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Stomatal Studies on Colchicine Treated Bajra Napier Hybrids (*Pennisetum glaucum* × *P. purpureum*)

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ABSTRACT

Background: This experiment was done to analyze the colchiploids obtained in two bajra napier hybrid genotypes named CO6 and TNCN 1534. Stomatal size is considered as one of the main parameters in analysing the variants developed by colchicine application.

Methods: Two methods were adopted for inducing polyploidy BN hybrids. In case of whole immersion method, budded setts were completely immersed in concentrations of 0.05%, 0.10%, 0.15%, 0.20% and 0.25% for 3 and 6 hours duration. In case of the cotton swab method, the nodded setts were covered with a thin layer of cotton and same five concentrations were given continuously for a period of over two days. After transplanting them in the field for 30 days, evaluation of stomatal length and width was done.

Result: The stomatal measurements changed with various range of concentrations and the method of application. In the case of whole immersion method, 0.20% and 0.25% concentration for 3 and 6 hours produced variants having largest width and length of stomata and also covered the largest range for mean values. In cotton swab treatment, highest range for stomatal measurements were seen at 0.20% and 0.25% concentrations. The whole immersion method was able to generate a wide range for stomatal length and width as compared to cotton swab method. Hence, it can be deciphered that whole immersion method was more effective than cotton swab method in this study. The highest concentration of 0.25% was the most effective in inducing changes in the treated plants in both the methods. Through this, we were able to identify some putative colchiploids in the study. These screened polyploids were forwarded for further analysis which were then used to identify genotypes having desirable forage and quality parameters.

Key words: Colchicine, Colchiploids, Concentration, *Pennisetum,* Stomata.

INTRODUCTION

Poaceae family contains *Pennisetum* which is considered as one of its key genera. Pearl millet [*Pennisetum glaucum* (L.) R. Br.] and Napier grass (*Pennisetum purpureum* (K.) Schum) are the two economically significant cultivars. Napier grass is an allotetraploid species with 2n = 4x = 28 and Pearl millet is a diploid with 2n = 2x = 14 (genomes AA). Because of their close kinship and strong genetic compatibility, these two species can produce interspecific hybrids with 2n= 3x = 21 (genomes A'AB). This kind of hybridization was an attempt to combine the traits of pearl millet and Napier grass, such as disease resistance, high vigour, fodder quality and perennial nature, with the traits of Napier grass, such as high biomass, aggressiveness and high dry matter production. Cattle have preferred the interspecific hybrids that were produced over Napier grass. However, their infertility as a result of meiotic abnormalities has been viewed as a significant barrier for breeding programmes. The term "polyploidy" describes the presence of multiple chromosomal sets. Autopolyploids are produced by a species' genome doubling, whereas allopolyploids are produced *via* interspecific hybridization and genome doubling. Genome duplication is one of the most crucial evolutionary events leading to the huge expansion of genetic diversity; thus, allowing species to adapt to varying environmental habitats. The evolutionary success of polyploids has been greatly influenced by various adaptive modifications, including increased tolerance to extreme environmental fluctuations, effective vegetative reproduction methods, apomixes, disease resistance and changes in

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morphology, flowering period and biomass. As a result, they are an excellent choice for many agricultural and breeding practises (Ramsey and Schemske, 2002).

A powerful approach for creating unique germplasm appropriate for selective breeding is inducing polyploidy. Autopolyploid plants have a number of potential benefits over allopolyploid plants, which are more common, since they have more genetic variability and higher gene doses (Soltis and Soltis, 2009). Additionally, polyploid plants typically have thicker and bigger leaves, stalks, flowers, fruits and seeds, which results in a higher yield (Verma *et al*., 2017). Recent research has revealed that polyploidy genomes are very dynamic and exhibit quick structural and functional changes (Soltis and Soltis, 2009; Dar *et al*., 2013).

The most widely used antimitotic drug, colchicine, is derived from the angiosperm *Colchicum autumnale* plant and binds selectively to tubulins to block microtubule polymerization and hence cause polyploidy in cells (Murali *et al*., 2013). Chromosome doubling has been used to restore fertility in sterile hybrids between napier grass (*Pennisetum purpureum*) and pearl millet (*Pennisetum glaucum*) (Falerio *et al*., 2015). Stomata count and size measurements are a reliable screening method for determining the ploidy level effectively. Stomatal size has been used successfully as an indicator of altered ploidy by Murali *et al*. (2013) in sorghum, Quesenberry *et al*. (2010) in bahia grass, Compos *et al*. (2009) in pearl millet napier hybrids, Ramesh *et al*. (2014) in mulberry and Falerio *et al*. (2015) in pearl millet napier hybrids. This was kept in mind when the current work was carried out to induce polyploidy in two kinds of bajra napier hybrids using two distinct techniques of colchicine application and to analyse the putative polyploids using the stomatal measurements.

MATERIALS AND METHODS

During the year 2021-2022, this study was carried out in the forage department of CPBG, TNAU, Coimbatore. Two alternative techniques were used to administer the colchicine treatment to one nodded setts of pearl millet napier hybrids. One nodded setts were given various treatments utilising cotton swabs and the complete immersion procedure. The planting material (setts) were prepared and planted individually in polybags filled with soil. The buds were cleaned with distilled water before the application of colchicine. Buds were covered with dry swabs of cotton and colchicine solution was applied (concentrations mentioned in materials and methods) from 6.00 A.M to 6.00 P.M for two continuous days at three hour intervals while the buds of control were given distilled water. The concentrations used were 0.05%, 0.10%, 0.15%, 0.20% and 0.25%. The growth parameters and stomatal dimensions (30 days after transplanting) were recorded and subjected for analysis. Several one nodded setts were also used in the full immersion technique and kept for 3 and 6 hours in five different colchicine concentrations: 0.05%, 0.10%, 0.15%, 0.20% and 0.25%. In this way, a total of 10 treatments were done. Growth metrics and stomatal investigations were carried out in the same manner as in the cotton swab approach.

Stomatal studies

Approximately one cm^2 strips from the middle of the leaf's epidermis were coated with a thin layer of quick fix or natural nail polish. The stomata impressions were carefully removed with forceps or nails when dried and put on the glass slide. Longitudinal and transverse dimensions were recorded for adaxial epidermal stomata in the leaves of all survived and established plants at 30 days after transplanting in the field. Scope Image programme was used to measure five stomata from each leaf and determine the mean value.

RESULTS AND DISCUSSION

Putative colchiploids were identified by preliminary screening based on stomatal size so that they could be chosen for further field experiments. When the adaxial stomatal length and width of each plant were examined, a significant degree of variance was found among these colchiploids. In case of the whole immersion method, the control plant of the genotype CO 6 recorded an average stomatal length and width of 52.927±0.579 µm and 22.046±0.290 µm respectively (Table 1). The highest mean value of 55.470±1.128 µm for length of stomata was observed at 0.20% for 6 hours treatment with a range of 46.008-61.334 µm, followed by 0.25% for 3 hours treatment with a mean value of 52.9740.510 µm having the range between 43.010-59.201 µm. The highest stomatal length of 61.334 µm was observed in plants that were treated with 0.20% colchicine for 6 hours (Fig 1a) indicating that this concentration was capable of producing variants in this genotype. In case of width of stomata, the highest value of 23.768±0.643 µm was observed at 0.25% concentration for 3 hours treatment where the stomatal width ranged from 17.326-27.77 µm. As the concentration and period of the treatment increased, the range of stomatal length and breadth also expanded. The highest range for stomatal length (36.005-54.116) (Fig 1c) and width (17.628-28.135) were observed for 0.25% concentration for 6 hours duration. It indicates that as the concentration increased, the stomatal size started to fluctuate. Hence, it can be seen as a reliable factor for determining variants produced during colchicine application.

In case of genotype TNCN 1534 (Table 1), the control plant had the mean value of 46.2480.834 µm and 24.2860.376 µm for stomatal length and width respectively. As the concentration increased, the range of variation for stomatal length and width increased. The highest range of stomatal length and width of 31.003-50.353 µm and 17.334-31.201 µm was observed at 0.25% concentration for 6 hours duration. The second highest range of stomatal length (33.334-46.702 µm) was recorded at 0.25% concentration for 3 hours duration (Fig 1d). The mean of stomatal length and width decreased with increase in concentration and duration, the lowest being seen in plants treated with 0.20% concentration for 6 hours duration (40.3360.732 µm). The increase in range of variants

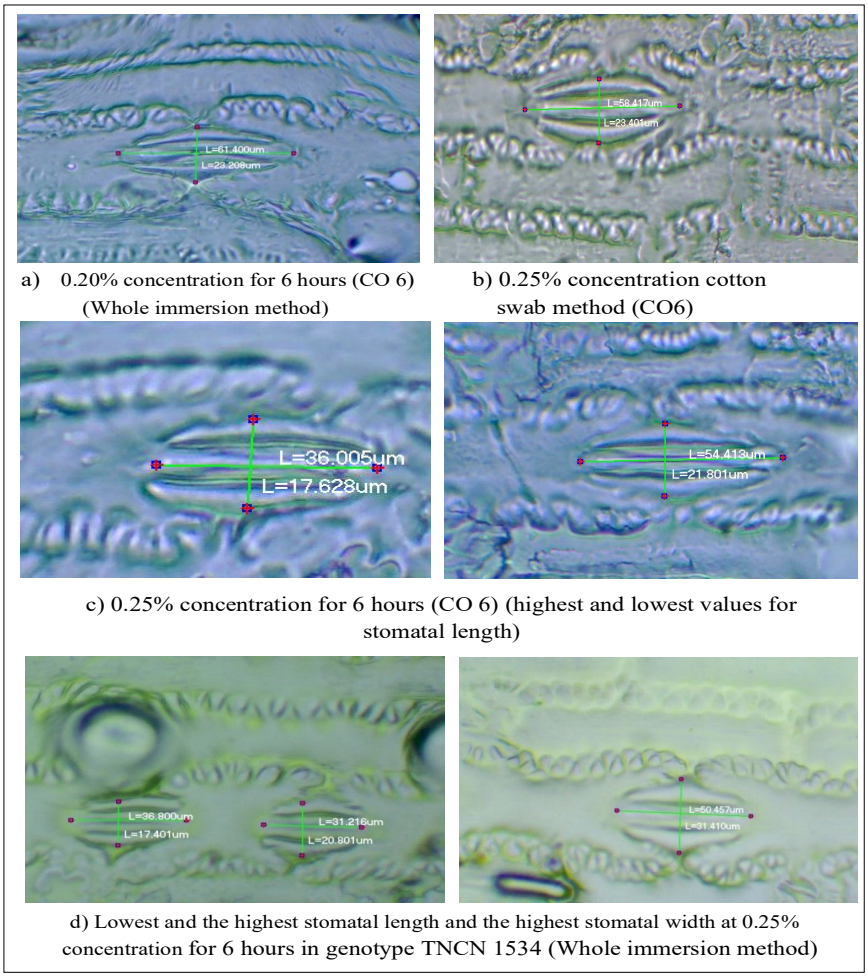
decreased the mean of stomatal length and width of plants which were treated at higher concentration.

The swab method also generated some variations in genotypes CO 6 and TNCN 1534. In case of genotype CO 6, all of the concentrations had similar range of stomatal length when compared to the control (530.759 µm) (Table 2). The highest range of stomatal length was observed for 0.25% concentration (45.188-58.034 µm) (Fig 1b) followed by 0.20% concentration where the plants had the values between 46.021-56.100 µm. The highest mean value for stomatal width of 23.124±0.648 µm was recorded for 0.15% concentration. In the case of genotype CO 6, the range of stomatal length and width gradually increased with increase in colchicine concentration, hence, proving the fact that stomatal measurements can be used as an indirect tool to analyse induced polyploids in plants.

In case of genotype, TNCN 1534 (Table 2), the highest mean value for stomatal length of 49.8680.919 µm was recorded at 0.20% concentration followed by 0.25% concentration of 49.186±1.083 µm. The highest stomatal range between 42.187-56.021 µm was observed at 0.25% concentration. The highest mean value for stomatal width was also observed at 0.25% concentration (23.080±0.399 µm). However, the highest stomatal range between 18.320-28.116 µm was observed at 0.20% concentration. This indicated increment in variations as concentrations increased.

The scatter plot diagram indicated that the genotype CO 6 produced a whole range of variation when the setts were treated with whole immersion method (Fig 2a). The genotype TNCN 1534 had plants which showed a less scattered pattern (Fig 2b) and also didn't had much range as compared to genotype CO 6. Majority of the genotypes in CO 6 were concentrated at a particular range of stomatal length and width but some genotypes were scattered more than TNCN 1534.

The genotypes (CO6 and TNCN 1534) when treated with cotton swab method showed a range between 48 µm to 52 µm. The plants in both the genotypes were much scattered over ranges but didn't had a wide range as compared to the plants treated with whole immersion method (Fig 3a and b). This indicates that even the method used for colchicine treatment can cause variations in the plants. The longer the chemical comes in contact with the plant material, more will be the variation induced.



a) 0.20% concentration for 6 hours (CO 6) (Whole immersion method)

b) 0.25% concentration cotton swab method (CO6)

c) 0.25% concentration for 6 hours (CO 6) (highest and lowest values for stomatal length)

d) Lowest and the highest stomatal length and the highest stomatal width at 0.25% concentration for 6 hours in genotype TNCN 1534 (Whole immersion method)

Fig 1: Stomatal measurements of colchiploids.

Table 1: Mean performance and range of pearl millet Napier colchiploids treated with whole immersion method for adaxial stomata.

Colchicine concentration (%)	Duration of treatment	CO6				TNCN 1534			
		Stomatal length (µm)		Stomatal width (µm)		Stomatal length (µm)		Stomatal width (µm)	
		Mean±SE	Range	Mean±SE	Range	Mean±SE	Range	Mean±SE	Range
Control	3 hr	52.92±70.579	48.28-55.203	22.046±0.290	19-24.42	46.248±0.834	44.43-47.413	24.286±0.376	21.41-27.40
	6 hr	52.76±0.919	49.13-56.300	21.760±0.258	18.567-25.477	47.640±0.827	44.330-50.330	22.484±0.159	19.33-25.15
0.05%	3 hr	52.286±0.348	47.204-58.805	21.690±0.251	19.004-26.457	46.230±0.266	42.112-51.402	24.337±0.142	21.557-26.712
	6 hr	52.034±0.377	47.118-58.034	21.616±0.120	19.009-25.458	46.269±0.513	42.168-52.003	24.207±0.132	22.085-26.728
0.10%	3 hr	50.802±0.593	46.411-58.121	20.978±0.289	18.204-25.201	45.536±0.331	41.084-49.237	24.440±0.148	22.008-26.452
	6 hr	51.094±0.302	45.200-56.669	21.476±0.265	18.401-25.115	45.521±0.365	41.208-49.279	24.735±0.256	21.308-27.115
0.15%	3 hr	49.252±0.548	45.932-56.303	21.040±0.290	18.204-25.112	46.679±0.818	41.297-52.226	24.414±0.301	20.541-27.528
	6 hr	50.082±0.761	45.208-56.220	19.752±0.488	15.266-25.187	45.54±01.08	41.337-52.145	24.995±0.197	21.485-28.205
0.20%	3 hr	48.190±0.787	44.538-55.462	19.822±0.508	15.119-25.501	43.108±0.734	37.119-49.003	22.774±1.052	18.180-27.625
	6 hr	55.470±1.128	46.008-61.334	23.058±0.272	16.552-28.182	40.336±0.732	36.021-45.235	22.805±0.860	19.385-29.135
0.25%	3 hr	52.974±0.510	43.010-59.201	23.768±0.643	17.326-27.77	40.421±0.942	33.334-46.702	22.928±0.401	18.801-29.498
	6 hr	41.848±1.770	36.005-54.116	22.618±1.447	17.628-28.135	41.836±0.631	31.410-50.353	23.514±0.460	17.334-31.201

Table 2: Mean performance and range of pearl millet Napier colchiploids treated with cotton swab method for adaxial stomata.

Colchicine concentration (%)	CO 6				TNCN 1534			
	Stomatal length (µm)		Stomatal width (µm)		Stomatal length (µm)		Stomatal width (µm)	
	Mean±SE	Range	Mean±SE	Range	Mean±SE	Range	Mean±SE	Range
Control	53.018±0.759	49.253-57.210	21.147±0.215	18.353-24.567	50.182±0.807	46.557-54.023	24.650±0.301	21.007-27.407
0.05%	53.478±0.669	50.025-56.813	22.968±0.788	19.001-25.758	47.696±0.476	44.118-51.208	20.916±0.476	19.054-23.168
0.10%	52.262±0.568	48.771-55.858	21.910±0.585	19.203-26.029	48.052±0.566	45.002-53.164	21.928±0.311	19.335-25.325
0.15%	51.302±0.424	49.025-55.307	23.124±0.648	19.203-25.628	48.434±0.713	45.002-52.213	22.558±0.532	20.116-25.445
0.20%	51.376±0.226	46.021-56.100	22.058±0.308	17.315-26.110	49.868±0.919	45.483-55.228	22.956±0.301	18.320-28.116
0.25%	50.668±0.560	45.188-58.417	20.574±0.418	18.132-26.002	49.186±1.083	42.187-56.021	23.080±0.399	18.905-26.332

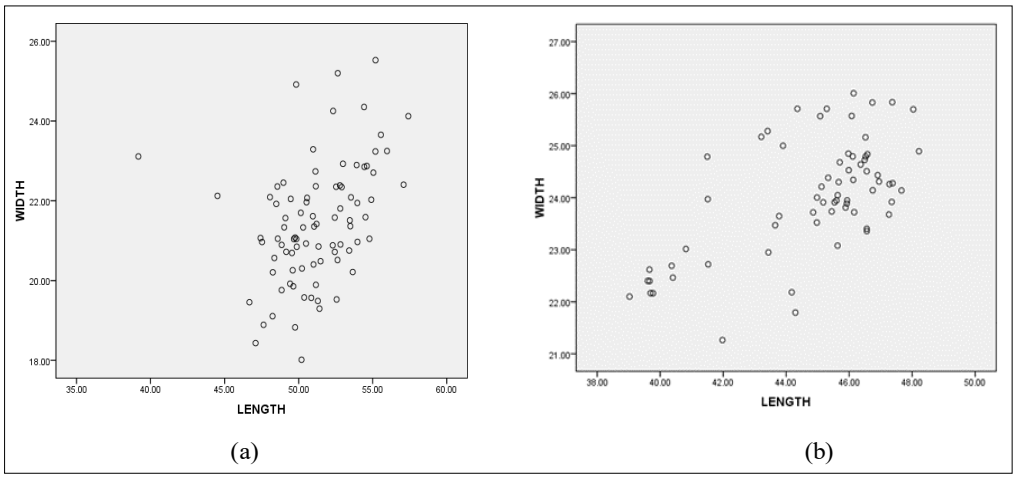


Fig 2: Scatter graph showing stomatal measurements of genotype (a) CO 6 and (b) TNCN 1534 treated with whole immersion method.

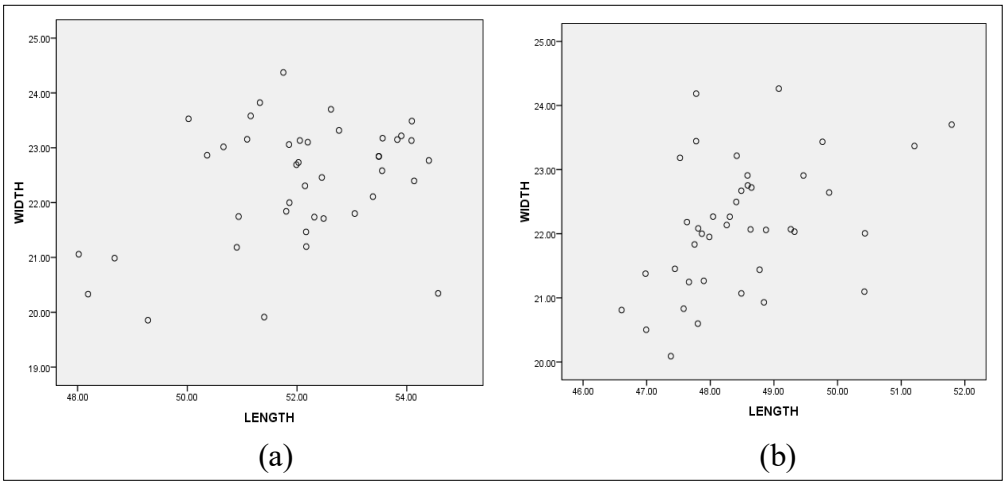


Fig 3: Scatter graph showing stomatal measurements of genotype (a) CO 6 and (b) TNCN 1534 treated with cotton swab method.

Stomatal size has been used to distinguish between diploid and polyploid regenerants of several plants, including orchids, *Stevia rebaudiana* and many varieties of *Lilium* (Nouraddin *et al*., 2019). According to Raghunath *et al*. (2014), the stomatal dimension (length and width) of the colchicine-treated plants in African marigold seemed to be higher than the untreated control. According to Dario and Paul (2009), guard cells in *Vaccininium darrowii* treated with colchicine grew longer and were bigger, making them a useful tool for checking colchiploid alterations. It can be summarized that colchicine was efficient in generating variants at higher concentrations which was evident from the above results. In whole immersion method, 0.20% and 0.25% concentration for 3 and 6 hours generated considerable variants having largest width and length of stomata and also covered the largest range for mean values. In cotton swab treatment, highest range for stomatal measurements were seen at higher concentrations of 0.20% and 0.25%. However, genotype CO 6 recorded the highest stomatal width at 0.15% concentration. Although stomatal dimensions can be utilized as a primary selection criteria for colchiploids, further verification is necessary as there might be a chance of occurrence of mixoploids as was indicated by Zhang *et al* (2010) in crape myrtle (*Lagerstroemia indica*). Similar results were revealed by Campos *et al*. (2009) in bajra napier hybrids as well. It can be stated that colchicine can be used as a potential antimitotic agent to produce variability in triploids and can be seen as a key tool in overcoming the sterility of these triploid hybrids to produce fertile hybrids and also generate variants that are having superior forage characteristics.

CONCLUSION

Colchicine application usually results in increased stomatal length and width. This revealed that variation in the stomatal traits due to colchicine treatments can act as an indicator for the variation induced through colchiploidy. Different genotypes react differently to colchicine treatments as from the results, it could be interpreted that genotype CO 6 showed wide variation for stomatal measurements as

compared to TNCN 1534 genotype. This indicates how some genotypes are better adapted to chemical treatments that can induce changes in their cellular functions and structure. The method applied to induce polyploidy in these genotypes also generated a wide difference in the stomatal measurements. Whole immersion method was more effective in generating variants than the cotton swab method. Similarly, the concentration also plays a vital role in inducing polyploidy. It is established from previous studies as well as from the present study that more the concentration of the chemical used, more are the chances for inducing polyploidy in the plants. This indicates that when appropriate methods and concentrations are selected for inducing polyploidy, it can generate useful and true variants.

Conflict of interest: None.

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