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INDUCED MUTATIONS IN TRITICALE: FREQUENCY AND SPECTRUM OF CHLOROPHYLL MUTATIONS

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ABSTRACT

Mutations were induced in four hexaploid triticale varieties (Beagle, Coorong, TL 419 and Welsh) by gamma rays (10, 20, and 30 kR) and EMS (0.5%; 8, 12, 16 h). Ten different kinds of monogenic chlorophyll mutants, including two new types— redina and white-virescens— were observed in M_2 and M_3 generations. Mutation frequency increased with increase in mutagenic dose. The chemical EMS produced high frequency of mutations in comparison to gamma rays.

Key words: Hexaploid triticale, chlorophyll mutants, frequency and spectrum of mutations.

Chlorophyll mutations are one of the most dependable indices for evaluation of genetic effects of different mutagens and are used as genetic markers in basic and applied research. Different types of chlorophyll mutations have been produced in several crop plants by using both chemical and physical mutagens [1] Chlorophyll mutations occur with different frequencies and spectrum in different plant species [2–5]. Frequency of chlorophyll mutations has been observed to decrease with increasing ploidy level in plants [6, 7].

MATERIALS AND METHODS

Seeds of four hexaploid triticale varieties (Beagle, Coorong, TL 419 and Welsh) were irradiated with 10, 20 and 30 kR gamma rays at Jawaharlal Nehru University, New Delhi, and 0.5% aqueous solution of ethylmethane sulphonate (EMS) of Eastman Kodak chemicals for 8, 12 and 16 h, taking 100 seeds per treatment. Individual spikes of M_1 plants were harvested separately in all the treatments and sown as spike progenies to raise M_2 generation.

Chlorophyll mutations were recorded at seedling stage, following Gustafsson [8]. The characteristic features of the new redina and white-virescens mutation are presented in Table 1.

Mutation frequency was calculated as percentage of mutated M_1 plants, M_1 spikes and M_2 plants. For each mutant type, 1-3 mutated M_2 progenies were selected

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Table 1. Characteristic features of two chlorophyll mutants redina and white-virescens along with control in hexaploid triticale

Character	Control	Redina	White-virescens
Seedling colour	Dark green colour; viable; normal chlorophyll pigments present in leaves and stem	Pinkish colour; lethal; survive up to 15–20 days; chlorophyll pigments absent but anthocyanins present	Light green colour; viable; chlorophyll pigments present but gradually disappear in leaves as the plant grows and restricted to floral parts
Leaves/plant	5–13	1	3–9
Plant height (cm)	80–120	5	30–40
Tillers/plant	3–15	—	1
Spikelets/spike	13–33	—	3–7
Florets/spikelet	3–5	—	3–5
Days to maturity	58–65	—	> 70 days

irrespective of the variety and treatment and sown as single plant progenies in M_3 generation. Segregation in M_3 was recorded within as well as between the rows. The χ^2 test was applied to confirm the goodness of fit for segregation within and between rows.

RESULTS AND DISCUSSION

FREQUENCY OF MUTATIONS

Triticales have not been used for a systematic study of chlorophyll mutations. Therefore, we have induced chlorophyll mutations in four hexaploid triticale varieties. The frequency of chlorophyll mutations was estimated by three parameters (Table 2). Studies in barley and other crops [5] suggested that mutation frequency expressed as mutated plant or spike progenies gives an underestimate at higher dose, therefore, for efficient screening of individual spike progenies gives a more reliable index of mutation frequency. It is known that in triticale, a spike consists of a single sector [9], so that the question of reduction in the number of sectors in a spike at high doses does not arise. Further, a large number of seeds were obtained from a single spike, so that reduced fertility at higher doses will not influence the results. It can, therefore, be suggested that, with the exception of 16 h EMS treatment, all the three methods should give a reliable estimate of mutation frequency in triticale.

MUTATION SPECTRUM

Mutations maculata and chlorina were the most frequent, and white-virescens and striata least frequent. Striata were observed only in one variety, and white-virescens in three varieties. In all the four varieties taken together, different chlorophyll mutations were induced in the following order: maculata > chlorina > xanthoviridis >

redina > virescens > xantha > albina > alboviridis > white-virescens > striata (Table 2). However, within the variety, minor deviations from this sequence were observed, as some of the mutants were found only in gamma-ray while others only in EMS treatments.

It is generally believed that ionizing radiations produce high frequency of albina mutations and the chemical mutagens produce other types of chlorophyll mutations [10]. However, in the present experiment, the frequency of all chlorophyll mutations including albina was high in the EMS treatments of all the four varieties studied. Many studies have reported EMS to induce a large spectrum and high frequency of chlorophyll mutations in ragi [11], rice [12], wheat [13,14] and barley [5]. However, in the present experiment, the frequency of albina mutations in the EMS treated populations was higher in comparison to the gamma-ray treatments. This is contrary to the earlier observations, where radiations were shown to induce more albina mutations [2, 15]. This may be due to the fact that most of the earlier observations were recorded in stabilised varieties of diploid cereal crops. Even when the ploidy level is taken into consideration, the frequency of albina mutations is higher in diploid crops than in tetraploid or hexaploid ones. For instance, it has been shown earlier that there was high frequency of albina mutations in durum wheat (AABB) compared to breadwheat (AABBDD) [16]. However, triticale is an intergeneric hybrid between wheat and rye of relatively recent origin and has an unstable genomic constitution. Therefore, it is possible that the genes responsible for chlorophyll mutations, including albina, are more sensitive to EMS. Swaminathan et al. [1] and Sree Ramulu [17] suggested that differences in the mutation spectrum and rate in different genotypes may due to difference in the location of genes in relation to the centromere. In the present study, striata chlorophyll mutation was observed only in variety Welsh, while white-virescens mutation was absent in TL 419. These varietal differences in the frequency of chlorophyll mutations indicate that the number of genes controlling chlorophyll development may differ in different varieties. Such a conclusion gets support from the earlier reports that at least 250–300 loci for chlorophyll synthesis exist in barley [18]. Gustafsson [10] reported 125–150 loci for albina, 125 loci for viridis, and only 15–50 loci for other type of chlorophyll mutations.

Mutation frequency increased with increase in mutagenic dose irrespective of the method used (Table 2), with the only exception of 10 kR gamma rays in the variety Welsh. These results are in conformity with earlier studies in barley [5, 19], ragi [11], and rice [20]. High frequency of chlorophyll mutations in EMS treatment is perhaps due to preferential action of EMS on genes for chlorophyll development located near the centromere [17], or preferential effect of EMS on guanine in the GC rich chlorophyll genome [16].

In triticale, being a polyploid cereal (6x=42), low frequency of chlorophyll mutations is expected, as was reported in oats and barley [6, 21, 22], rice [23], and wheat [6, 7, 24]. Contrary to these reports, both spectrum and frequency of chlorophyll mutations were fairly high in all the four triticale varieties used in this study. Unfortunately, no reports are available in tetraploid, octoploid or decaploid triticales to compare the mutation frequencies in relation to their ploidy level. However,

Table 2. Frequency and spectrum of different chlorophyll mutants in various treatments of four hexaploid triticale varieties in M_2 generation

Variety and treatment	Mutation frequency (%)			Spectrum of chlorophyll mutations (%)									
	plant progenies	spike progenies	M_2 plants	albina	chlorina	redina	xantha	albo-viridis	xantho-viridis	maculata	striata	virescens	white virescens
Welsh:													
Gamma													
rays, kR	—	—	1.03	—	0.16	0.15	0.09	0.02	0.22	0.37	—	0.02	—
10	12.0	2.6	0.50	—	—	0.05	—	—	0.15	0.30	—	—	—
20	29.2	4.9	1.07	—	0.25	0.09	0.12	0.05	0.18	0.34	—	0.04	—
30	52.0	12.7	1.60	—	0.20	0.07	0.22	—	0.49	0.62	—	—	—
EMS, 0.5%	—	—	1.28	0.04	0.30	0.28	—	0.10	0.31	0.14	—	0.08	0.03
8 h	36.2	18.2	0.91	—	0.07	0.23	—	0.11	0.23	0.19	—	0.05	0.03
12 h	98.0	25.5	4.94	0.39	1.84	0.77	—	—	1.16	0.39	—	0.39	—
16 h	50.0	14.3	2.60	—	2.60	—	—	—	—	—	—	—	—
Coorong:													
Gamma													
rays, kR	—	—	0.49	0.02	0.09	0.01	0.02	—	0.12	0.14	—	0.09	—
10	26.8	3.5	0.54	0.02	0.07	0.02	—	—	0.14	0.22	—	0.07	—
20	13.5	2.7	0.55	0.04	0.14	—	—	—	0.13	0.09	—	0.15	—
30	1.8	0.6	0.10	—	—	—	0.10	—	—	—	—	—	—
EMS, 0.5%	—	—	1.95	0.11	0.57	0.19	0.07	0.09	0.36	0.42	—	0.07	0.07
8 h	31.8	7.7	1.44	0.02	0.28	0.07	0.05	—	0.47	0.50	—	—	0.05
12 h	56.9	15.4	2.73	0.25	1.04	0.38	0.11	0.23	0.19	0.23	—	0.19	0.11
16 h	50.0	33.3	4.55	—	—	—	—	—	—	4.55	—	—	—
TL 419:													
Gamma													
rays, kR	—	—	0.34	0.03	0.07	0.01	0.04	—	0.10	0.04	—	0.05	—
10	6.7	2.1	0.37	—	0.13	—	—	—	0.11	0.13	—	—	—
20	3.8	0.9	0.24	—	—	—	—	—	0.11	—	—	0.13	—
30	7.3	2.7	0.37	0.09	0.08	0.03	0.11	—	0.06	—	—	—	—
EMS, 0.5%	—	—	1.07	0.04	0.19	0.09	0.11	0.05	0.29	0.28	—	—	0.02
8 h	16.0	5.1	0.83	—	0.22	0.06	—	0.06	0.28	0.31	—	—	—
12 h	19.1	18.2	1.13	0.09	0.16	0.14	0.19	0.05	0.32	0.18	—	—	0.05
16 h	50.0	33.3	4.55	—	—	—	—	—	—	4.55	—	—	—
Welsh:													
Gamma													
rays, kR	—	—	0.60	—	0.25	0.04	0.11	—	0.08	0.12	—	—	—
10	10.3	2.3	0.76	—	0.27	0.05	0.24	—	—	0.20	—	—	—
20	6.7	1.9	0.36	—	0.24	0.05	—	—	—	0.07	—	—	—
30	9.1	3.7	0.71	—	0.24	—	—	—	0.47	—	—	—	—
EMS, 0.5%	—	—	1.32	0.04	0.60	0.04	0.03	0.06	0.20	0.05	0.03	0.18	0.09
8 h	34.4	8.8	1.62	0.06	0.38	0.06	0.05	0.25	0.26	0.08	0.05	0.03	—
12 h	25.0	12.8	2.08	—	1.04	—	—	—	0.08	—	—	0.63	0.33
16 h	50.0	10.0	2.72	—	2.72	—	—	—	—	—	—	—	—
All													
varieties	—	—	1.12	0.03	0.23	0.09	0.06	0.03	0.28	0.33	0.001	0.05	0.02
Gamma													
rays	—	—	0.62	0.01	0.13	0.06	0.06	0.01	0.13	0.18	—	0.04	—
EMS	—	—	2.08	0.06	0.42	0.16	0.06	0.08	0.56	0.61	0.01	0.08	0.05

Froier [25] observed higher frequency of chlorophyll mutations in hexaploid *Avena* than at diploid level. Natarajan et al. [24] also reported similar results in tetraploid and hexaploid wheats. Similarly, Gaul [5] observed a high frequency of some chlorophyll mutations in tetraploid barley which are completely absent in diploid barley. The wide spectrum and high frequency of chlorophyll mutations in triticale, which are considerably lower in either of the parents, i.e. wheat and rye, can be explained as follows: first, hexaploid triticale (AABBRR) is the first synthetic intergeneric hybrid between wheat (AABBDD) and rye (RR), and it is possible that the genes responsible for chlorophyll mutations are present in either of the two triticale genomes, but for the expression of these genes the other parental genome, is necessary; second, if the same type of chlorophyll mutation is controlled by the genes of wheat and rye genomes separately, the frequency and spectrum obviously will be high in triticale. Only monosomic analysis can clarify the position of genes located on the chromosomes of wheat or rye, or both.

It is evident from the data in Table 2 that the total frequency of chlorophyll mutations was 11.62% in variety Beagle, 9.9% in Coorong, 7.5% in TL 419, and 8.25% in Welsh. The different mutation frequencies in different genotypes in the present study may also be attributed to differences in moisture content of seeds. The moisture content of seeds increased in the order of Beagle, Coorong, Welsh, TL 419; while mutation frequency decreased in the same order, suggesting an inverse relationship between moisture content and chlorophyll mutation induction as observed earlier by Conger et al. [26] in barley.

The segregation ratios in M_2 and M_3 confirmed that all the ten chlorophyll mutations observed in the present study are recessive. No deviation from 3:1 segregation ratio ($P=0.05$–0.80) in M_2 and M_3 generations within the segregating line, and from 1 : 2 : 1 ($P=0.50$–1.00) in M_3 among the segregating lines suggests that all the chlorophyll mutants were inherited monogenically without any deficit. Most of the earlier investigations reported that there was a significant deficit of recessive homozygotes either due to elimination of male or female gametes [27] or at zygotic and embryonic levels [28]. This indicates that triticale, due to its polyploid nature, can tolerate chromosomal rearrangements to a greater extent.

The frequency of albina mutations was relatively high in the present study relative to that in wheat, barley and rice. The redina mutant (chlorophyll pigment completely absent) has not been reported in wheat and rye. Therefore, one may conclude that the expression of redina mutant gene may depend on the presence of the two genomes (wheat and rye). The isolation of 'red stem seedling' a morphological viable mutant in M_3, and its subsequent confirmation in M_4[9] (characterized by pinkish culm) further supports the existence of redina mutants, thereby suggesting that there are genes for synthesis of anthocyanin pigment in triticale. Red stem seedling mutant was earlier reported in *Vicia* by Sjodin [29], where the mutant seedlings are distinctly red pigmented — a pigmentation which is reportedly due to the presence of anthocyanins [30]. Goyal [31] observed that in *Vicia*, anthocyanin pigmented flower and stem were recessive characters. Similarly, induced mutation for anthocyanin pigment in barley was also reported [32, 33]. Okonkwo and Clayberg [34] also reported a mutant in *Phaseolus*, where anthocyanin

pigment, present in both flowers and pods, was found to be controlled by a single gene. Similarly in another recent study, Kulakow et al. [35] observed that red pigment in seedlings of several *Amaranthus* species was controlled by a single gene with recessive inheritance. Monogenically controlled anthocyanin pigment was also reported in rice [36, 37]. Therefore, the existence of 'redina' mutant and its segregation pattern are in agreement with the above studies.

The second chlorophyll mutant, white-virescens, is also monogenic recessive where the mutant plants are characterized by light green leaves at earlier stages (seedling with 3-6 leaves). Green colour gradually disappears at maturity and chlorophyll pigment is restricted to spikelets and completely absent in foliar parts including culm at maturity. Anatomical studies reveal that there are very few tiny plastids in the palisade tissue above the midrib and lateral veins and these pigments also disappear at maturity in most of the plants. In barley, Gaul [5] observed that lack of chlorophyll pigment in mutant seedlings at earlier stages is due to low temperature, they develop chlorophyll pigment as the temperature increases. However, in the present study, these plants did not develop chlorophyll as the plant matured. Therefore, these white-virescens mutants and their segregation in M_2 and M_3 and the breeding behaviour in M_3 suggest that it is a gene mutant, and the presence of light green leaves at seedling stage (3–6 leaves) and gradual reduction of chlorophyll towards maturity is due to secondary effects of more rapid degradation of chlorophyll at later stages of development.

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