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Research Article

Studies on colchicine induced changes in bajra napier hybrid (*Pennisetum glaucum* x *P. purpureum*)

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Abstract
An experiment was conducted to estimate the lethal concentration of the polyploidy inducing chemical Colchicine in two genotypes of Bajra Napier hybrid CO 6 and TNCN 1534. Single nodded setts of the genotypes CO 6 and TNCN 1534 were treated with colchicine using two different methods *viz.,* whole immersion with concentrations of 0.05%, 0.10%, 0.15%, 0.20% and 0.25% for 3 and 6 hours and the cotton swab method using the same five different concentrations. The LC_{50} values were observed based on growth reduction of seedlings after the planting of treated setts by the two methods. The sprouting percentage and survival percentage of both genotypes gradually reduced with increasing doses of colchicine and it also differed based on the method used. The LC_{50} values differed for both the methods used. The cotton swab method had the LC_{50} value of 0.21% for the genotype CO 6 and 0.19% for TNCN 1534. The whole immersion method had the LC_{50} value of 0.14% in case of CO 6 and 0.15% colchicine concentration was found to be optimum for TNCN 1534 indicating the differential response of genotypes for colchicine treatment. Correlation between different concentrations and stomatal parameters indicated positive correlation when the varieties were treated for 3 hours duration and a negative correlation was observed when they were treated for 6 hour duration in case of whole immersion method. As the duration of exposure increased, the parameters showed decreased growth. In case of cotton swab method, both the varieties showed positive correlation for stomatal width with the increasing concentration while stomatal length was seen having negative correlation when the concentrations were increased.

Keywords: *Pennisetum*, colchicine, sprouting, survivability, Probit analysis, correlation

INTRODUCTION

Livestock sector is one of the most promising sectors in agriculture and is providing livelihood to majority of households in India. India has one of the largest livestock population in the world and despite of around 35% deficit in green fodder, it leads in milk production globally. The livestock in India comprises of 16% cattle population, 20% goat, 5% sheep and 55% of world's buffalo population (IGFRI). Not only it fulfils the milk and meat requirement, but also provides manure, draught power, fuel and rural transport. Due to urbanisation, there has been a shrinkage in agricultural land and therefore, there is a need to increase the livestock population so as to fulfil the

human requirement of milk and meat. Average milk and meat production of Indian animals is low as compared to the global average, which offers considerable scope of enhancement. The major cause of low productivity is lack of good feed and fodder. Hence, there is urgent need to increase feed and fodder potential to increase milk production.

Commonly grown fodder crops such as sorghum, maize, pearl millet, berseem, oat etc. are seasonal in nature and hence, cannot provide year round green fodder in sufficient manner. Therefore, there is a need to cultivate fodder crops which are perennial in nature and have high green biomass production potential. In this way, Bajra Napier Grass, which is the hybridization product between bajra (*Pennisetum glaucum*) and Napier grass *(P. purpureum*), is having the characteristics of bajra that is good palatability and taste along with perennial nature and deep root characteristics of Napier. Combined characters of high productivity and good palatability makes bajra Napier hybrid an ideal fodder crop for round the year fodder production. It contains 7-10 % crude protein, 28-30 % crude fibre and 10-11.5 % ash on dry matter basis. Once planted, it can give 8-10 cuts in a year and if managed properly, it can provide fodder for 5-6 years. The farmers can cultivate them for over a decade with proper agronomic management and maintenance of moisture level and nutrient management.

Induction of polyploidy in crops is an important tool for the production of new sources of germplasm that are applicable for plant breeding (Tang *et al*., 2010). Besides, polyploidy can enhance the quality parameters and also improves resistance to biotic and abiotic stresses (Ahloowalia, 1967). Colchicine is widely used for induction of polyploidy and is 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* and BINDS specifically to tubulins to prevent polymerization of microtubules and hence, induces polyploidy (Ramachandran, 2013).

MATERIALS AND METHODS

The study was carried out in the Experimental farm of the Department of Forage crops, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore during the year 2021-2022. Setts of two bajra Napier hybrids *viz*., CO 6 and TNCN 1534 were used for the colchicine treatment. Whole immersion method and cotton swab method were the two methods used for the treatment of setts.

Whole immersion method: About 20 setts each of the genotypes CO 6 and TNCN 1534 were used for this treatment. Single nodded setts were first wiped with 70% ethanol and were immersed in five different concentrations of colchicine: 0.05%, 0.10%, 0.15%, 0.20% and 0.25% each for 3 hours and 6 hours. A total of 10 treatments were used for this study and a total of 200 setts were used for this treatment. After the time period, these setts were washed thoroughly under tap water and the setts were then planted immediately in the field.

Cotton swab method: Setts were planted individually in polybags filled with soil. The buds were cleaned with water before the application of colchicine. The cleaned buds were covered with dry cotton swab and five different concentrations (0.05%, 0.10%, 0.15%, 0.20% and 0.25%) of colchicine solution was applied from 6 A.M to 6 P.M. for two consecutive days at an interval of 3 hours, while the control buds were watered normally with fresh water. Cotton swabs were removed after the two day treatment and buds were watered with distilled water to remove any excess chemical remaining on the buds. After the treatment, the buds were covered with a thin layer of coir pith so that the buds do not dry. The sprouted setts were transferred to the field after 30 days. The aim of this study was to fix the optimum dose for large-scale treatment using probit analysis by two different treatment methods on two varieties. Sprouting percentage and survivability percentage (Scott *et al*., 1984) were calculated 15 and 30 days after planting respectively by using the following formula:

$$\text{Sprouting percentage} = \frac{\text{Number of setts sprouted}}{\text{Total number of setts planted}} \times 100$$

$$\text{Survivability percentage} = \frac{\text{Number of plants survived after 30 days}}{\text{Number of plants setts planted}} \times 100$$

LC_{50} value for colchicine was calculated based on Probit analysis (Finney, 1978). Probit analysis was carried out in OPSTAT by following procedure:

1. Mortality percentage of setts was calculated for all the doses and the value was rounded to the nearest whole number.
2. Corrected mortality percentage was calculated using Abbott's formula:

$$\text{Corrected \% mortality} = \frac{\text{Observed \% mortality - average control \% mortality}}{\text{100\% - average control \% mortality}}$$

3. All the corrected values obtained were rounded to the nearest whole number.
4. Probit value was worked for the corresponding corrected mortality percentage value.
5. Probit graph was drawn using probit values on Y-axis against treatment concentration on X- axis.
6. The log LC_{50} is estimated from the line as *m (intercept)*, the dosage at which Y = 5.

RESULTS AND DISCUSSION

Colchicine is a chemical that is used both for polyploidy induction in plants and mutation induction. The mutagen, as a bioactive alkaloid and a poisonous compound, is extracted from seeds and corms of the meadow saffron (*Colchicum autumnale* L.) (Sattler *et al*., 2016). The chemical was used to standardize the concentration which can be used for treating plants to produce polyploids at higher levels. Probit analysis was carried out based on the survivability of plants in the field after colchicine treatment.

The sprouting percentage is one of the factors which is affected adversely after the colchicine treatment. In the case of whole immersion treatment, it decreased with increase in the concentration and time of application of chemical. In the variety CO 6, the maximum sprouting percentage of about 90% was recorded in 0.05% colchicine which was treated for 3 hours with colchicine, while the lowest sprouting percentage of 40% was observed for 0.25% concentration treated for 6 hours. Regarding the genotype TNCN 1534, the highest sprouting percentage of about 90% was observed in 0.05% concentration which was treated for 6 hours before planting in the field while the concentration 0.25% for 6 hours gave the lowest sprouting percent of about 35%. (**Fig. 1**). In case of cotton swab method, the highest sprouting percentage of 95% was recorded at 0.05% colchicine treatment whereas the lowest sprouting percent (55%) was observed in 0.25%. The concentrations of 0.10% and 0.15% recorded sprouting percent of about 90% (**Fig. 2**).

The survivability percent was taken 30 days after the planting of setts in the field. Survivability of treated setts showed similar trend like the sprouting percent, where plants treated with lower concentration were well established in the field as compared to the plants that were treated with higher concentration. Both the genotypes showed similar range of survivability percent in the case of whole immersion method (**Fig. 1**).

In cotton swab method, variety CO 6 showed a little higher survival percent when compared with TNCN 1534. The survival percent of CO 6 and TNCN 1534 was 75% and 70% respectively, when treated with 0.05% colchicine. When treated with 0.25% colchicine, the survivability percent of CO 6 was 45% as compared to TNCN 1534 which had only 40% survivability (**Fig. 2**).

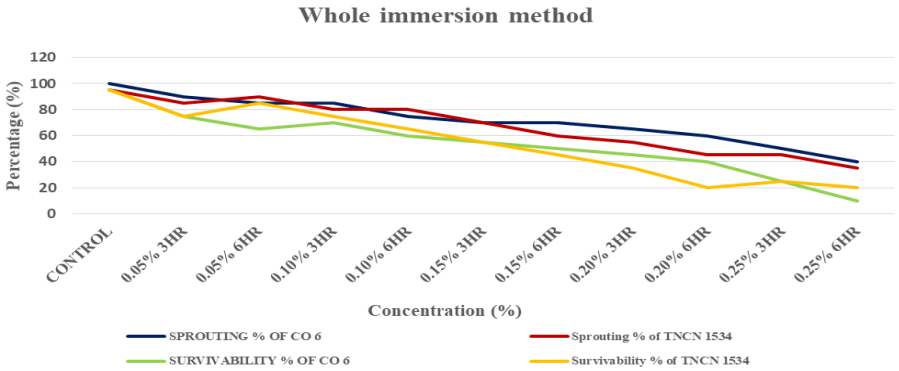

Fig. 1. Sprouting and survivability percentage of CO 6 and TNCN 1534 plants treated with whole immersion method

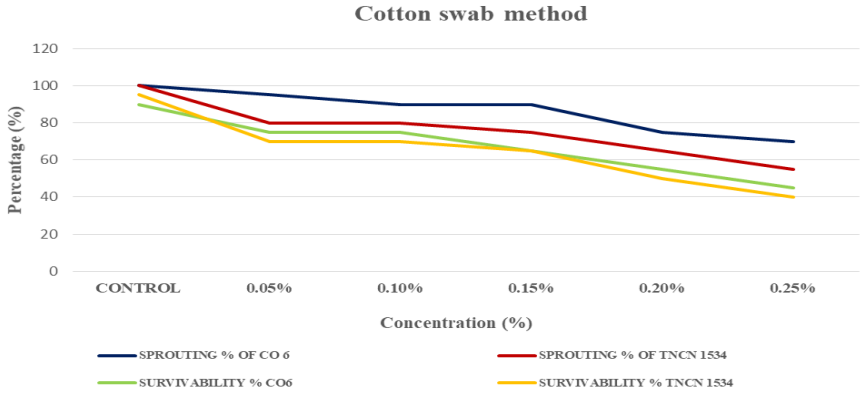

Fig. 2. Sprouting and survivability percentage of CO 6 and TNCN 1534 plants treated with cotton swab method.

Colchicine not only disturbs cell division, but also spreads through the cell, interfering with cellular mechanism and hence, causes toxicity when applied at high concentrations. It also impacts the viscosity of cytoplasm so the cell will not be able to function normally. Han *et al.* (1999) reported that when high dose of colchicine was used as a mutagen in plants, phytotoxicity and abnormality became main cause of death. Similar observations were reported in Japanese mulberry genotypes (Tojyo, 1966), *Platanus Acerifolia* (Liu *et al*., 2007), and *Quercus aegilops* L. (Toma, 2015), which indicated that a high concentration of colchicine and longer duration of immersion lead to reduction in the rate of seed germination and plant survival (Suliman, *et al*., 2019).

LC_{50} for colchicine were determined with the help of probit analysis based on the sprouting percent of both varieties. Optimum dose is the dose that cause maximum of mutation with minimum of damage to the plant. In the present investigation, the probit analysis was done to standardize the correct dose to proceed with colchicine application. The LC_{50} value for both the methods differed for both the varieties.

The LC_{50} value for whole immersion method was found to be 0.14% for variety CO 6 with the line intercepting at Y=5. In case of the variety TNCN 1534, the optimum dosage to be treated with was 0.15% which had the probit value of 4.977 (**Table 1).**

In case of cotton swab method, variety CO 6 had the probit graph indicating the optimum dose as 0.21% concentration. The cotton swab method for TNCN 1534 showed that the LC_{50} dose was 0.19%. The LC_{50} value optimum in case of whole immersion method was 0.14% and 0.15% for CO 6. Hence, it can be said that for whole immersion method, the colchicine dose of 0.15% can be followed (**Table 1**) whereas in case of cotton swab method, 0.20% can be the optimum concentration (**Table 2**). These concentrations can be used in further application of colchicine so as to develop colchiploids having minimum mortality rate and having maximum variations due to doubling of chromosomes.

The sprouting and survival percent by the two methods (whole immersion method and cotton swab method) showed different trends in the genotypes used. In the case of whole immersion method, the genotypes CO 6 and TNCN 1534 had similar sprouting percent when treated with lower concentration of 0.05% and 0.10%. The sprouting percent was having the similar range for both these genotypes upto 0.15% concentration for 3 hours. The difference was observed from 0.15% concentration for 6 hours where the sprouting of CO 6 (70%) genotype

Table 1. Probit analysis for variety CO 6 and TNCN 1534 for whole immersion method

	CO 6					TNCN 1534				
DOSAGE	LOG DOSE	% DEAD	% CORRECTED	PROBIT VALUE	LC_{50} VALUE	LOG DOSE	% DEAD	% CORRECTED	PROBIT VALUE	LC_{50} VALUE
0.05% 3HR	-1.3	25	21	4.143		-1.301	25	17	3.553	
0.05% 6HR	-1.3	35	32	4.143		-1.301	15	6	3.553	
0.10% 3HR	-1	30	26	4.717		-1.00	25	17	4451	
0.10% 6HR	-1	40	37	4.717	0.14%	-1.00	35	28	4.451	0.15%
0.15% 3HR	-0.82	45	42	5.054		-0.824	45	39	4.977	
0.15% 6HR	-0.82	50	47	5.054		-0.824	55	50	4.977	
0.20% 3HR	-0.7	55	53	5.292		-0.699	65	61	5.35	
0.20% 6HR	-0.7	60	58	5.292		-0.699	80	78	5.35	
0.25% 3HR	-0.6	75	74	5.477		-0.602	75	72	5.693	
0.25% 6HR	-0.6	90	89	5.477		-0.602	80	78	5.693	

Table 2. Probit analysis for variety CO 6 and TNCN 1534 for cotton swab method

	CO 6					TNCN 1534				
DOSAGE	LOG DOSE	% DEAD	% CORRECTED	PROBIT VALUE	LC_{50} VALUE	LOG DOSE	% DEAD	% CORRECTED	PROBIT VALUE	LC_{50} VALUE
0.05%	-1.301	25	25	4.134		-1.301	30	30	4.284	
0.10%	-1	25	25	4.546		-1	30	30	4.649	
0.15%	-0.824	35	35	4.787	0.21%	-0.8	35	35	4.863	0.19%
0.20%	-0.699	50	50	4.958		0.699	50	50	5.014	
0.25%	-0.602	60	60	5.091		0.602	65	65	5.132	

was higher as compared to TNCN 1534 (60%). Sprouting percent of both these genotypes decreased gradually with the increase in concentration and was least at 0.25% for 6 hours. The survivability percentage also showed similar trend where higher survivability was seen at lower concentrations and vice versa. For cotton swab method, CO 6 showed higher sprouting percent than TNCN 1534. The sprouting percent gradually decreased with increase in concentration of colchicine. However, both the genotypes CO 6 and TNC 1534 had similar survivability range indicating that both these genotypes were able to survive when transplanted in the field after 30 days.

The LC_{50} value for whole immersion method was found to be 0.14% for genotype CO 6 whereas for genotype TNCN 1534, the LC_{50} value was found to be 0.15%. In case of cotton swab method, 0.21% was the optimum LC_{50} value observed for genotype CO 6. The genotype TNCN 1534 had the LC_{50} value of 0.19%. Stomatal length and frequency and number of chloroplasts are a few of the most widely used indicators of polyploidy (Geok-Yong Tan & Dunn, 1973). Stomatal lengths have been successfully used in *Trifolium pratense*, (Evans, 1955), *Lolium multiflora*, *L. perenne* and *Bromus inermis* (Geok-Yong Tan & Dunn, 1973) and for *Actinidia deliciosa* by Przywara, Pandey & Sanders (1988). The number of stomata has proved to be a good indicator of ploidy in a number of species. Colchicine also acts as a mutagen and hence, can cause changes in number of stomata and its shape, aperture length and its dimension, characteristics of guard cells in both dorsal and ventral surfaces of leaf which can vary from treatment to treatment (Rajib *et al*., 2011).

Stomatal studies of the treated colchiploids were done to find out the effect of these concentrations on the length and width of the stomatal apertures. It also acts as a reliable indicator to analyze the ploidy induced changes in colchicine treated plants. Correlation analysis was done to analyse how different concentrations are affecting the stomatal morphology.

In case of whole immersion method, correlation analysis was done to find out the difference in stomatal length and width in variety CO 6 when the setts were treated with five concentrations for 3 hours. The length (0.52) and width (0.61) were having a positive correlation with concentration indicating the size of stomata increased as the concentration increased (**Fig. 3**).

In case of setts treated with different concentrations for 6 hours, stomatal length (-0.22) and stomatal width (-0.23) both had negative effect with increasing concentration and thus, it could be concluded that as the concentration and duration of treatment increased in variety CO6, the stomatal length and width decreased (**Fig. 4**).

In case of variety TNCN 1534, a different trend was observed for plants which were treated for 3 hours. The concentration was positively correlated to width of the stomata (0.31) which was treated for 3 hours. The length of stomata was negatively correlated (-0.45) with concentration indicating that as the concentration increased, the length of stomata decreased while the width of stomata was increasing with high concentration (**Fig. 5**).

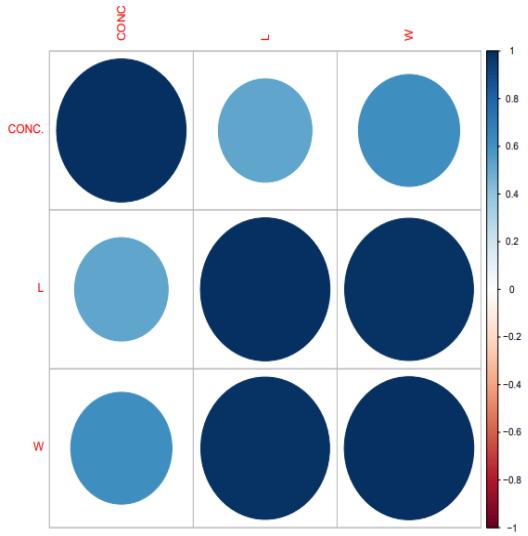

Fig.3. Correlation graph for variety CO6 treated under whole immersion method for 3 hours

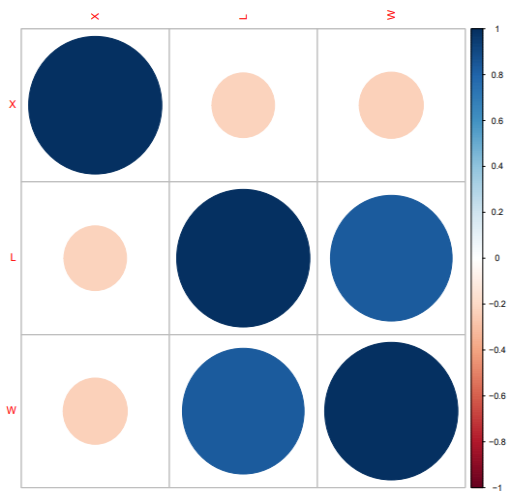

Fig. 4. Correlation graph for variety CO6 treated under whole immersion method for 6 hours

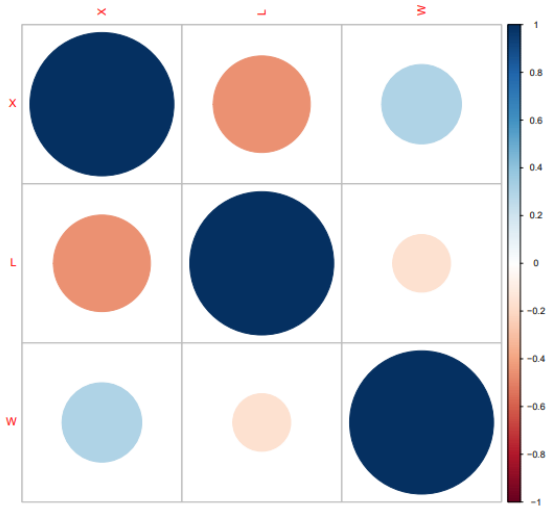

Fig. 5. Correlation graph for variety TNCN 1534 treated under whole immersion method for 3 hours

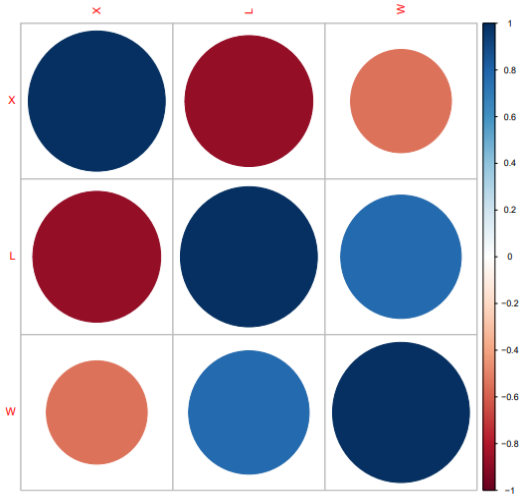

Fig. 6. Correlation graph for variety TNCN 1534 treated under whole immersion method for 6 hours

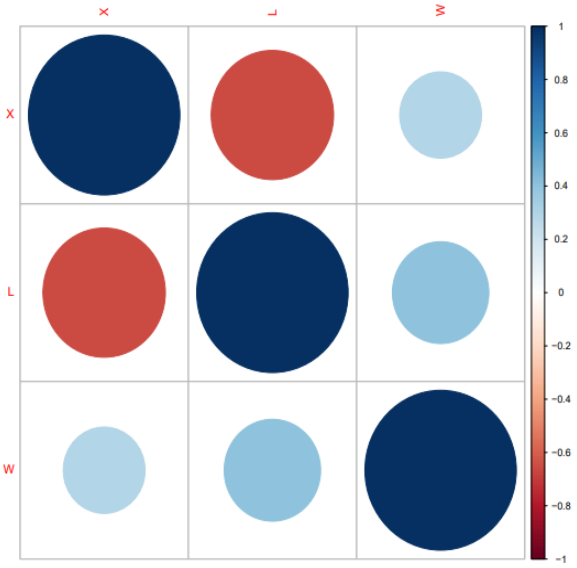

Fig. 7. Correlation graph for variety CO 6 treated under cotton swab method

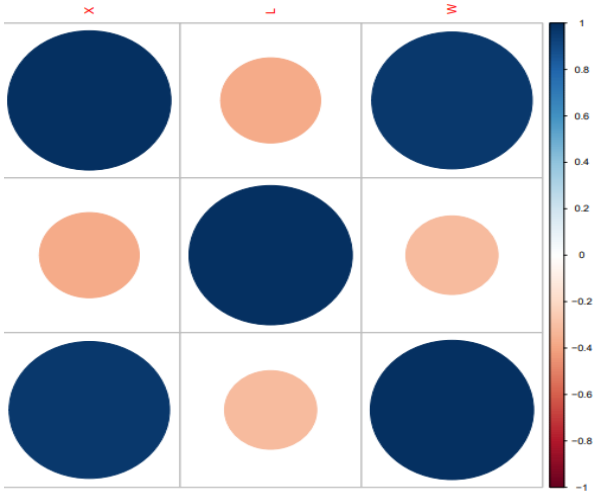

Fig. 8. Correlation graph for variety TNCN 1534 treated under cotton swab method

When the setts of variety TNCN 1534 were treated with different concentrations of colchicine for 6 hours, length and width were negatively impacted by the increasing colchicine concentration. The stomatal length had a high negative correlation coefficient of -0.87 while the width had the negative correlation coefficient of -0.54. This showed that as the concentration and duration of concentration increased, it negatively impacted the stomatal length and width of the colchiploids. This result followed the same trend that was observed in variety CO6 when treated for 6 hours under the same different concentrations (**Fig. 6**).

When the variety CO6 was treated with cotton swab method, the stomatal length decreased with increase in colchicine concentration. A negative correlation coefficient of -0.65 was observed for stomatal length indicating that as the concentration increased, there was a gradual decrease in stomatal length. The stomatal width was positively correlated (0.29) with the concentration (**Fig. 7**).

In case of variety TNCN 1534, similar trend was observed as in case of variety CO6. The stomatal length was negatively correlated (-0.37) with the increasing

concentration while the width was positively correlated and indicated a highly significant relationship with the increasing concentration (0.97). Hence, it indicated that length and width are the two parameters which can be used to identify variation caused by colchicine in plants (**Fig. 8**).

Hence, in case of whole immersion method, the length and width of stomata were observed to have a positive correlation with increasing concentration when they were treated for 3 hours and then planted in the field. However, when the duration of exposure was increased to 6 hours, both the parameters showed a negative effect on the increasing concentration indicating that stomatal parameters are the first to be altered when polyploidy is induced in plants.

In case of whole immersion method, stomatal length had negative correlation while stomatal width had a positive correlation with increase in colchicine concentrations. There have been studies where colchicine treatment was able to generate variations in stomatal length and width when sett treatment was done in bajra napier hybrids (Swathi *et al*., 2019).This indicates that polyploids can be effectively identified by using stomatal measurements as an indicator.

Hence, it can be concluded that different genotypes respond differently to chemicals that interfere with their cellular functions as some have more endurance to these chemicals than others. The response also differed with the method used for application of these chemicals, enabling the identification of the efficient method. From the experiment, it is clear that even though survivability of cotton swab method treated plants was comparatively much higher, the variability with respect to stomatal length and width in different concentrations was exhibited by plants that were treated *via* whole immersion method. Hence, to induce polyploidy, whole sett immersion method can be more useful than cotton swab method. The standardized concentrations along with stomatal analysis can further be used for producing and identifying polyploids having desirable forage characters that can be utilised for crop improvement programmes.

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