomen persicum* Mill. and *C. hederifolium* Aiton induced through colchicine treatment of ovules *in vitro*. Breed. Sci., **44**: 161-166.

Jahuar P. P. and Hanna W. W. 1998. Cytogenetics and genetics of pearl millet and related species. Adv. Agron., **64**: 1-26.

Kadota M. and Niimi Y. 2002. *In vitro* induction of tetraploid plants from diploid Japanese pear cultivar (*Pyruspyrifolia* N. cv. Hosui). Plant Cell Rep., **21**: 282-286.

Manzoor A., Ahmad T., Bashir M. A., Hafiz I. A. and Silvestri C. 2019. Studies on Colchicine Induced Chromosome Doubling for Enhancement of Quality Traits in Ornamental Plants. Plants (Basel), **8**(7): 194-210

Mo L., Chen J., Lou X., Xu Q., Dong R., Tong Z., Huang H. and Erpei L. 2020. Colchicine-Induced Polyploidy in *Rhododendron fortunei* Lindl Plants. **9**: 424-437.

Nalawade A. S. and Gurav R. V. 2017. Stomatal studies in genus *Chlorophytum* (Asparagaceae). Biosci. Discov., **8**(3): 574-581.

Nayeem K. A. and Dalvi D. G. 1989. Rapid technique of obtaining leaf prints with the help of Fevicol. Current Sci., **58**: 641-642.

Nimura M., Kato J., Horaguchi H., Mii M., Sakai K. and Katoh T. 2006. Induction of fertile amphidiploids by artificial chromosome-doubling in interspecific hybrid between *Dianthus caryophyllus* L. and *D. japonicas* Thunb. Breed Sci., **56**(3): 303-10.

Pasakinskiene I. 2000. Culture of embryos and shoot tips for chromosome doubling in *Loliumperenne* and sterile hybrids between *Lolium* and *Festuca*. Plant Breed., **119**: 185-187.

Petersen K. K., Hagberg P., Kristiansen K. and Forkmann G. 2002. *In vitro* chromosome doubling of *Miscanthus sinensis.* Plant Breed., **121**(5): 445-50.

Quesenberry K. H., Dampier J. M. and Lee Y. Y. 2010. Doubling the chromosome number of bahiagrass via tissue culture. Euphytica1, **75**: 43-50.

Ramulu K. S., Verhoeven H. A. and Dijkhuis P. 1991. Mitotic blocking, micronucleation and chromosome doubling by oryzalin, amiprophos-methyl and colchicine in potato. Protoplasma1, **60**: 65-73.

Segraves K. A. and Anneberg T. J. 2016. Species interactions and plant polyploidy. Am. J. Bot., **103**: 1326-1335.

Singh S. and Sethi G. S. 1995. Stomatal size, frequency and distribution in *Triticum aestivum, Secalecereale* and their amphiploids. Cereal Res. Commun., **23**: 103-108.

Soetopo L. and Hosnia D. 2018. *In vivo* Polyploid-Induction by Colchicine on Orchids *Phalaenopsis pulcherrima* (Lindl.) Biosci. Res., **15**(2): 941-949.

Solangi A. H. 2001. Characteristics of 20 coconut (*Cocusnucifera* L.) varieties based on leaf morpho-physiological markers. M.Sc. thesis, University of Phillippine, Phillippines.

Tavan M., Mirjalili M. H. and Karimzadeh G. 2015. *In vitro* polyploidy induction: Changes in morphological, anatomical and phytochemical characteristics of *Thymus persicus* (Lamiaceae). Plant Cell Tiss. Organ Cult., **122**(3): 573-583.

Tuyl V. J. M., Meijer B. and Van Dien M. P. 1992. The use of oryzalin as an alternative for colchicine *in vitro* chromosome doubling of *Lilium* and *Nerine.* Acta. Hortic., **325**: 625-630.

Vandenhout H., Ortiz R. and Vuylsteke D. 1995. Effect of ploidy on stomatal and other quantitative traits in plantain and banana hybrids. Euphytica, **83**: 117-122.

Wu J. H., Ferguson A. R. and Murray B. G. 2011. Manipulation of ploidy for kiwifruit breeding: *in vitro* chromosome doubling in diploid *Actinidiachinensis* Planch. Plant Tiss. Organ Cult., **106**(3): 503-511.

Yu C. Y., Kim H. S., Rayburn A. L., Widholm J. M. and Juvik J. A**.** 2009. Chromosome doubling of the bioenergy crop *Miscanthus* × *giganteus.* Gcb Bioenergy, **1**(6): 404-412.