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*Original scientific article*

# POLYPLOIDISATION EFFECT ON TRICHOME DENSITY IN INTERSPECIFIC HYBRIDS OF *Pennisetum glaucum* (L.) R. BR. × *Pennisetum purpureum* (K.) SCHUM

Arshpreet KAUR[1], Rahul KAPOOR[1]*, Anu KALIA[2]

[1]Department of Plant Breeding and Genetics, PAU, Ludhiana, Punjab, India
[2]Department of Soil Science, PAU, Ludhiana, Punjab, India

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This study is aimed at testing the efficiency of colchicine on inducing polyploidy in *Pennisetum glaucum* (L.) R. Br. × *P. purpureum* (K.) Schum (BNH) and to investigate the effect of polyploidy on trichome density. Root cuttings of 4 hybrids and two check varieties were treated with different concentrations of colchicine (0, 0.05%, 0.1% and 0.2%) for different time durations (12 and 24 hrs). The ploidy level of plants was confirmed by cytogenetic studies using conventional protocol. Colchicine concentration of 0.1% for 24 hrs was found to induce polyploidy in PBN 233. Trichome density was investigated using Scanning Electron Microscopy at 100X. During the study, it was found that the trichome density decreases with increase in ploidy level.

*Key words:* BNH, Colchicine, Interspecific hybrid, Polyploid, Trichomes

## INTRODUCTION

The prime confront for agriculture in the present and future is to meet the food and fibre needs of the increasing globe population. As livestock rearing is a fundamental part of rural livelihood but India faces a net deficit of 61.1 per cent green fodder and 21.9 per cent dry crop residues. In India, pearl millet occupies 8.68 million ha area which comes fourth among fodder crops after sorghum, berseem and alfalfa. To increase the area under cultivation of fodder crops is tedious because of preferential human food, although opportunities exist to advance the productivity (ICAR 2012). In Punjab, about 0.88 million hectare area comes under fodder crops and there was 68.0 million tonnes annual production of green forages (ANONYMOUS 2023). The average requisite of green fodder is 40 kg per animal per day against the availability of 29.8 kg per animal per day. So in the present, fodder production per unit area should be increased by cultivating the high yielding multicut fodder crops akin to Napier bajra hybrid and guinea grass.

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] has been also classified as *Pennisetum typhoideum*, *Pennisetum americanum* or *Pennisetumspicatum* and is locally known as bajra in

*Corresponding author:* Rahul Kapoor, Department of Plant Breeding and Genetics, PAU, Ludhiana, Punjab, India, email: rahulkapoor@pau.edu, +919815585599

India. It is a diploid, annual, allogamous species with large chromosomes (2n = 2x = 14, AA) coupled with 4.72 pg genomic DNA content. Its forage has better nutritional quality in terms of crude protein (%) and dry matter digestibility (%) along with good palatability (MEENA and JAIN 2013). Whereas Napier grass (*Pennisetum purpureum* Schumach.) is a perennial, allogamous species commonly known as elephant grass or Uganda grass. It has high productive potential, carrying capacity, nutrient quality and low water and nutrient requirements that have highlighted it as the chief tropical forages used for dairy grazing system enhancement. Genetically it is tetraploid (2n = 4x = 28, A'A'BB) species coupled with 4.60 pg genomic DNA content (MARTEL *et al* 1997).

Najra Napier Hybrid is an interspecific hybrid of pearl millet and Napier grass which constitutes high quality traits and faster growth combined with the deep root system and multicient behaviour of Napier grass. Pearl millet Napier hybrid is a triploid grass, so it does not produce seeds. Thus it cannot be propagated without the danger of becoming weed, as in Napier grass (GUPTA and MHERE 1997).For the induction of polyploidy in plants, an alkaloid that is Colchicine is widely used for chromosomal doubling (PASAKINSKIENE 2000, SEGRAVES and ANNEBERG 2016). KADOTA and NIIMI in 2002 used this *in vitro* treatment of explants with colchicine in many cases. Chromosomal count and trichome study has been used for confirmation of amphiploidy.

## MATERIAL AND METHODS

The present investigation was conducted in the experimental area of Forage research farm and forage evaluation laboratory at Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. The experimental site is situated at latitude of 30˚54`N and longitude of 75˚48`E at a mean height of 247 meters above mean sea level in the trans-Gangetic agro climatic zone of India. The area experiences an average rainfall of 700 mm mostly during the monsoon season and approximately 80% is received during the south-west monsoon period (July – September).

### *Induction of polyploidy*

The four interspecific hybrids and two checks (Table 1) 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 (Fig 1 a, b). After treatment, the plants were transplanted in the field. The $F_1$ plants were evaluated morphologically and cytologically for confirmation of hybridity and for colchiploid sectors.

*Table 1. Interspecific hybrids used in the experiment*

| Sr. No. | Interspecific Hybrid | Sr. No. | Interspecific Hybrid |
|---|---|---|---|
| 1. | FBC 16 × M 30086 | 4. | PIB 339 × K 59347 |
| 2. | PIB 394 × M 30086 | 5. | PBN 233 |
| 3. | PIB 394 × K 52440 | 6. | PBN 346 |

(a)

(b)

Fig. 1 (a) treatment of colchicine at different concentrations in field, (b) plants for washing after treatment with colchicine

*Cytogenetic studies*

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.

One percent acetocarmine stain was prepared by adding one gram of acetocarmine powder to boiling 100 ml of 45 percent glacial acetic acid. The solution was allowed to cool at room temperature. Then the solution was filtered and the filtrate was used as stain. Small traces of ferric chloride were added to the stain to act as mordant. For different stages of microsporogenesis, minimum of 10 well spread and stained pollen mother cells were observed.

*Trichome studies*

Five leaves from each hybrid were collected from the field and examined for the trichome density at five different random locations using Scanning Electron Microscope (GOMTI *et al* 2016). CD and CV were calculated.

*Scanning electron microscopy*

The selected leaf samples were taken and washed with tap water to remove the impurities and then halved along the mid rib with a razor blade. A rectangular section was cut from the curved portion of each halved leaf. The samples were mounted on the SEM stub with the support of an aluminium stand. The stand allows the leaf sample to sit on the stub at an angle that enabled a view of horizontal section of the leaf surface. The specimens were mounted on a viewing stub with an adhesive tape and were then coated with gold (20-30nm). The stub then placed in the Scanning electron microscope and photographs were taken in the EMN lab of Punjab Agricultural University, Ludhiana. Trichome density was viewed at 100Xmagnification.

## RESULTS AND DISCUSSION

The cytological analysis of the *Pennisetum* species and pearl millet Napier hybrids showed normal meiosis. The somatic chromosome number of parents i.e. *Pennisetum glaucum* (2n = 2x = 14) and *Pennisetum purpureum* (2n = 4x = 28) with normal bivalent formation. The derived hybrid and the colchicine induced amphiploids revealed a somatic chromosome number of 2n = 3x = 21 and 2n = 6x = 42 respectively. Each of the pearl millet accessions showed 14 chromosomes at metaphase and seven on each pole during anaphase. From all hybrids treated with colchicine at different concentrations (0.05%, 0.1% and 0.2%) for two different time durations that is 12 hours and 24 hours. There was only one putative hybrid after a cytogenetic study which was the check variety PBN 233 following 0.1% colchicine concentration for 24 hours to root slips (as in Fig. 2).

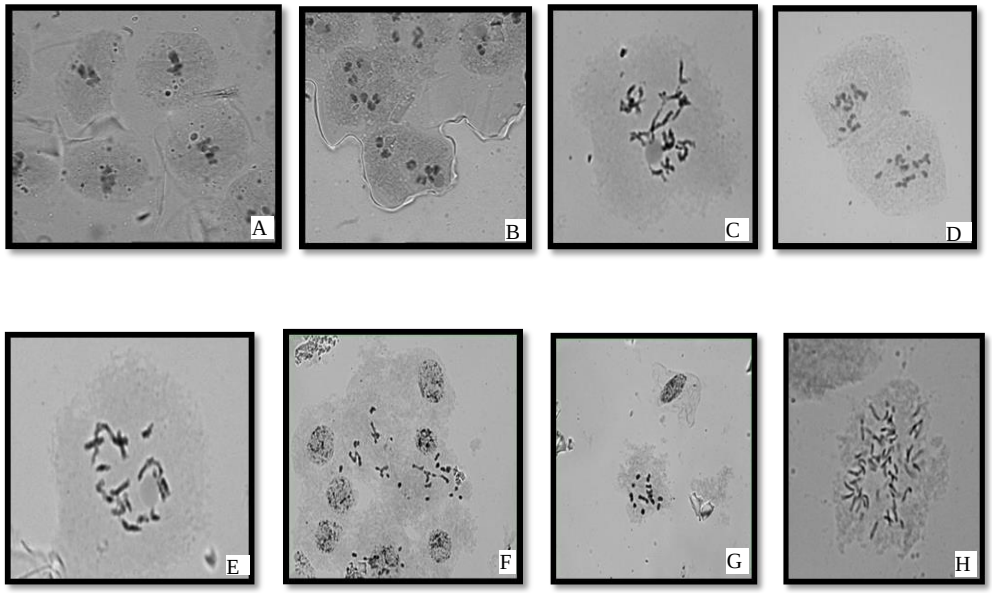


Fig 2.Cytological investigation of (A) *Pennisetum glaucum* (2n = 2x = 14) showing chromosomes at metaphase plate, (B) *P. purpureum* (2n = 4x = 28) showing 14 bivalents , (C) Interspecific hybrid (2n = 3x = 21) showing crossing over at prophase (D) Interspecific hybrid (2n = 3x = 21) showing telophase, (E) Interspecific hybrid PIB 394 × K 52440 [Control showing triploid (2n = 3x = 21)], (F) Interspecific hybrid PIB 394 × K 52440 (0.05% for 24 hrs showing mixoploid); (G) Hybrid PBN 233 (Control showing 21 chromosomes) (H) Hybrid PBN 233 (0.1% for 24 hrs showing more number of chromosomes)

Trichome counts were made on five random plants of each parent on young as well as on the mature leaves. The female parent *Pennisetum glaucum* was found to have the dense trichomes while the pollen parent *Pennisetum purpureum* did not have any trichome on its surface (KREITNER and SORENSEN 1979, OGUNKUNLE *et al* 2013). The resultant hybrids have the intermediate number of trichomes between two parents.

The colchicine treatment has been given to 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) at three different concentrations 0.05%, 0.1% and 0.2% for two different time durations 12 and 24 hours and compared with the control plants to induce the amphiploidy. The data for trichome density (per $mm^2$) has been presented in (Table 2) and (Fig. 3). From the table, it was concluded that the young leaves were found to have more number of trichomes than the mature leaves in all interspecific hybrids and check varieties (PEREZ-ESTRADA *et al* 2000).

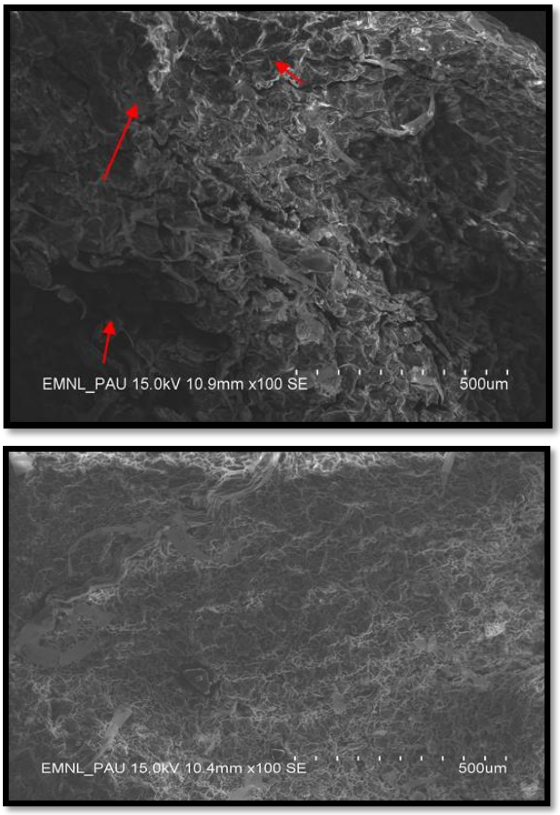


Fig 3. Scanning electron microscope showing trichome density for check variety PBN 233 on young leaf (A): control (having more trichome density), (B): treated with 0.1% colchicine concentration for 24 hrs (having less trichome density)

*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 Trichome frequency (mm$^2$) of new interspecific crosses and check varieties*

| Trichome density (mm$^2$) | | | | | | |
|---|---|---|---|---|---|---|
| Sr No. | BNH hybrid | Colchicine concentration | Mature leaf | | Young leaf | |
| | | | 12 hr | 24 hr | 12 hr | 24 hr |
| 1. | FBC 16 × M | 0 | 47.77 ± 4.19 | 47.77 ± 4.19 | 143.31 ± | 143.31± 1.45 |
| | | 0.05% | 79.61 ± | 63.69 ± | 175.15 ± | 127.38 ± 3.29 |
| | | 0.1% | 95.54 ± | 111.46 ± | 111.46 ± | 191.08 ± 3.7 |
| | | 0.2% | 47.77 ± 7.84 | 95.54 ± | 143.31 ± | 127.38 ± 4.21 |
| | CD (p=0.05) | | NS | NS | NS | NS |
| 2. | PIB 394 × M | 0 | 175.15 ± | 175.15 ± | 254.77 ± | 254.77 ± 3.18 |
| | | 0.05% | 143.31 ± | 191.08 ± | 222.92 ± | 222.92 ± 0.00 |
| | | 0.1% | 191.08 ± | 191.08 ± | 366.24 ± | 302.54 ± 9.86 |
| | | 0.2% | 191.08 ± | 170.70 ± | 350.31 ± | 382.16 ± 2.43 |
| | CD (p=0.05) | | NS | NS | NS | NS |
| 3. | PIB 394 × K | 0 | 111.46 ± | 111.46 ± | 159.23 ± | 159.53 ± 2.79 |
| | | 0.05% | 127.46 ± | 115.5 ± 5.52 | 165.25 ± | 154.58 ± 2.45 |
| | | 0.1% | 111.46 ± | 111.46 ± | 143.31 ± | 143.31 ± 20.3 |
| | | 0.2% | 143.31 ± | 111.46 ± | 159.23 ± | 230.54 ± 8.12 |
| | CD (p=0.05) | | NS | NS | NS | NS |
| 4. | PIB 339 × K | 0 | 127.38 ± | 127.38 ± | 270.70 ± | 270.70 ± 4.24 |
| | | 0.05% | 95.54 ± | 143.31 ± | 191.08 ± | 270.70 ± 8.35 |
| | | 0.1% | 159.23 ± | 143.31 ± | 238.85 ± | 286.62 ± 5.59 |
| | | 0.2% | 159.23 ± | 175.15 ± | 234.39 ± | 318.47 ± 1.93 |
| | CD (p=0.05) | | NS | NS | NS | NS |
| 5. | PBN 233 | 0 | 318.46 ± | 318.46 ± | 636.94 ± | 636.94 ± 1.78 |
| | | 0.05% | 366.24 ± | 318.46 ± | 657.32 ± | 614.01 ± 0.08 |
| | | 0.1% | 350.31 ± | 143.31 ± | 652.86 ± | 366.24 ± 6.28 |
| | | 0.2% | 382.16 ± | 334.39 ± | 668.78 ± | 589.17 ± 0.79 |
| | CD (p=0.05) | | NS | 24.12 | NS | 17.84 |
| 6. | PBN 346 | 0 | 318.47 ± | 318.47 ± | 382.16 ± | 382.16 ± 2.02 |
| | | 0.05% | 254.77 ± | 270.70 ± | 366.24 ± | 398.08 ± |
| | | 0.1% | 238.85 ± | 222.92 ± | 414.01 ± | 477.70 ± 2.08 |
| | | 0.2% | 318.47 ± | 334.39 ± | 509.55 ± | 414.01 ± 7.06 |
| | CD (p=0.05) | | NS | NS | NS | NS |

From the six hybrids treated with 3 concentrations of colchicine i.e. 0.05%, 0.1% and 0.2% for two different time durations 12 and 24 hours, only one check variety were found to be significantly different at $P \leq 0.05$ for trichome density. The maximum number of trichomes was found in check variety PBN 233 that is 318.46 ± 28.52 and 636.94 ± 1.78 mm$^2$ on mature and

young leaves respectively. The hybrid PBN 233 has the average trichome density 318.46 ± 28.52 and 636.94 ± 1.78 (per $mm^2$) on the mature and young leaves respectively. While the hybrid following 0.1% colchicine treatment for 24 hours has the average trichome density 143.31 ± 13.76 and 366.24 ± 6.28 on the mature and young leaves. So the results indicate that there is 42.49 and 52.06% reduction in the trichome density on mature and young leaves respectively with an increase in the ploidy level. The study concluded that there is higher trichome density on young leaves as compared to mature leaves and also as the ploidy level of the plant increases, the trichome density decreases.

## CONCLUSION

From the present study, it can be concluded that the interspecific hybrids between *Pennisetum glaucum* × *Pennisetum purpureum* can be produced easily due to genomic similarities between two species. Also, the colchicine acts as an effective antimitotic agent to induce the amphiploidy at 0.1% concentration for 24 hours. With reference to the study, the relationship between ploidy level and trichome density has been derived. The trichome density decreases with an increase in ploidy level. Also, the research concludes that 0.1% colchicine concentration is more effective to induce polyploidy when given to roots for 24 hours.

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# EFEKAT POLIPLOIDIZACIJE NA GUSTINU TRIHOMA U MEĐUVRSNOM HIBRIDU *Pennisetum glaucum* (L.) R. BR. × *Pennisetum purpureum* (K.) SCHUM

Arshpreet KAUR[1], Rahul KAPOOR[1]*, Anu KALIA[2]

[1]Departmant za oplemenjivanje i genetiku, PAU, Ludhiana, Punjab, Indija
[2]Departmant za zemljište, PAU, Ludhiana, Punjab, Indija

IZVOD

Ova studija ima za cilj ispitivanje efikasnosti kolhicina na izazivanje poliploidije kod *Pennisetum glaucum* (L.) R. Br. × *P. purpureum* (K.) Schum (BNH) i efekat poliploidije na gustinu trihoma. Korenske reznice 4 hibrida i dve kontrolne sorte tretirane su različitim koncentracijama kolhicina (0, 0,05%, 0,1% i 0,2%) u različitom vremenskom trajanju (12 i 24 časa). Nivo ploidnosti biljaka potvrđen je citogenetskim studijama korišćenjem konvencionalnog protokola. Utvrđeno je da koncentracija kolhicina od 0,1% tokom 24 sata izaziva poliploidiju u PBN 233. Gustina trihoma je ispitivana korišćenjem skenirajuće elektronske mikroskopije na 100Ks. Tokom istraživanja je utvrđeno da gustina trihoma opada sa povećanjem nivoa ploidnosti.

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