Table 1. Frequency of viable mutations in the M_2 generation of *Portulaca grandiflora*

Cultivar	Mutant types	Total No. of plants	Mutated M_1 plants
PgBmj	dwarf	938	217
	late flowering	938	99
	female-sterile	252	2
	male-sterile	162	6
PfR	dwarf	459	144
	dwarf (altered leaves)	409	1
	late flowering	459	53
	female-sterile	370	7
	male-sterile	459	5

Five morphological mutations, namely, dwarf (Dw_1), late flowering (Flt), male-sterile (Sm), female-sterile (Sf) and dwarf mutant with altered leaves (Dwr_2) were selected from the M_2 population. Some mutants, identical with those scored in *Portulaca* were identified in another species [3, 4].

The higher frequency of mutants was obtained from treatment of seeds with 4.8% EMS concentration in variety PgR and from 20 mM NaN_3 treatment in variety PgBmj. In the M_3 generation, segregation for *Dwr_1*, *Dwr_2*, *Sm* and *Sf* revealed that these mutations are recessive to normal plants. The experiment proved that EMS and NaN_3 were effective in inducing phenotypic variation. This is the first report on inducing variability in *Portulaca* through mutagenic treatment. The new gene markers identified will be of great value for studying the genetics of *Portulaca grandiflora.*

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GENETIC ANALYSIS OF SUNFLOWER CHLOROPHYLL MUTANTS

The method of getting the chlorophyll mutations in sunflower was developed by Y.D. Beletskii in 1969 with the use of N-nitroso-N-methylurea (NMH) [1; 2]. Certain concentrations of NMH are known to induce plastid mutations in growing seeds, and their yield depends on the duration of the exposure [3]. The given work presented studieds on the influence of rifampicin (R) and 2,4-dinitrophenol (DNP) on the genetic activity NMH, as an inductor of plastid and nuclear mutations.

Seeds of a sunflower line 3629 were soaked under anaerobic conditions for 18 h and then treated with 0,015% NMH in combination with either 5 x 10^{-5} M DNP or 10^{-2} M rifampicin. Treatment with NMH was performed at 12-15 h of growth, for 3 h. DNP and R were applied at 0-6, 6-12, 0-12, 12-18 and 15-21 h of growth. Treated and control seedlings were washed with running water and seeded in the field. The frequency of plants with altered chlorophyll content in M_1, inheritance of traits in M_2 and in M_3 was studied. To analyze the genetic nature of chlorophyll mutants, reciprocal crosses with the original inbred line 3629 were made.

Used concentrations of DNP and R did not induce genetic changes. MNH induce variegated forms already in M_1 with the frequency 2,4-8% (in different years).

Pretreatment with R or DNP increased the effect of MNH (i.e., the frequency of chlorophyll mutants in M_2) (Table 1). After self-pollination of surviving M_2 chlorophyll mutants segregation in their progeny was observed. Variegated forms induced by MNH in combination with 6 h pre-treatment of R (variants 1, 2, 4 and 5) segregated to three types of seedlings: green, variegated and yellow-white (inviable). Non-Mendelian segregation was observed, which indicated the plastome nature of variegated forms. In the progeny of variegated plants, no *chlorina*-type forms were ever recorded.

Table 1. Frequency of chlorophyll mutations induced by MNH in combination with DNP or R

Variant of experiments	M_2 chlorophyll mutants (%)							
	Total		Variegation		*Chlorina*		Inviable	
	1995	1996	1995	1996	1995	1996	1995	1996
1. R(0-6)+MNH	7.2	1.5	2.9	1.5	1.4	0	2.9	0
2. R(6-12)+MNH	2.7	0.4	2.7	0	0	0	0	0.4
3. R(0-12)+MNH	2.8	0	0	0	1.4	0	1.4	0
4. R(12-18)+MNH	0	1.1	0	0.3	0	0	0	0.8
5. R(15-21)+MNH	0	15.9	0	1.2	0	13.5	0	1.2
6. DNP(0-6)+MNH	4.0	0	0	0	4	0	0	0
7. DNP(6-12)+MNH	0	0	0	0	0	0	0	0
8. DNP(0-12)+MNH	7.7	0	0	0	6.7	0	0.9	0
9. DNP(12-18)+MNH	0	2.6	0	0	0	0	0	2.6
10. DNP(15-21)+MNH	0	1.0	0	0.7	0	0	0	0.3
11. MNH(12-15)	2.2	2.9	1.1	1.5	1.1	0	0	1.5
12. Control	0	0	0	0	0	0	0	0

Chlorophyll *chlorina*-type mutations induced by MNH in combinations with R represent a heterogeneous group, although all plants were phenotypically similar. In variant 3, *chlorina*-type mutants segregated to only *chlorina* and green seedlings after self-pollination. The proportion of *chlorina* plants in M_3 was 96%. In M_4 – M_6 all progenies expressed the mutant phenotype. In progeny of *chlorina* variant 1, *chlorina* forms constituted only 53% in M_3. It is noteworthy that, only in this variant, a single variegated form was noted in the *chlorina* progeny in addition to green and *chlorina* plants. In general, plants of this variant showed a decreased viability, most of them perished before flowering. This made a detailed genetic analysis of these mutants impossible. In variant 5, *chlorina* plants produced only green and *chlorina* forms in M_3 after self-pollination. The proportion of *chlorina* plants was about 73%. *Chlorina* from variants 6, 8 phenotypically differed from each other. In M_3 the proportion of mutants was from 82 to 88%, while in M_4 – M_6 it was 100% in both variants. The absence of segregation indicated plastom homogeneity of these plants.

Variegated forms induced by MNH are most likely of different origin. The forms developed in 1995 produced variegated, green, and yellow-white plants after self-pollination in M_3 and M_4. The variegated M_2 forms developed in 1996 produced *chlorina* plants in addition to variegated and green forms in M_3 (5,6%). The proportion of variegated forms was 25%. This was a unique variant in which *chlorina*-type mutants appeared in the progeny of variegated plants after self-pollination. The mutational changes in cytoplasmic organelles are manifested due to plastid sorting during the period necessary to attain near-homoplastomic conditions. Segregation into two phenotypic classes after self-pollination – the original mutant and normal green – indicated the existence of two plastid types. In *chlorina*-type mutants, altered chloroplasts prevail and their proportion in a cell increases in generations. The appearance of a single variegated form in the *chlorina* progeny (M_3 in the variant 1) and *chlorina* plants in the progeny of variegated forms (M_3 in the variant 11) may indicate the existence of several types of plastids in the cells of these plants. The reciprocal crosses between variegated plants (variant 2) and the line 3629 showed that, when a variegated from was used as a female, segregation in F_1 hybrid progeny was similar to that after self-pollination. In reciprocal crosses, all progeny were green, both in F_1 and F_2 (Table 2). Results indicate the plastid nature of this mutation. Cross of *chlorina* mutants (variant 6) to the green line 3629 revealed the dominance of green phenotype in F_1, irrespective of cross direction. This provides evidence for the nuclear origin of these mutations. In F_2 segregation for green and *chlorina* plants was observed. This segregation corresponded to the expected 3:1 ratio.

Table 2. Result of crosses between mutants and the original line 3629

Cross combination	F_1 plants				F_2 plants			
	Green	*Chlorina*	Variegated	Inviable	Green	*Chlorina*	Variegated	Inviable
DNP(0-12)-MNH(var.8)x3629	74	29	0	0	55	13	0	6
3629xDNP(0-12)-MNH(var.8)	174	0	0	0	363	42	4	12
R(0-12)-MNH(var.3)x3629	91	29	0	0	41	13	0	2
3629xR(0-12)-MNH(var.3)	74	0	0	0	46	7	0	1
R(6-12)-MNH(var.2)x3629	18	0	20	2	-	-	-	-
3629xR(6-12)-MNH(var.2)	73	0	0	0	72	0	0	0
DNP(0-6)-MNH(var.6)x3629	97	0	0	0	99	22	0	0
3629xDNP(0-6)-MNH(var.6)	41	0	0	0	92	22	0	0

Different results were observed in reciprocal crosses between the line 3629 and *chlorina* plants induced by MNH in combination with pretreatment with DNP or R for 12 h (variants 8 and 3 accordingly). When plants of the line 3629 were used in crosses as females, only green plants were observed in F_1. In reciprocal crosses, segregation of *chlorina* and green plants was observed in F_1. The proportion of *chlorina* in progeny was 24-28%. The obtained data demonstrate that these two *chlorina* mutations are of complex nature due to nuclear-cytoplasmic interactions. The genetic analysis of induced mutations in sunflower revealed that MNH induces variegated sunflower forms of the plastome nature. The mutagen in combination with DNP induces *chlorina*-type mutations. Beletskii succeeded in isolating *chlorina* mutations of extra nuclear origin in sunflower using MNH at concentrations of 0,01% and 0,015%. Nuclear *chlorina* mutants were generated only at the mutagen concentration above 0,02% [3]. In the presented experiment, the pretreatment with DNP for 6 h (variant 6) induced nuclear *chlorina* mutations at MNH concentration of 0,015%.

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IN VITRO MUTAGENESIS AND PRODUCTION OF AGRONOMICALLY USEFUL POTATO VARIANTS

In vitro grown shoot cultures of two Indian potato varieties 'Kufri jyoti' and 'Kufri Chandramukhi' were subjected to gamma irradiation at 20 and 40 Gy. The irradiated shoot cultures were subcultured to yield a generation of plantlets. After 4-6 weeks of incubation, these shoots were transferred onto MS medium supplemented with benzylaminopurine, BAP (10mg/1) and sucrose (8% w/v) and incubated at 20^0C. The M_1V_3 plants were screened *in vitro* for late blight resistance by detached leaf method [4; 5]. The resistant plants were screened in M_1V_4 generation by artificial inoculation of sporangial inoculum on the pot sown plants. Chlorophyll persistence is a simple screening method for heat tolerance [3]. Chlorophyll persistence of different plantlets showed that the percentage of injury was less in the case of plants, which had been obtained from irradiated material. In the case of control plants, there was one hundred-percent damage to the plants. The mutation frequency was calculated for characters like late blight resistance and heat tolerance (*in vitro* microtuberisation and chlorophyll persistence). The gamma ray dose of 40 Gy was observed to produce a higher mutation frequency (Tab. 1).

Table 1. Radiation induced mutation frequency in potato microtuber progeny

Variety	Character	Treatment	Variation observed (%)
Kufri Chandramukhi	Late blight resistance	40 Gy	26.3
		20 Gy	14.5
Kufri Jyoti		40 Gy	17.6
		20 Gy	20.0
Kufri Chandramukhi	Heat tolerance	40 Gy	28.0
	(*in vitro*)	20 Gy	12.0
Kufri Jyoti		40 Gy	22.0
		20 Gy	8.0
Kufri Chankdramukhi	Heat tolerance	40 Gy	46.3
	(Chlorophyll resistance)	20 Gy	28.0
Kufri Jyoti		40 Gy	34.0
		20 Gy	30.6