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INDUCED MACROMUTATION IN *ANDROGRAPHIS PANICULATA* (BURM. F.) NEES

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ABSTRACT
Fourteen viable macromutants (*viridis*, *lax branching*, *bushy*, *unbranched I* and *II*, *dark green leaf*, *broad leaf I* and *II*, *narrow leaf I* and *II*, *drooping leaf I* and *II*, *dwarf* and *early maturity*) were induced in *Andrographis paniculata* (Burm. F.) Nees (Family: Acanthaceae) at M2 following EMS (Ethyl methanesulfonate) and dES (Diethyl sulfate) treatments (0.25, 0.50 and 1.00% for 2h and 4h durations) to dry seeds (moisture content 11.55%). Mutation frequency over M_2 population was 2.82% and *lax branching* mutant was maximum (0.51%). EMS induced relatively higher (3.12%, spectrum 1 to 4) frequency of mutation than dES (2.46%, spectrum 1 to 3). The mutant trait(s) were monogenic recessive mostly (*viridis* showed digenic mode of inheritance). All mutants bred true at M_4. Meiotic analysis revealed $2n$=50 chromosomes always in the plant types (control: 24.92II+0.51I/cell; mutants: 24.80II+0.40I to 25II/cell at MI; AI cells were cytologically balanced in all cases). Pollen fertility (control: 80.63%, mutants: 21.28% to 89.25%) and viability (control: 72.24%, mutants: 27.55% to 59.04%) were also assessed. DAPI staining was performed to assess pollen nuclei composition (3-nucleate stage) in *dark green leaf*, *broad leaf I* and *drooping leaf II* as the mutants showed considerably lower pollen fertility and viability than normal plants. Results indicated that pollen developmental phase may be significant for reproductive outcome. Andrographolide (estimated from matured leaves by HPTLC) content (control: 3.41%, mutants: 0.03 to 3.99%) was significantly higher in *bushy* and *broad leaf I* and *II* than normal plants. The mutants induced were considered to be important genetic resources in the plant species.
Keywords: *Andrographis paniculata*; DAPI staining; genetic segregation; macromutation; meiotic analysis

INTRODUCTION

Andrographis paniculata (Burm. F.) Nees (Family: Acanthaceae; Synonyms: *Justicea latebrossa* Russ., *J. stricta* Lam. Ex Steud – Hooker[1]; Anonymous[2]; common names: Kalmegh, Kariyat, Green chiretta, Kreat, Rice bitter; English name: King of Bitters) is an annual herb[3] as well as reported to be perennial shrub[4] possessing immense therapeutic uses (used parts- roots, leaves and aerial parts of mature twig) in Ayurvedic (26 Ayurvedic formulations – Indian Pharmacopoeia; in Traditional Chinese Medicine it is an important "cold property" herb used to release body heat in fever – Bobbarala *et al.*[5]) and in modern system of medicine. The therapeutic uses of the plant species are mainly due to diterpenoid andrographolide and related compounds[6,7]. The species is widely cultivated in tropical and subtropical Asia, South-East Asia and India[8] and also reported from different phytogeographical and edaphic zones of China, America, West Indies and Christmas Island in Indian Ocean[9].

Medicinal plant species are gift of nature and considering their global significance it would be worthwhile to raise novel plant types which may meet up the up surging demands of value added products in National and International markets. The methodology of induced mutagenesis may be adopted which provides an opportunity to create allelic variation at one or more gene loci of interest in a quick span of time, thereby widening the gene pool thereby offering scope of suitable selection exploring the existing germplasm(s). Kharkwal[10] reported that mutation breeding has contributed significantly to the global agriculture by producing more than 3000 mutant varieties with enhanced production and productivity in about 175 plant species. The objective of the present work is to raise macromutant(s) in *A. paniculata* which would be important genetic resources for exploration in human benefits. The present communication describes EMS and dES induced mutagenesis in *A. paniculata* and shed light on the types and frequency of macromutation, cytomorphological and genetical aspects of the mutants and andrographolide content in them.

MATERIALS AND METHODS

Plant material

Seeds of *Andrographis paniculata* (Burm. F.) Nees (moisture content: 11.55%; seed size: 1.403 mm ± 0.34 × 0.970 mm ± 0.25; 100 seed weight: 0.117 gm ± 0.04; seed colour: Golden brown, code 41332 British Atlas of Colour) were obtained from Medicinal Plant Garden, Narendrapur, Ramkrishna Mission, Govt. of West Bengal, India.

Mutagenic treatment and raising of M_1, M_2, M_3 and M_4 generations

Dry seeds of *A. paniculata* were treated with different concentrations (0.25, 0.50 and 1.00%; treatments for 2h and 4h durations) of ethyl methane sulphate (EMS – Sigma, USA; dilution were made in 0.2M Phosphate buffer) and diethyl sulphate (dES - Sigma, USA; aqueous solution) at 36°C ± 1°C at pH 6.8. The doses were applied after pilot trials. Control and treated (50 seeds in each lot) seeds were sown in the experimental field of Department of Botany, University of Kalyani (West Bengal plains, Nadia: latitude 22°50´N to 24°11´N, longitude 88°09´E to 88°48´E, elevation 48 ft above sea level, sandy loamy soil, organic carbon 0.76%, soil pH 6.85) to raise M_1 generation (spacing 30 cm between plants and 30 cm between lines) during the months of April to January (2008-09'). Selfed seeds of each surviving M_1 plant (2-3 inflorescences per plant were bugged) were harvested

separately and M_2 plants were raised as plant to row progenies (2009-10').

Macromutants were carefully screened from M_2 mutagenized plant population throughout the growth period and the frequency of the mutants was assessed as per 100 plants[11]. Selfed M_2 seeds of the mutants along with control were sown at M_3 (2010-11'). The mutants segregating in M_3 generation was noted and inheritance of the mutant trait(s) was estimated by using χ^2 test analysis. Selfed seeds of M_3 mutants were sown in 3 lines (for each mutant type) randomly along with control lines at M_4 (2011-12'). All the mutants bred true at M_4. Spacing (between lines and plants) and growth period (mid April to late January) of the plants were uniform over the generations. No fertilizers were applied during the growth period of the plants.

Morphometric traits were analyzed from M_4 plants (6 to 9 plants randomly selected from 3 lines in each case) on harvest. The seedling colour of chlorophyll mutant (classified as per Blixt[12]) and all colour codes were laid with reference to British Atlas of Colour (7th edition 2007). Chlorophyll content (chlorophyll mutant, *dark green leaf* mutant as well as in normal plants) was estimated from leaf tissue of identical maturity quantitatively as per Arnon[13].

Meiotic analysis

Suitable sized inflorescences from mutants as well as from control plants (randomly selected 3 plants for each plant type) at M_4 were fixed (6:30 am to 7:00 am during the months of October to November) in Carnoy's solution and minimum of 3 changes were given in the fixative at an interval of 48 hours and preserved in 70% alcohol under refrigeration. Anthers were squashed in 3% propinocarmine solution and meiotic data (pooled over the plants for each plant type) were scored from metaphase I (MI) and anaphase I (AI) plates. Photomicrographs were taken from temporary squash preparations.

Pollen characteristics

Pollen fertility (M_4 plants) was assessed following staining of pollen grains in 2% propinocarmine solution and fully stained pollen grains were considered fertile[14]. Pollen grain viability (aniline blue in lactophenol- detects the presence of callose on pollen wall, viable pollen turns blue- Bengtsson[15]) percentage was also analyzed.

Mutants namely *dark green leaf*, *broad leaf I* and *drooping leaf II* showed considerably lower pollen fertility and viability than control, and therefore in these plant types (M_4 generation) pollen nuclei composition (number of vegetative – v and generative – g nuclei present at 3-nucleate stage per pollen grain was assessed by staining with DAPI - 4',6-diamidino-2-phenylindole - Johnson and McCormick[16], under fluorescence microscope Carl Zeiss Axio Fluor 900EX, Carl Zeiss Mag Analytic 10.1, DAPI excitation range 350-360 nm, emission maximum 460 nm) was studied to ascertain the possible cause for sterility, if any.

Methodology for quantification of andrographolide

Sample preparation:1.5 gm of sun dried powdered leaf sample (reproductive stage - 95 to 100 days from sowing when leaves were showing purple colouration – Brilliant purple 219461; 3 replicas from each plant type; true breeding M_4 mutant plants and selfed control lines were considered; leaves from a single plant constituted single replica) were extracted in 50 ml methanol for overnight, sonicated at 60°C for 3 hours, extracts filtered by Whatman No. 42 filter paper and made up to 25 ml in a volumetric flask.

Standard preparation: 1000 ppm of stock solution was prepared by dissolving 10 mg ± 0.01 of andrographolide (analytical standard, Chroma Dex, California, U.S.A.) in 10 ml of methanol (certified 'A' class).

HPTLC analysis: Aluminium TLC plates pre-coated with silica gel $60F_{254}$ (E. Merck KgaA, 20×20 cm^2, 0.3 mm thick) was developed in Camag TLC twin through glass chamber pre-saturated (30 mins. prior to development in ambient temperature) with chloroform : methanol (7:1) v/v as mobile phase. Methanolic extracts of samples (1, 2, and 5 µl volume) and andrographolide standard solution (1, 2, 3 and 4 µl of volume) were applied on plate by using Linomat V automatic applicator (nitrogen flow 150 nl/sec. - Peak N_2 Generator, Renfrew, UK) to a 6 mm wide band, positioned 10 mm from the bottom of the plates (syringe delivery speed 0.01 µl/sec.; number of tracts 18). After development, the plates were dried and quantified with Camag TLC scanner- 3 equipped (slit dimension 5.00×0.45 mm, scanning speed 20 mm/sec., absorption-reflection scan mode) with winCATS software at a wavelength of 220 nm with D_2 lamp. Rf of standard was 0.34 and it matched with that of the extract thereby confirming that they were the same substances.

The linearity was examined by applying the calibration working standard solution for 3 consecutive days. The calibration curve, log-transformed peak area versus log-transformed concentration was calculated according to least square method (Y = a + bx) for compound under consideration (linear regression- Y = 162.4 + 0.09557x, r = 0.98069, sdv = 7.51 via height; Y = 196.2 + 2.709x, r = 0.99122, sdv = 6.54 via area). The calibration curve was linear in the range 100 ng to 1 µg. The accuracy and reproducibility of the method was established by means of recovery experiment as per Pawer *et al.*[17] and the mean recovery was 95.98% of the compound (close to 100%) suggesting the accuracy of the method.

RESULTS

Types and frequency of macromutation

Fourteen different (viable) macromutants (mutant traits confirmed at M_3 from selfed segregation; mutants bred true at M_4) were spotted (*viridis*, *lax branching*, *bushy*, *unbranched I* and *II*, *dark green leaf*, *broad leaf I* and *II*, *narrow leaf I* and *II*, *drooping leaf I* and *II*, *dwarf* and *early maturity*) at M_2 (Figure 1: B-F) mutagenized population (1168 plants scored). Leaf mutations were predominant. *Lax branching*, *bushy*, *unbranched I* and *broad leaf I* and *II* appeared only in EMS treatments; while, *narrow leaf I*, *drooping leaf I* and *dwarf* mutants were recovered only from dES treatments.

Viridis (colour: yellowish green, 125249; normal plants – Figure 1:A possessing leaf colour - leaf green [s.g.3] 15627(3); chlorophyll content mg/gm of tissue in *viridis*: chlorophyll a 0.16, chlorophyll b 0.17, control: chlorophyll a 0.27, chlorophyll b 0.24) plant type attained

62.0 cm ± 2.16 (control: 74.2 cm ± 4.3) height at maturity and yielded 4.71 ± 3.64 seeds per capsule (8.67 ± 1.51/capsule in control plants) and were recovered from 1.0%, 2h EMS (1.0%) and 0.25%, 2h dES (0.56%) treatments. *Lax branching* (angle between main axis and branches, range - 54° to 72°, mean 64.6°; control: range - 37° to 48°, mean 42.2°) mutant plants were with higher number (42.0 ± 1.6; control: 24 to 38, mean 36.0 ± 3.4) of primary branches per plant (0.50%, 2h EMS – 3.51%, 1.0%, 4h EMS – 3.57%). *Bushy* mutant plants were relatively taller in height (94.6 cm ± 2.8) with enhanced total branches (265-281, mean 272 ± 0.6; control plants had primary branches only) and seed yield (4.02 gm ± 0.2; control: 2.05 gm ± 1.1) and were recovered from 1.0%, 2h EMS (1.0%) and 0.5%, 4h EMS (0.86%) treatments. *Unbranched* (*I*: late flowering – 221 to 234 days from sowing compared to 153 to 178 days in untreated control, only 4 capsules were produced, 5.83 ± 3.5 seeds/capsule, recovered only form 0.5%, 4h EMS treatment; *II*: normal flowering range – 144 to 162 days, occurrence – 0.25%, 4h EMS and dES treatments), *broad* (size: range – 7.1 to 11.3 cm × 1.5 to 2.2 cm, mean 9.3 cm × 1.9 cm; control: range – 6.1 to 7.5 cm × 0.9 to 1.6 cm, mean 6.8 cm × 1.3 cm) *leaf* (*I*: normal leaf colour; *II*: dark green - Jade green 126891; chlorophyll a 0.41, chlorophyll b 0.38, occurred only in 1.0%, 2h EMS treatment – 2.0%), *narrow* (size: range – 3.52 to 5.5 cm × 0.5 to 0.8 cm, mean 4.5 × 0.6 cm) *leaf* (*I*: normal flowering range; appeared only in 0.5%, 4h dES treatment – 1.47%; *II*: early flowering – 121 to 138 days from sowing; maturity 139 to 146 days compared 161 to 177 days in control; maximum occurrence in 0.5%, 4h dES – 1.47%) and *drooping leaf* (*I*: stem colour normal – Oriental green 126242; occurrence – 0.50%, 2h dES, 0.61%; semi dwarf 51.0 to 63.0 cm in height; seed yield 1.84 gm ± 0.9; *II*: pigmented stem - dark maroon 181216; occurrence – 0.50%, 4h EMS – 0.86% and 0.50%, 2h dES – 1.22%; seed yield low – 0.44 gm ± 0.6) mutants were characterized on the basis of their associated traits. *Dark green leaf* (pearl green 113422; chlorophyll a 0.54, chlorophyll b 0.53), dwarf (42.2 to 58.6 cm in height at maturity, mean 53.6 cm ± 2.72) and *early maturity* (119 to 136 days from sowing) mutants occurred in 0.26, 0.17 and 0.17% respectively over M_2 population.

Estimated total mutation frequency was not does dependent in either EMS (0.79 to 5.0%, maximum frequency – 1.0%, 2h treatment) or dES (0.56 to 10.0%; maximum frequency – 1.0%, 4h; 10.0% mutation frequency was possibly due to low turnover of M_1 progenies at M_2) treatments. EMS induced relatively higher (3.12%; spectrum 1 to 4; 640 plants scored) frequency of mutation than dES (2.46%; spectrum 1 to 3; 528 plants estimated). Mutation frequency over the M_2 population was 2.82% and the mutant types occurred in the following order: *lax branching* (0.51%) > *unbranched II* (0.43%) > *dark green leaf* (0.26%) = *drooping leaf II* > *viridis* (0.17%) = *bushy* = *broad leaf II* = *narrow leaf II* = *dwarf* = *early maturity* > *unbranched I* (0.09%) = *broad leaf I* = *narrow leaf I* = *drooping leaf I*. Concomitant traits associated with the macromutants persisted uniformly at M_3 as well as in M_4, thereby suggesting possible pleiotropic action of the mutant gene(s).

Segregation of the macromutants

Segregation patterns were assessed at M_3 of mutants as either 1:1 or 3:1 (*lax branching*: normal 24, mutant 23, χ^2=0.022 for 1:1, p>0.80; *bushy*: normal 14, mutant 11, χ^2=0.36 for 1:1, p>0.50; *unbranched I*: normal 25, mutant 20, χ^2=0.556 for 1:1, p>0.30; *unbranched II*: normal 12, mutant 9, χ^2=0.046 for 1:1, p>0.80; *broad leaf I*: normal 11, mutant 7, χ^2=0.888 for 1:1, p>0.30; *broad leaf II*: normal 16, mutant 14, χ^2=0.134 for 1:1, p>0.70; *dark green leaf*: normal 15, mutant 6, χ^2=0.143 for 3:1, p>0.20; *narrow leaf I*: normal 30, mutant 26, χ^2=0.286 for 1:1, p>0.50; *narrow leaf II*: normal 36, mutant 33, χ^2=0.13 for 1:1, p>0.70; *drooping leaf I*: normal 11, mutant 7, χ^2=0.888 for 1:1, p>0.30; *drooping leaf II*: normal 18, mutant 17, χ^2=0.13 for 1:1, p>0.70; *early maturity*: normal 17, mutant 6, χ^2=0.013 for 3:1, p>0.90; *dwarf*: normal 21, mutant 8, χ^2=0.336 for 3:1, p>0.50) thereby indicating possible monogenic recessive inheritance of the mutant trait(s); however, *viridis* showed digenic (normal 33, mutant 3, χ^2=0.267 for 15:1, p>0.50) mode of inheritance.

Meiotic analysis

Meiotic analysis revealed 2*n*=50 chromosomes always in the plant types (Figure 2: A-B). Mean chromosome association at MI was 24.92 II + 0.15 I in control (52 PMCs studied), 24.85 II + 0.30 I in *viridis* (27 cells), 24.93 II + 0.15 I in *lax branching* (40 PMCs), 24.94 II + 0.13 I in *bushy* (47 meiocytes), 24.87 II + 0.26 I in *unbranched I* (23 cells), 24.93 II + 0.14 I in *unbranched II* (44 cells), 24.45 II + 1.10 I in *dark green leaf* (58 PMCs), 24.95 II + 0.09 I in *broad leaf I* (44 meiocytes), 24.90 II + 0.21 I in *broad leaf II* (58 PMCs), 24.80 II + 0.40 I in *drooping leaf I* (20 PMCs), 24.82 II + 0.37 I in *drooping leaf II* (38 cells); while, *narrow leaf I* (44 cells scored) and *II* (23 PMCs), *dwarf* (35 cells) and *early maturity* (23 PMCs) mutants showed 25II formation. Though bivalent frequency per cell was random (p>0.05) over the plant types, univalent per cell was inconsistent (p<0.001) as evidenced from χ^2 test of heterogeneity (DF 14). The AI chromosome segregation was always equal (25:25, 52 cells scored in control and 23 to 72 cells in mutants). Pollen fertility and viability assessed in normal plants was 80.63% (888 pollen grains analyzed) and 72.24% (893 pollen assessed) respectively. In mutants pollen fertility ranged from 21.28% (*broad leaf I*, 1001 pollen studied) to 89.25% (*narrow leaf I*, 1113 pollen scored) and that of viability varied between 27.55% (*broad leaf I*, 940 pollen observed) and 59.04% (*bushy*, 437 pollen estimated). Mostly, pollen viability was less than pollen fertility.

Pollen nuclei composition

Data presented in Table 1 suggested that *dark green leaf* and *broad leaf I* mutants were with lower pollen fertility and viability than control but seed set per capsule, seed yield per plant and abnormal (0v+1g, 1v+0g, v dispersed and 1v+2-5g) pollen nuclei composition (Figure 3: A-F) were more or less comparable in the plant types. *Drooping leaf II* possessing low pollen fertility and viability had low seed set per capsule and seed yield per plant and the percentage (40.04) of abnormal pollen nuclei composition was much higher than control (20.2%).

Andrographolide content

The yield of andrographolide (Figure 4: A-D) in matured leaves was 3.41% in normal plants and it was noted to be 2.33% in *viridis*, 3.45% in *lax branching*, 3.99 in bushy, 0.40% in *unbranched I*, 0.37% in *unbranched II*, 0.30% in *dark green leaf*, 3.76% in *broad leaf I*, 3.84% in *broad leaf II*, 0.11% in *narrow leaf I*, 1.26% in *narrow leaf II*, 0.64% in *drooping leaf I*, 0.02% in *drooping leaf II*, 0.03% in *dwarf* and 1.28% in *early maturity*. *Bushy* (t=26.85, DF=4, p<0.001), *broad leaf I* (t=14.71, DF=4, p<0.001) and *II* (t=19.91, DF=4, p<0.001) mutants were with significantly higher amount of andrographolide content than normal plants.

Table 1: Pollen characteristics and reproductive parameters in control and mutant plants of *A. paniculata*

Attributes	Plant types			
	Control	*Dark green leaf*	*Broad leaf I*	*Drooping leaf II*
Pollen fertility (%)	80.63 (888)	25.64 (1205)	21.28 (1001)	59.67 (1168)
Pollen viability (%)	72.74 (735)	33.49 (618)	27.55 (940)	45.67 (648)
Pollen nuclei composition (%)				
1v+2g	32.32	46.21	26.93	27.13
0v+2g	47.43	30.52	47.29	32.83
0v+1g	9.16	17.37	16.09	18.93
1v+0g	6.27	1.85	5.75	13.57
v-dispersed	4.50	3.88	2.96	3.52
1v+(2-5)g	0.32	0.17	0.99	4.02
Pollen grain analyzed	622	593	609	597
Seed per capsule	8.67±1.51	7.50±2.07	8.40±1.34	6.20±1.30
Capsule length (cm)	1.74±0.31	1.64±0.15	1.80±0.14	1.60±0.14
Total seed weight per plant (gm)	2.05	2.01	2.21	0.45

Data in parenthesis indicate number of pollen grains studied

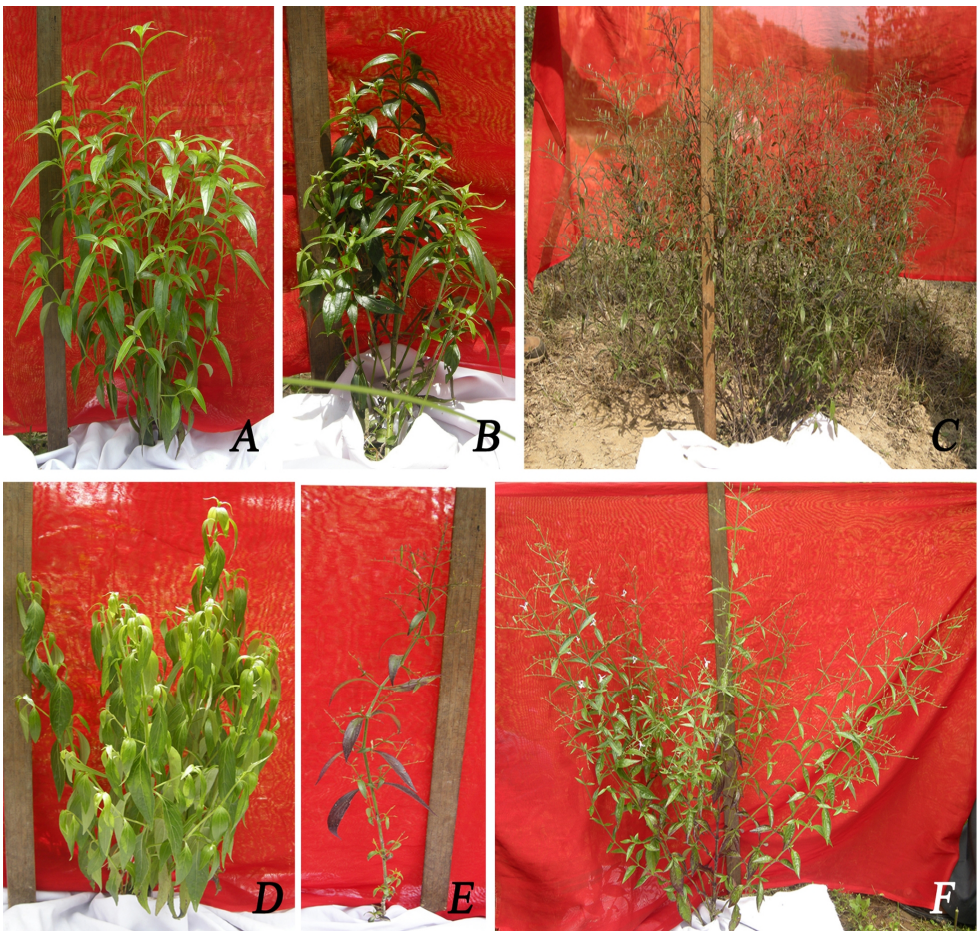

Figure 1: Normal and mutant (B) plant types of *A. paniculata*. A- Normal plant; B- *Dark green leaf*; C- *Bushy*; D- *Drooping leaf II*; E- *Unbranched I*; F- *Lax branching*

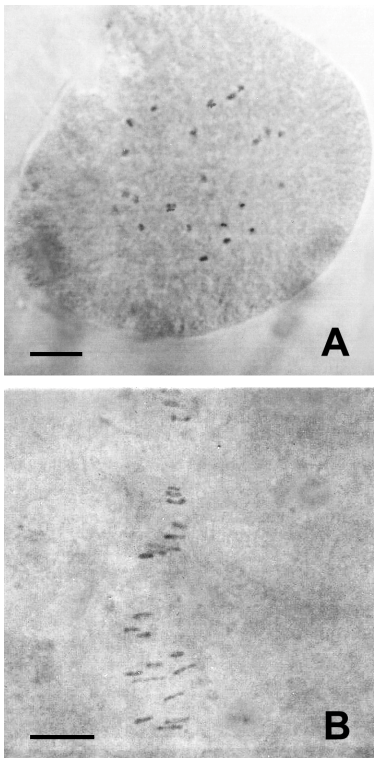

Figure 2: Meiotic chromosome configurations (A and B) in *A. paniculata* (2*n*=50). Scale bar = 20 µm.

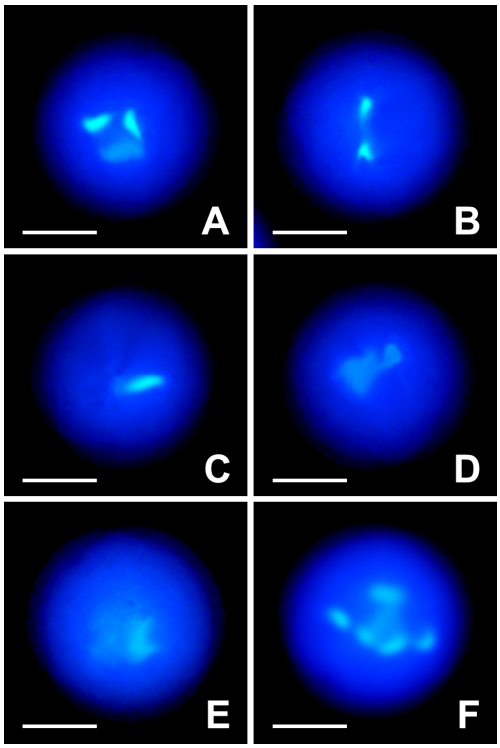

Figure 3: Pollen nuclei composition following DAPI staining in plant types of *A. paniculata*. A- 1v+2g; B- 0v+2g; C- 0v+1g; D- 1v+0g; E- v-dispersed; F- 1v+5g. Scale bar = 20 µm.

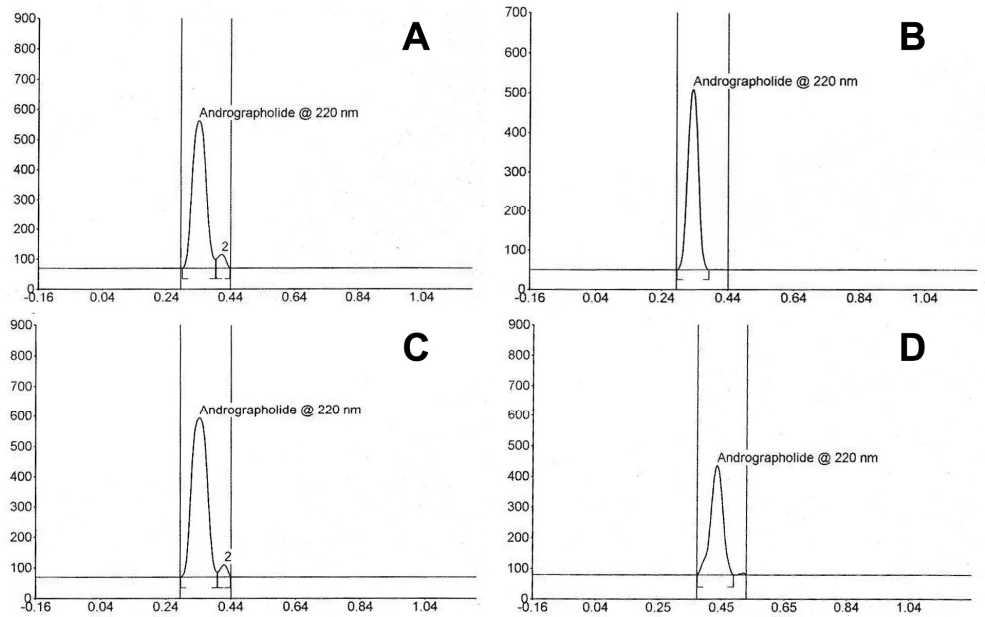

Figure 4: Chromatograms reflecting andrographolide content. A- Standard; B- Control; C- *Bushy* D- *Narrow leaf II*.

DISCUSSION

EMS and dES induced mutations have affected various plant parts of *A. paniculata* and brought about gross morphological changes in seedling colour (*viridis*), branching pattern (*lax branching*, *bushy* and *unbranched I* and *II*), growth habit (*dwarf*), leaf morphology (*dark green leaf*, *broad leaf I* and *II*, *narrow leaf I* and *II* and *drooping leaf I* and *II*) and maturity (*early maturity*), resulting into alteration of the plant ideotype. The mutants evolved as the consequence of gene mutation (all the mutants were cytologically balanced as normal plants) seems to be in the direction of the objective underlined (*bushy* and *broad leaf I* and *II* were with significantly enhanced andrographolide contents). *Early maturing* mutant type corresponds closely to the ideotype being looked for in the plant species. *Viridis*, *dark green leaf*, *narrow leaf I* and *II*, *drooping leaf I* and *II* and *lax branching* mutants induced were important genetic resources in the plant species and the mutant trait(s) may be explored as marker(s) in efficient breeding. Kharkwal[18] was of opinion that macromutations appearing in the segregating M_2 population were consequences of mutational changes of the 'major genes'. In the present investigation, mutant traits were mostly (excepting *viridis*

which showed digenic mode of inheritance) monogenic recessive to normal plants.

Concomitant occurrence of certain characteristics with the mutant trait(s) in some mutant lines (*broad leaf*, *narrow leaf*, *unbranched* and *drooping leaf*) gives an indication of pleiotropism or linkage or possibly the mutagens have affected different sites of the same gene; which, however, needs further investigation to derive to a specific conclusion. Emery *et al.*[19] presumed that all induced mutations have varying degree of pleiotropic influence on other characters. Kreft[20] reported high lysine genes in barley and genes for the determinant growth in buckwheat (*Fagopyrum esculentum*) were examples to show that the effects of single mutation could be complex or pleiotropic.

Pollen grains, product of male meiosis, play key role in fertility and reproduction. Analysis (pollen fertility and viability, pollen nuclei composition and reproductive parameters) of few mutants namely, *dark green leaf* and *broad leaf I* and *drooping leaf II* in relation to control revealed that pollen fertility and viability (*dark green leaf* and *broad leaf I*) were not detrimental in determining the reproductive outcome; however, pollen developmental phases (nuclei composition in relation to germination and fertilization) may also play significant role (as was noted in *drooping leaf II*) in the reproductive process. Ferri *et al.*[21], Lyra *et al.*[22] amongst others were of opinion that pollen germination is an important criterion for reproductive biology. Sedgley and Griffin[23] suggested that pollen germination may be controlled by vegetative nucleus in the absence of viable generative nucleus; however, such condition may not be appropriate for proper fertilization and subsequently seed setting[24].

Andrographolide content in control was 3.41% and in mutants it varied from 0.02 to 3.99%. Saxena *et al.*[25] demonstrated variations (1.77 to 2.66%) in andrographolide content from leaves using different solvent system for extraction and methanol was found most appropriate and it was also corroborated by Srivastava *et al.*[26]. Sanyal *et al.*[27] quantified andrographolide content from matured leaves as 1.40 to 2.14% in New Alluvial zone, 2.28 to 2.91% in Red Lateritic zone and 2.16 to 2.25% in Coastal zone in West Bengal plains following HPTLC methods and the authors were of opinion that soil and climatic factors were important parameters for biosynthesis of secondary metabolites. Vijaykumar *et al.*[28] reported andrographolide content (HPTLC method) from four different herb samples (0.47 to 1.67 mg/100 mg) and different leaf extracts (21.31 to 85.88 mg/100mg). Pawar *et al.*[17] found that the quantity of andrographolide from two samples (whole plant) from Ghaziabad, U.P., India was 0.77133% w/w and 0.75189% w/w (solvent: 5 parts toluene: 4.5 parts ethyl acetate: 0.5 part formic acid v/v as mobile phase). Ghosh *et al.*[29] reported yield of andrographolide to be 0.93% in young leaves and 3.41% in matured leaves under similar agro climatic conditions and were of opinion that possibly some key enzyme(s) may be triggered during reproductive phase of growth. Thus, maturity of the plant, germplasms under consideration, soil and climatic factor(s) seems to be important attributes for the biosynthesis and accumulation of andrographolide.

Released variation through induced mutation have widened the gene pool and enriched genetic diversity in *A. paniculata*, which did not exist previously. Mutational approach to develop important genetic resources in medicinal plant species has been rather meagre[30-33] and the present endeavour may provide some insight on the aspect.

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