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## SUMMARY

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Characterization of 2 germplasms (I and II) using different attributes has been made in *Abelmoschus moschatus* (L.) Medik (family: malvaceae; musk mallow; yields ambrette oil of commerce). Hybridization between the germplasms is performed to enhance genetic diversity in the species. Further, the possibility of improvement (to raise desirable plant types) through induction of mutation has been attempted and the consequences of induced mutagenesis are explored. A concise account of the study performed is presented below:

1. Seeds of 2 germplasms (germplasm I: seed moisture content 3.5%, 100 seed weight  $1.94 \text{ g} \pm 0.03$ , seed size  $3.90 \text{ mm} \pm 0.13 \times 3.88 \text{ mm} \pm 0.15$ ; germplasm II: seed moisture content 1.5%, 100 seed weight  $1.25 \text{ g} \pm 0.01$ , seed size  $3.27 \text{ mm} \pm 0.10 \times 4.02 \text{ mm} \pm 0.14$ ) of *Abelmoschus moschatus* were grown in the experimental plots of University of Kalyani (West Bengal plain;  $22^{\circ}99' \text{ N}$ ,  $88^{\circ}45' \text{ E}$ , elevation 48 feet above sea level, sandy loamy soil, soil pH 6.85) during the rainfed seasons of 2009 - 10, 2010 - 11, 2011 - 12 and 2012 - 13 (July to January) and a comprehensive study has been performed using morphological (taxonomical details), anatomical (transverse sections of ovary, stem and root), stomatal, cytological (meiotic chromosome behavior), palynological (fertility and viability), biochemical (qualitative: SDS- PAGE; quantitative: protein, essential oil and soluble sugar contents) and molecular (RAPD profile) attributes. Result indicates marked differences between the germplasms and based on the observations germplasm I has been recommended to be as tall, branched whereas germplasm II as dwarf, unbranched type.
2.  $F_1$  hybrid plants are raised following crossing between germplasm I (male parent; tall, branched, light yellow flower, oblong conical fruit, rounded seeds) and germplasm II (female parent; dwarf, unbranched, yellow flower, ovoid spindle capsular fruit, reniform seeds). Reciprocal crosses produce small size fruits with dusty seeds. The  $F_1$  hybrid plants resembles female parent for qualitative traits excepting for calyx color; while, quantitative traits are either lower or intermediate to parents. The hybrid shows 42.86% normal pairing of chromosomes ( $2n = 72$ ; 36 II formation; association/cell at MI:  $33.03 \text{ II} + 5.92 \text{ I}$ ) at MI, which is rather less than either of the parents (germplasm I:  $35.81 \text{ II} + 0.38 \text{ I}$ , 86.11%; germplasm II:  $35.80 \text{ II} + 0.40 \text{ I}$ , 82.86%). Pollen fertility and viability (stain tests used- lugol's iodine and aniline blue) are nearly comparable in between parents (germplasm I-

## SUMMARY

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pollen fertility: 33.81%, viability: lugol's iodine- 42.09%, aniline blue- 36.36%; germplasm II- pollen fertility: 30.86%, viability: lugol's iodine- 38.79%, aniline blue- 34.48%) and hybrid (pollen fertility: 29.04%, viability: lugol's iodine- 41.16%, aniline blue- 31.79%). Molecular analysis following the use of RAPD (random amplification of polymorphic DNA) markers reveal distinctiveness between the parents as well as authenticates trueness of the raised hybrid. Upon considering different genetic efficiency parameters, efficient and effective RAPD markers (OPA 01, OPA 02, OPA 10, OPB 02, OPB 04, OPB 05, OPB 08, OPC 03, OPC 05 and OPC 10) are developed for screening *A. moschatus* germplasms including hybrid(s).

3. Seeds of germplasm I and II of the species are treated with different concentrations (0.25%, 0.50% and 1.00%; treatments for 3h and 6h durations) of ethyl methane sulphonate (EMS- Sigma, USA; dilutions made in 0.2M phosphate buffer, pH 6.8) and hydroxylamine (NH<sub>2</sub>OH- aqueous solution) at 37°C ± 1°C. The doses are applied after pilot trials.
4. Fifty seeds from each treatment along with control are given in Petri plates (on moist filter paper) to assess germination (growth of radical following bursting of seed- coat is taken as an index of germination) and seedling growth (measured on a millimeter graph paper on the 8<sup>th</sup> day from treatment) under uniform laboratory condition(s). Biological damages like lethality and injury are assessed from seed germination frequency and seedling growth (as percent of control) respectively. LD<sub>50</sub> has also been ascertained from reduction in germination frequency.
5. LD<sub>50</sub> seems to lie beyond 0.50%, 6h EMS in both germplasm I and II; while, in NH<sub>2</sub>OH treatments germination frequency was rather not related to doses of treatments and therefore lethal doses could not be ascertained in either of the germplasm studied.
6. Under field condition(s) at M<sub>1</sub>, control plants of germplasm I shows 28.0% of germination compared to 44.0% in germplasm II. In treatments it ranges from 8.0% to 40.0% (EMS) and 8.0% to 20.0% (NH<sub>2</sub>OH) in germplasm I and 20.0% to 64.0% (EMS) and 40.0% to 64.0% (NH<sub>2</sub>OH) in germplasm II. All germinated plants have survived till maturity.
7. Flower (control- germplasm I: 72.92%, II: 42.86%; treatments- I: 59.52% to 85.71% in EMS, 43.06% to 82.14% in NH<sub>2</sub>OH; II: 23.29% to 47.83% in EMS, 25.71% to 50.00% in NH<sub>2</sub>OH) and capsule (control- germplasm I: 33.33%, II:

## SUMMARY

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- 16.67%; treatments- I: 13.89% to 33.33% in EMS, 12.93% to 33.33% in NH<sub>2</sub>OH; II: 9.09% to 29.41% in EMS, 4.55% to 36.36% in NH<sub>2</sub>OH) sterilities and seed yield (control- germplasm I: 10.82 g, II: 14.43 g; treatments- I: 6.00 g to 13.60 g in EMS, 5.08 g to 13.36 g in NH<sub>2</sub>OH; II: 5.65 g to 17.60 g in EMS, 9.04 g to 20.98 g in NH<sub>2</sub>OH) has been assessed in M<sub>1</sub> plants.
8. Selfed seeds of each surviving M<sub>1</sub> plants (2 to 3 flowers are bagged) has been harvested separately and are used to raise M<sub>2</sub> (plant to row; 30 cm between plants and lines) generation. No fertilizer application is made during growth period of the plants.
  9. Five and 3 viable mutant plant types are scored in germplasm I ('lax branching', 'pigmented stem', 'long petiole', 'large flower' and 'early flowering') and II ('funnel', 'thick stem' and 'late flowering') respectively from the M<sub>2</sub> mutagenized population.
  10. In both the germplasms mutation frequency is not dose dependent in either EMS (I: 2.04% to 6.29%; II: 0.48% to 3.66%) or NH<sub>2</sub>OH (I: 1.82% to 4.35%; II: 0.35% to 2.51%) treatments. EMS induces relatively higher (I: 3.31%, spectrum 2; II: 1.75%, spectrum 1 to 2) frequency of mutation than NH<sub>2</sub>OH (I: 2.90%; spectrum 1 to 3; II: 1.27%, spectrum 1 to 3).
  11. Mutation frequency estimated over the M<sub>2</sub> population is 3.11% in germplasm I and 1.46% in germplasm II.
  12. Mutation frequency across the M<sub>2</sub> population is found to occur in the order: 'pigmented stem' (1.68%) > 'long petiole' (0.75%) > 'lax branching' (0.56%) > 'large flower' (0.06%) = 'early flowering' in germplasm I and 'late flowering' (1.02%) > 'funnel' (0.27%) > 'thick stem' (0.17%) in germplasm II.
  13. Angle of divergence in 'lax branching' mutant is recorded to be 87.2° (70.0° to 110.0°) as compared to 64.4° (40.0° to 70.0°) in control. 'Long petiole' plant type has enhanced petiole length (40.73 cm ± 0.68) than control (32.40 cm ± 0.61) which is significant (t= 6.321, DF 18, P < 0.001). 'Long petiole' plant type is concomitantly associated with broader and larger leaves. 'Large flower' mutant plants are with enhanced flower sizes (length: 6.3 cm ± 0.11, breadth: 5.1 cm ± 0.34) than the control (length: 4.8 cm ± 0.18, breadth: 3.7 cm ± 0.11). 'Early flowering' mutant plants flowers within 81 to 97 days from sowing in comparison to 104 to 121 days in control. The stem diameter in 'thick stem' plant type is significantly thicker (2.96 cm ± 0.07) than stem of control plants (1.52 cm ± 0.13).

## SUMMARY

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Branching of 'funnel' mutant initiates 14.0 cm above base compared to unbranched nature in control plants (germplasm II). Stem color in 'pigmented stem' mutant is significant for genetic analysis in breeding endeavor.

14. Attributes like lethality, injury and sterility from M<sub>1</sub> generation and M<sub>2</sub> viable mutation frequency are used to determine mutagenic effectiveness and mutagenic efficiency.
15. Upon considering comparable doses it seems that in both germplasms mostly EMS is more effective than NH<sub>2</sub>OH. The most effective doses recorded are 0.25%, 3h and 6h EMS in both germplasm I and II and 0.25% and 0.50%, 3h NH<sub>2</sub>OH in germplasm II. The degree of mutagenic efficiency varies depending upon the selected criteria and it seems that employed doses of EMS and NH<sub>2</sub>OH at 3h in both germplasms are efficient.
16. Inheritance pattern of the mutant trait(s) is studied from selfed M<sub>2</sub> seeds segregating into normal and mutant plants at M<sub>3</sub>.  $\chi^2$ - test has revealed mutant trait(s) are mostly monogenic recessive (segregating into 3:1 ratio) and rarely digenic ('funnel' trait segregating into 15:1 ratio) in nature.
17. Germination frequency of control (germplasm I: 8.33%; II: 62.22%) and mutants (germplasm I: 6.67% to 13.89%; germplasm II: 46.67% to 68.33%) has been studied at M<sub>4</sub>.
18. Mean values of true breeding M<sub>4</sub> plants are compared with respective controls for a specific trait (7 and 6 different quantitative parameters are assessed in germplasm I and II respectively) following the use of CD at 5% level. Result indicates that 'lax branching', 'large flower' and 'early flowering' mutants are with enhanced number of capsules/plant and seed yield/plant. Seed yield/plant also enhances significantly in 'early flowering' mutant. 'Lax branching' and 'long petiole' mutants are with larger capsules than control. Number of primary branches/plant is found to increase significantly in 'pigmented stem' than control. 'Long petiole', 'large flower' and 'early flowering' mutants are significantly taller plants than control. In germplasm II both 'funnel' and 'thick stem' mutants are taller plants with enhanced number of capsules/plant, seeds/capsule and with higher seed yield/plant than control. 'Thick stem' mutant also possesses broader capsules than control. Thus, the mutants induced are significant and in the positive direction to plant ideotype being looked for.

## SUMMARY

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19. ANOVA reveals significant variations ( $P < 0.01$  to  $0.001$ ) for most traits (excepting: I- capsule breadth; II- seeds/capsule) analyzes in 6 (control and mutants of germplasm I) and 4 (control and mutants of germplasm II) plant types of the germplasms.
20. Heritability percentage (broad sense) has been found to be higher for plant height, number of capsules/plant and seed yield/plant in both germplasm I and II. In germplasm II heritability is also of higher magnitude for plant height and capsule length and breadth. Heritability coupled with genetic gain is found to be maximum for plant height and number of capsules/plant in germplasm II; while, in germplasm I genetic gain for all parameters is low to moderate. Result obtained from genetic analysis may be effectively used as selection criteria.
21. Seed traits (seed size- length and breadth and 100 seed weight) do not vary among the plant types (control and mutants) of both germplasms.
22. Meiotic chromosome analysis reveals  $2n = 72$  chromosomes always in meiocytes of controls and mutants of both germplasms with predominant formation of 36 II (germplasm I: control- 86.11%, mutants- 55.56% to 100.00%; germplasm II: control- 82.86%, mutants- 71.05% to 100.00%). Average chromosome association at MI is noted to be  $35.81 \text{ II} + 0.39 \text{ I}$  (mutants:  $35.26 \text{ II} + 1.48 \text{ I}$  to  $36.00 \text{ II}$ ) in germplasm I and  $35.80 \text{ II} + 0.34 \text{ I}$  (mutants:  $35.45 \text{ II} + 1.11 \text{ I}$  to  $36.00 \text{ II}$ ) in germplasm II. Equal AI segregation of chromosomes (36/36) is noted to be 82.76% to 100.00% and 88.24% to 100.00% in germplasm I and II plant types respectively.
23. Pollen fertility (germplasm I- control: 33.81%, mutants: 31.68% to 40.41%; germplasm II- control: 30.86%, mutants: 34.66% to 38.36%) and viability (germplasm I- control: lugol's iodine- 42.09%, aniline blue- 36.36%, mutants: lugol's iodine- 31.65% to 57.06%, aniline blue- 31.12% to 45.89%; germplasm II- control: lugol's iodine- 38.68%, aniline blue- 34.48%, mutants: lugol's iodine- 44.34% to 48.28%, aniline blue- 36.04% to 44.93%) have been assessed in plant types of germplasm I and II.
24. Quantitative biochemical parameters namely (mg/g and % yield), seed protein (germplasm I- control: 19.167, mutants: 18.082 to 22.021; germplasm II- control: 14.235, mutants: 13.342 to 16.602), total soluble sugar (germplasm I- control: 0.363, mutants: 0.296 to 0.392; germplasm II- control: 0.648, mutants: 0.532 to 0.621) and essential oil (germplasm I- control: 0.208, mutants: 0.188 to 0.221; germplasm II- control: 0.204, mutants: 0.188 to 0.216) contents are assessed in the

## SUMMARY

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plant types. Statistical analysis reveals that the parameters do not vary significantly among the plant types of germplasm I and II.

25. Seed viability percentage are also assessed in the plant types (germplasm I- control: 80.0%, mutants: 60.0% to 96.0%; germplasm II- control: 88.0%, mutants: 60.0% to 92.0%).
26. Mutants induced have enrich genetic diversity in ambrette which did not exist previously in the studied germplasms.