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MUTAGENIC RESPONSE IN TWO CULTIVARS OF MORNING GLORY *IPOMOEA PURPUREA* (L.) ROTH

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ABSTRACT

Induced mutation studies were carried out in violet blue (VB) and red purple (RP) cultivars of *Ipomoea prpurea*, differing markedly in corolla morphology. Chemical mutagens selected were ethyl methane sulfonate (EMS), sodium azide (SA) and N-methyl-N'- nitro-N-nitrosoguanidine (NMG). Treatments were administered by soaking dry, 6 h pre-soaked water (PSW) and 8 h PSW seeds in aqueous mutagenic solutions. M_1 generation seeds were collected capsule wise and sown to raise M_2 generation. M_2 population was screened for chlorophyll, flower colour and floral morpological mutations. SA and NMG have induced useful new mutations in both the cultivars. The differences in the mutagenicity observed in two cultivars has been attributed to the variations in the microgenomic architecture.

Key word: *Ipomea purpurea,* mutagene response

Since the discovery of chemical mutagens, large number of investigations on methodology, effectiveness and efficient mutagenesis have been dealt with [1-3]. Such studies have resulted in useful mutations in crop and ornamental plants. *Ipomoea purpurea* is polymorphic for its flower colour. Genetics of flower colour polymorphism has been already worked out [4]. In the present studies comparative response of the two cultivars of *I. purpurea* to ethyl methanesulfonate (EMS), sodium azide (SA) and N-methyl-N' nitro-N-nitrosoguanidine (NMG) has been investigated. The two cultivars differ markedly in flower colour and corolla morphology. Violet blue (VB) is with typical convolvulous corolla while red purple has imperfectly gamopetalous corolla provided with a corona of petalloid appendages on outer side. It has been described as petallomaniatic.

MATERIALS AND METHODS

Freshly harvested physiologically similar and genetically uniform seeds of both the cultivars were used. For chemical mutagenic treatments seeds were either treated dry or pre-soaked in distilled water (PSW) for 6 and 8 h. The (surface dried) and

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dry seeds were soaked in 50 ml aqueous mutagenic solutions along with control. at 23 ± 2°C with intermitant shaking. The doses selected were 0.05, 0.1, 0.2 and 0.3% of EMS and NMG for dry seed treatments and 0.1, 0.2, 0.3 and 0.4% for pre-soaked seeds. Concentrations of SA were 0.005, 0.01, 0.015, and 0.02% for dry and 0.02, 0.03, 0.04 and 0.05% for 6 and 8 hours presoaked seed treatments. Every treatment was replicated twice with 250 seeds.

The mutagenic treated seeds were washed and post-soaked in 50 ml of distilled water for 2 h. Out of 250 seeds, 100 were sown in the plot to raise M_1 generation and remaining 150 were used for studying various M_1 parameters. M_2 population, raised from first ten capsules of each M_1 plant, was screened for chlorophyll, flower colour and floral morphological mutations. Mutation frequencies were calculated as per 100 M_2 plant basis following Gaul [5]. Total mutation frequency was the sum of these frequencies.

RESULTS AND DISCUSSION

M_1 population raised from mutagen treated seeds were studied for germination, seedling height, chlorophyll deficient-chimeras, mitotic chromosomal aberrations, pollen sterility and seed set. Since this paper is devoted to the mutagenic response only, data pertaining to M_2 chlorophyll, flower colour and floral morphological frequencies along with their total are presented in Table 1 to 6.

Table 1 and 2 includes EMS induced mutation frequencies of VB and RP, respectively. It was high when dry seeds of VB were exposed to 0.2% EMS. For higher concentrations of EMS, 6 and 8 h presoaking were not very effective in VB. Mutation frequency ranged from 2.9 to 3.9 for dry seed treatments in RP. Of the pre- soaked treatments, 6 h pre-soaking was either ineffective or lethal for higher doses of EMS (0.3 and 0.4%), however with 8h pre-soaking marginally higher mutation rates were obtained in RP.

Sodium azide induced mutations data are presented in Table 3 and 4. In both the cultivars 6 h and 8 h PSW treatments responded better as compared to dry seed treatments. Of the pre- soaked treatments 8 h pre-soaking gave enhanced mutations in VB. However, in RP cultivar no particular trend was observed among them. Petallomaniatic morphotype in VB and white and pink- streak flower colour mutants in RP were induced with SA. These mutations represented significant alterations in corolla morphology and flower colour in VB and RP, respectively.

Table 1. EMS induced mutation frequencies in M_2 generation of *I. purpurea* (Violet blue cultivar)

Treatments	No. of M_2 plants scored	Mutation frequency in M_2 plants (%)			
		Chlorophyll	Flower colour	Flower morpho-logical	Total
Dry-Control	751	0.00	0.00	0.00	0.0
Dry 18 h EMS 0.05%	1243	0.64	0.56	0.24	1.4
Dry 18 h EMS 0.1%	1784	1.46	1.68	0.62	3.8
Dry 18 h EMS 0.2%	1351	3.77	3.48	1.99	9.2
Dry 18 h EMS 0.3%	1330	0.90	1.80	0.90	3.6
6 h PSW-Control	907	0.00	0.00	0.00	0.0
6 h PSW 8 h EMS 0.1%	1016	1.97	2.07	0.98	5.0
6 h PSW 8 h EMS 0.2%	1092	2.75	1.56	0.73	5.0
6 h PSW 8 h EMS 0.3%	891	1.57	1.01	1.12	3.7
6 h PSW 8 h EMS 0.4%	844	1.54	0.83	0.71	3.1
8 h PSW-Control	963	0.00	0.00	0.00	0.0
8 h PSW 6 h EMS 0.1%	1338	1.12	1.19	0.67	3.0
8 h PSW 6 h EMS 0.2%	1266	2.84	1.18	1.58	5.6
8 h PSW 6 h EMS 0.3%	1158	2.24	2.16	0.43	4.8
8 h PSW 6 h EMS 0.4%	1212	1.98	1.07	0.49	3.5

Table 2. EMS induced mutation frequencies in M_2 generation of *I. purpurea* (Red purple cultivar)

Treatments	No. of M_2 plants scored	Mutation frequency in M_2 plants (%)			
		Chlorophyll	Flower colour	Flower morpho-logical	Total
Dry-control	706	0.00	0.00	0.00	0.0
Dry 18 h EMS 0.05%	1167	0.94	0.77	1.19	2.9
Dry 18 h EMS 0.1%	1261	1.51	0.79	1.35	3.6
Dry 18 h EMS 0.2%	1000	1.70	1.00	1.00	3.7
Dry 18 h EMS 0.3%	903	1.11	1.44	1.33	3.9

6 h PSW-control	727	0.00	0.00	0.00	0.0
6 h PSW 8 h EMS 0.1%	629	0.48	1.11	1.43	3.0
6 h PSW 8 h EMS 0.2%	265	1.13	0.75	1.13	3.0
6 h PSW 8 h EMS 0.3%	44	0.00	0.00	0.00	0.0
6 h PSW 8 h EMS 0.4%	-	-	-	-	-
8 h PSW-Control	901	0.00	0.00	0.00	0.0
8 h PSW 6 h EMS 0.1%	1150	2.26	1.22	0.52	4.0
8 h PSW 6 h EMS 0.2%	1065	3.00	2.53	0.85	6.4
8 h PSW 6 h EMS 0.3%	1027	3.11	1.46	1.10	5.7
8 h PSW 6 h EMS 0.4%	980	2.65	1.43	0.41	4.5

§ Did not survive till maturity

Table 3. SA induced mutation frequencies in M_2 generation of *I. purpurea* (Violet blue cultivar)

Treatments	No. of M_2 plants scored	Mutation frequency in M_2 plants (%)			
		Chlorophyll	Flower colour	Flower morpho-logical	Total (%)
Dry-Control	1323	0.00	0.00	0.00	0.0
Dry 18 h SA 0.005%	1525	0.72	0.66	0.52	1.9
Dry 18 h SA 0.01%	1277	1.25	0.94	0.63	2.8
Dry 18 h SA 0.015%	1348	0.44	0.74	0.52	1.7
Dry 18 h SA 0.02%	1254	0.00	0.88	0.48	1.4
6 h PSW-Control	1057	0.00	0.00	0.00	0.0
6 h PSW 8 h SA 0.02%	1270	2.20	1.18	0.71	4.1
6 h PSW 8 h SA 0.03%	1032	2.42	1.55	1.07	5.0
6 h PSW 8 h SA 0.04%	1075	2.51	2.14	1.21	5.9
6 h PSW 8 h SA 0.05%	1090	1.83	0.83	0.92	3.6
8 h PSW-Control	1123	0.00	0.00	0.00	0.0
8 h PSW 6 h SA 0.02%	1055	4.36	1.99	1.99	8.3
8 h PSW 6 h SA 0.03%	1033	2.90	1.55	2.81	7.3
8 h PSW 6 h SA 0.04%	1330	2.26	1.80	1.95	6.0
8 h PSW 6 h SA 0.05%	1179	1.27	1.36	1.52	4.1

Table 4. SA induced mutation frequencies in M_2 generation of *I. purpurea* (Red purple cultivar)

Treatments	No. of M_2 plants scored	Mutation frequency in M_2 plants (%)			
		chlorophyll	Flower colour	Flower morpho-logical	Total
Dry-control	837	0.00	0.00	0.00	0.0
Dry 18 h SA 0.005%	1032	0.00	0.58	0.68	1.3
Dry 18 h SA 0.01%	1065	1.03	0.75	1.03	2.8
Dry 18 h SA 0.015%	1050	0.86	0.86	0.66	2.4
Dry 18 h SA 0.02%	981	0.81	0.91	0.40	2.1
6 h PSW-Control	961	0.00	0.00	0.00	0.0
6 h PSW 8 h SA 0.02%	1251	1.35	1.58	1.68	4.6
6 h PSW 8 h SA 0.03%	1020	2.74	2.16	0.88	5.8
6 h PSW 8 h SA 0.04%	1124	2.84	1.16	1.60	5.6
6 h PSW 8 h SA 0.05%	1096	3.28	0.91	1.00	5.2
8 h PSW-Control	975	0.00	0.00	0.00	0.0
8 h PSW 6 h SA 0.02%	1192	1.76	1.00	0.92	3.7
8 h PSW 6 h SA 0.03%	1140	2.71	1.84	1.84	6.4
8 h PSW 6 h SA 0.04%	998	3.10	1.60	1.40	6.1
8 h PSW 6 h SA 0.05%	911	2.30	1.52	0.60	4.4

NMG treatments in both the cultivars were characterised by low chlorophyll mutation rates (Table 5 and 6). In M_1 generation also very few chlorophyll deficient chimeras were recorded. Maximum chlorophyll mutation frequencies 0.36 and 1.05% were recorded in VB and RP, respectively, when 6 h PSW seeds were exposed to 0.1% NMG. In contrast NMG induced high frequency flower colour and floral morphological mutations (horticultural importance) in both the cultivars. Pre-soaked treatments gave marginally higher mutation frequencies in both the cultivars. An unusual bicolour mutant where corolla limb consisting of two colours distributed in a regular fashion and a magenta colour mutant were obtained in VB. NMG also produced petallomaniatic morphotype in VB and white and variegated flower colour mutants in RP.

In the present study mutagenic potentials in two cultivars of *I. purpurea* were explored, under uniform treatment conditions. Total mutation frequency values in VB with EMS and SA showed a reflection of the trend observed in chlorophyll

Table 5. NMG induced mutation frequencies in M_2 generation of *I. purpurea* (Violet blue cultivar)

Treatments	No. of M_2 plants scored	Mutation frequency in M_2 plants (%)			
		Chlorophyll	Flower colour	Flower morpho-logical	Total
Dry-Control	988	0.00	0.00	0.00	0.0
Dry 18 h NMG 0.05%	1117	0.00	1.34	0.54	1.9
Dry 18 h NMG 0.1%	1041	0.00	1.06	0.58	1.6
Dry 18 h NMG 0.2%	1136	0.00	2.55	1.05	3.6
Dry 18 h NMG 0.3%	1035	0.00	0.78	1.91	2.7
6 h PSW-Control	1175	0.00	0.00	0.00	0.0
6 h PSW 8 h NMG 0.1%	1389	0.36	2.36	0.35	3.1
6 h PSW 8 h NMG 0.2%	1263	0.00	1.74	0.72	2.5
6h PSW 8 h NMG 0.3%	1272	0.31	1.57	0.94	2.8
6 h PSW 8 h NMG 0.4%	1298	0.00	1.54	0.29	1.8
8 h PSW-Control	1020	0.00	0.00	0.00	0.0
8 h PSW 6 h NMG 0.1%	1047	0.00	1.24	0.46	1.7
8 h PSW 6 h NMG 0.2%	1007	0.00	1.39	1.75	3.1
8 h PSW 6 h NMG 0.3%	1092	0.00	2.19	2.11	4.3
8 h PSW 6 h NMG 0.4%	1165	0.09	3.26	2.06	5.4

mutation frequencies, while no such corelation was observed in case of NMG treatments. In RP cultivar, such relationship was exhibited by presoaked treatments of SA and 8 h pre-soaked treatments of EMS.

EMS is one of the most potent mutagen. Enhanced mutations frequencies with EMS and treatments of pre-soaked as compared to dry-seeds were reported in barley and other cereals [6, 7]. However, both the cultivars with 6 h pre-soaking did not exhibit any increase in the mutational rates and this could be due to higher deleterious effects at M_1 level.

SA is safe, inexpensive and a convenient mutagen [8]. It was reported to be effective and efficient mutagen in terms of chlorophyll deficient, morphological and biochemical mutations in barley. Its pH dependancy has been reported in barley [9]. However, its mutagenicity when dissolved in glass distilled water was reported in

Petunia hybrida [10]. They have also reported enhanced mutation frequency with pre-soaking and ascribed it to the metabolically active state of the embryonic cells and larger availability of sites for mutagenic reactions [11]. In the present investigations also SA was dissolved in glass distilled water and mutation frequencies were higher with pre- soaked seed treatments.

Table 6. NMG induced mutation frequencies in M_2 generation of *I. purpurea* (Red purple cultivar)

Treatments	No. of M_2 plants scored	Mutation frequency in M_2 plants (%)			
		Chlorophyll	Flower colour	Flower morpho-logical	Total
Dry-control	969	0.00	0.00	0.00	0.0
Dry 18h NMG 0.05%	1041	0.00	1.44	0.57	2.0
Dry 18h NMG 0.1%	1000	0.00	1.50	0.60	2.1
Dry 18h NMG 0.2%	1071	0.00	1.31	0.84	2.1
Dry 18 h NMG 0.3%	1029	0.00	1.65	1.55	3.2
6 h PSW-Control	882	0.00	0.00	0.00	0.0
6 h PSW 8 h NMG 0.1%	1237	1.05	1.13	0.97	3.1
6 h PSW 8 h NMG 0.2%	1102	0.45	1.45	0.99	2.9
6 h PSW 8 h NMG 0.3%	1234	0.81	1.38	1.22	3.4
6 h PSW 8 h NMG 0.4%	1124	0.62	1.60	0.89	3.1
8 h PSW-Control	935	0.00	0.00	0.00	0.0
8 h PSW 6 h NMG 0.1%	1062	0.00	1.88	1.41	3.3
8 h PSW 6 h NMG 0.2%	1164	0.60	1.28	1.46	3.3
8 h PSW 6 h NMG 0.3%	1021	0.00	1.37	1.57	2.9
8 h PSW 6 h NMG 0.4%	1145	0.17	1.31	1.31	2.8

In both the cultivars NMG was ineffective in inducing chlorophyll deficient mutations. Both high mutagenecity of NMG as well as its ineffectiveness had been reported in barley [12, 13]. Although EMS and NMG are alkylating agents, it was suggested that seed metabolism of plant species may be related to the differential mutagenecity at species level [14].

Discrepancies were observed regarding mutation frequencies for similar mutagenic treatments in two cultivars of *I. purpurea*. Reports on mutagenecity of a particular mutagen in diverse systems are frequent. However, comparative mutagenicity of different mutagens in the present two cultivars of a single system are infrequent. These discrepancies in mutational response between two different species or even strains of the same species may only reflect differences in the processing of primary genetic damage [15]. In the mutagenic treatments whole genome is vulnerable to the attack of mutagen. Therefore, differential response of the two cultivars in *I. purpurea* may be attributed to the differences in genomic architecture. Thus the mutagenicity is ultimately determined by the genome itself.

Based upon mutagenic response on chlorophyll deficient mutations only does not seem to be reliable in ornamental plants. The data indicated the necessity of screening flower colour and morphological mutations for addressing both academic and applied application. Further, induction of new and rare characteristics are of great interest in ornamental plants like *I. purpurea*. From this viewpoint SA and NMG have induced useful new mutations in both the cultivars and would be worth exploitable in other ornamentals as well.

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