CHAPTER VI

SUMMARY

The present study was conducted to restore the fertility status in triploid sterile PMN hybrids via chromosomal doubling of either triploid hybrids directly or by doubling the chromosomes of the diploid pearl millet parent which can be further used for conducting hybridization with tetraploid napier lines. For induction of doubling in pearl millet the seeds of variety CO 8 were used. For seed treatment in PMN hybrid, the F1 seeds of the cross combination IP 20594 x FD 471 and for sett treatment the setts of CO (BN) 5 were used. Prior to doubling of pearl millet and PMN hybrid, chromosomal behavior of [CO (BN) 5] and its respective parents (IP 20594 and FD 437) was observed. Screening of colchiploids for putative polyploids was done by morphological and cytological observations in pearl millet, while in PMN hybrids besides morphological and cytological observations, flow cytometry analysis and molecular characterization was also attained. The salient findings of the present investigation are summarized below

- Studies on meiotic behavior of pearl millet (IP 20594) showed normal bivalent formation and in napier grass (FD 437), besides normal bivalent (14 II) formation, rare occurrence of univalent and laggards was observed.

- Observations on meiotic behavior of PMN hybrid CO (BN) 5 showed several abnormalities viz., association of bivalents with the nucleoli during diakinesis, early metaphase with pentavalent, metaphase I with 19I + 1II and irregular chromosomal arrangement of the chromosomes on the plate leaving behind the univalent. Various anaphase abnormalities such as laggards, early migration of chromosomes, unequal separation of chromosomes, and univalent in the post anaphase were observed. Telophase with micro nuclei was also documented.

- With regard to standardization of concentration of colchicine for induction of tetraploids in pearl millet, six concentrations viz., 0.05, 0.06, 0.07, 0.08, 0.09 and 0.10% for four durations viz., 6, 8, 12 and 24hrs were employed and the concentration of colchicine and duration of treatment had inverse relationship with germination and survival per cent. Analysis of variance for variation due to
concentration, duration and their interaction effects was recorded to be highly significant for both germination and survival per cent.

- Among the 24 treatment combinations, 0.05% for 6 hrs treatment recorded the significant and highest germination per cent, while 0.10% for 12 and 24 hrs recorded the least. Pertaining to survival per cent the treatment combination 0.05% at 6 hrs recorded the significant and highest survival per cent, while 0.08% at 24 hrs and 0.10 % at 8 hrs recorded the least per cent. In contrast, 0.09 and 0.10% for 12 and 24 hrs lead to complete mortality.

- The generated colchiploids of pearl millet were subjected to preliminary screening based on stomatal measurements and maximum mean (51.7 ± 4.6 µm) stomatal length was obtained at 0.07% for 8 hrs duration and highest mean value (30.4 ± 1.8 µm) for width was obtained at 0.07% for 6 hrs duration treatment, which were greater than the control with length and width of 45.0 ± 0.70 µm and 28.1± 0.4, respectively.

- One variant (PM 36) was recovered at colchicine concentration of 0.07% treated for 8 hrs and was confirmed to be aneuploid by meiotic studies. It possessed 24 chromosomes, which were observed as bivalents (12 II) and univalent ranging from 1 to 4 as laggards. The pollen fertility and seed set of this variant was 53 and 5%, respectively. Besides this variant, another variant (PM 37) with bristled panicle was recovered at 0.07% treated for 12 hrs.

- All the concentrations showed discernible variations for morphological and stomatal measurements however, significant variations for obtaining selective variants were observed at 0.07% concentration of colchicine in pearl millet.

- Pertaining to standardization of protocol for chromosomal doubling of PMN hybrids via. seed treatment of cross combination (IP 20594 x FD 471), seven colchicine concentrations viz., 0.04, 0.05, 0.06, 0.07, 0.08, 0.09 and 0.10% for four durations viz., 6, 8, 12 and 24 hrs was employed and analysis of variance for germination and survival per cent recorded the existence of high significant difference between treatments, concentration, duration and the interaction of concentration vs duration.
Similar to pearl millet, as the concentration and duration of treatment increased, the germination and survival per cent decreased in this study also. The highest germination (%) was recorded at 0.04% colchicine treatment for 6hrs treatment, while it was least at 0.08% for 24 hrs and 0.09% for 12 hrs treatment. In contrast, the treatments, 0.09% for 24 hrs duration and 0.10% for 12 and 24 hrs recorded zero per cent of germination. Similarly, for survival per cent the highest and significant value was recorded at 0.04% for 6 hrs, while the treatments viž., 0.08% for 24 hrs and 0.09% for 12 hrs resulted in complete morality.

For standardizing the concentration of colchicine through sett treatment of PMN hybrid [CO (BN) 5], 10 concentrations viž., 0.05, 0.06, 0.07, 0.08, 0.09, 0.10, 0.15, 0.20, 0.25 and 0.30% were attempted at three hours interval for 2 consecutive days. The analysis of variance showed the existence of significant difference between different concentrations of colchicine for germination and survival per cent. For germination and survival per cent, the concentrations 0.05, 0.06, 0.07 and 0.08% showed on par performance; however their performance varied with 0.09% concentration. Least germination and survival per cent were observed at 0.30% colchicine.

With regard to stomatal measurements of the seed treated PMN hybrids, the control recorded the length and width of 46.1±0.2 and 24.04±0.8, respectively and the highest mean value (61.03±7.44) for stomatal length was observed at 0.06% for 24 hrs treatment, while the range was highest (46.97–66.84) at 0.07% for 6 hrs treatment. In contrast, the least mean (45.41±4.01) was observed at 0.06% for 12 hrs treatments. For stomatal width the highest mean value (28.82±0.45) was recorded at 0.07% concentration for 12 hrs with wider range (23.08–34.46), while least mean value (23.62±0.48) was observed at 0.05% for 8 hrs treatment.

In sett treatment, the variation in stomatal measurements generated upon treatment with 0.05, 0.06, 0.07, 0.08 and 0.10% was very less and was almost similar to the control with length and width of 46.27±0.57 and 24.61±0.6, respectively. However, the highest mean for stomatal length (56.72±4.0) and width (26.16±1.09) was obtained at 0.20% and 0.15%, respectively.
The standardized concentration of colchicine 0.15%, that a generated maximum value for stomatal measurements and with considerable survival per cent was used for bulk treatment. The germination and survival per cent of 82.2 and 60.0, respectively were observed.

The hybrid CO (BN) 5 and its parental lines were subjected to flow cytometry analysis, besides the variants. The histograms of the 2C and 4C of the pearl millet line and napier are locations close on X axis due to their similarity in DNA content. The ploidy level of the surviving variants was analyzed and an induction per cent of 43.7 of mixoploids was recorded.

These mixoploids generated considerable variations for various morphological traits and stomatal measurements in comparison with the control.

For molecular characterization of selected mixoploids, 13 EST- SSR markers that are specific to PMN hybrids were used of which two markers showed amplification, depicting the existence of variations among the colchiploids.

Following the morphological observations, stomatal measurements, molecular characterization and flow cytometry analysis, mitotic confirmation of the ploidy level of selected mixoploids viz., CH 30, CH 61 and CH 71 was carried out and 15, 26 and 32 chromosomes respectively, were observed against the control with 21 chromosomes.

Therefore, it can be concluded that colchicine could be used as a potential anti mitotic agent in generating variation and is highly dependent on the concentration and duration of the treatment and plant parts being utilized for treatment. In seed treatment, lower concentrations were efficient in generating variants, however higher concentrations (0.15% and beyond) generated variations in sett treatment. Besides typical polyploidy morphological traits, stomatal measurements could be used for preliminary putative ploidy screening though it does not provide accurate results. However, flow cytometry though does not give the exact chromosomal number could be used as a reliable method for ploidy screening.
Summarizing the current experiment, considerable mixoploids were obtained in pearl millet (PM 36 and 37) and PMN hybrid CO (BN) 5 (CM 30, CM 61 and CM 71) and could be utilized for further studies to evaluate their behavior and stability in future forage breeding programme.