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RECOVERY OF DESIRABLE MUTATIONS IN WHEAT

C. S. KALIA, M. C. KHARKWAL AND M. P. SINGH

Division of Genetics,
Indian Agricultural Research Institute, New Delhi 110 012

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ABSTRACT

Mutagens of environmental significance have gained special attention on account of their environmental pollution and possible genetic hazards. Three groups of structurally different environmental chemical mutagens viz., Aziridines (Metepa and Thio-Tepa), Nitroso-compounds (MNG and NMU) and esters (MMS and EMS) were used to study their comparative mutagenic efficiency and also to induce mutations for specific traits of economic importance e.g., protein content and rust resistance in two varieties, one belonging to tetraploid (var. HD 4502) and another to hexaploid (var. NP 880) wheat. With effective mutagenesis, it was possible to induce mutations and with rigorous screening in M_2 and M_3 generations, isolate mutant plants with higher grain yield potential, protein content, desirable quality and better rust resistance in both the varieties. Rectification of mottling effect of kernels of var. HD 4502 was achieved with EMS treatment, which were found to be most efficacious. The experimental evidences revealed that environmental chemical agents are potent mutagenic agents.

Key words: Wheat, mutation, environmental mutagens, effectiveness, protein content, rust resistance

With the discovery of ionising radiations and mutagenic chemicals, there was considerable optimism for practical utilization of mutagenesis particularly for specific defects. Subsequently, the results obtained were not comparable with the expectations, thus sceptism was expressed about the relevance of mutagenic techniques [1]. Induced mutations for specific characters such as disease resistance and quality aspects in crop plants occur generally at low frequency. Genes for disease and pest resistance available in the germplasm are mostly associated with several undesirable linkages, hence their utilization has certain physical limitations. In order to enlarge gene pool for disease resistance in high yielding varieties, induced mutagenesis has been advocated as an important methodology [2]. The role of induced mutations in crop improvements is evident from a large number of improved high yielding varieties of several crops developed through mutation breeding and released for commercial cultivation in India [3].

In the present investigation, efforts were made to ascertain the mutagenic efficiency and relative response of different chemical mutagens of environmental significance with the scope of crop improvement. Aziridiens, Thio-Tepa and Metepa are known as chemosterilants and are used as mordant in the textile industry.. Nitroso compounds N- methyle-N-nitro- N-nitroso guanidine and N-methyl- N-nitroso urethane (MNG and NMU) are considered as carcinogens and anti-leukemic agents. Esters methyl methane sulphonate and ethyl methane sulphonate (MMS and EMS) have also been listed in the environmental mutagens, being used as chemosterilant in housefly, leukemogenic agents and in cancer chemotherapy.

MATERIALS AND METHODS

Three groups of chemical mutagens viz. Aziridines (Metepa 0.4 and 0.2%, Thio-Tepa 0.2 and 0.1%), nitroso-compounds namely MNG (0.1 and 0.05%), NMU (0.1 and 0.05%); esters viz. MMS (0.1 and 0.05%), and EMS (0.1 and 0.05%) were used. Two hundred seeds of uniform size of each of the tetraploid var. HD 4502 and hexaploid var. NP 880 of wheat were treated with aquous mutagenic solutions with pH 5.2 either in direct single treatment of 2 h or in sequence of one h each at a room temperature of 20$\pm$1 °C. Dry seeds were used as controls. Treated seeds were washed immediately with running tap water and dried under shade. Treated and control seeds were sown same day at a spacing of 5 cm in single rows of 5 m long and 30 cm apart.

Ears of M_1 plants were bagged and harvested individually to raise M_2 plant progenies. M_2 seeds were obtained from 25 normal looking plants at random in each treatment along with control in completely randomized design. About fifty thousand M_2 plants of tetraploid var. HD 4502 and hexaploid wheat var. NP 880 were separately screened to isolate visible mutations pertaining to alterations in height, maturity, single plant yield, 100 kernel weight and adult plant resistance. Subsequently, fifty progenies emerging from different treatments were selected on the basis of their performance. Emphasis for selecting promising progenies was associated with selection of desirable traits of uniform maturity, compact longer spike, erect leaves and reduced mottling in kernels. Further screening and selection in M_4 ultimately culminated in the recovery of eighteen promising M_5 mutant lines, which were further studied for their yield potentials, protein percentage, quality characters and rust resistance.

For estimation of seedling rust reaction, seeds from M_4 generation were used to raise the seedlings in small pots containing 5-6 seeds each in three replications. The inoculation was done at one leaf stage on the abaxial side in the glass house. These were later transferred to humid chamber maintained at 90-100% humidity. Inoculum mixture of eighteen stem rust races collected from Division of Mycology and Plant Pathology, I.A.R.I., New Delhi was used for seedling infection. After 48 h of inoculation the pots were transferred to glass house benches with sufficient diffused light available throughout the day with optimum temparature and humidity required for rust development. Two weeks after inoculation, observations on rust reaction were recorded on three replications and infection types classified as described by Stakman and Levine [4]. Adult plant test for rust reaction in selected mutant lines from M_3 onward, was undertaken during summer season at IARI Regional Station, Wellington (Nilgiri hills), a hot spot for rust studies. The plant reactions for stem, leaf and stripe rusts were seaprately recorded as per the modified Cobb's scale [5].

The protein percentages of the mutant lines selected from M_4 along with their control lines were measured with near infrared analyser (NIR) model 102 (Neotec) in M_5 generation of selected mutant lines grown in R.B.D. with two replications at I.A.R.I., New Delhi.

RESULTS AND DISCUSSION

Yield and protein content : Mutant selection 246 showed the highest yield of 40.3 q/ha (Table 1) which was significantly higher than the control var. HD 4502 (26.9 q/ha). Other mutant lines also exhibited better yield performances in comparison to the control. For 100 kernel weight, substantial increase was observed in mutant lines 314, 276 and 104-122. Protein content was not significantly affected in mutant lines of tetraploid wheat but moderate increase in mutant selection 104-122, 246 and 268 was observed. These mutants also showed significant increase in yield over the control var. HD 4502. It is noteworthy that with the increase in yield, there was no corresponding decrease in kernel weight. In fact there was significant increase in mutant lines 104-122 & 276. It is thus evident that some of the negative linkages have been broken and new character associations have been established in some mutant lines. Protein content was not significantly affected in mutant progencies of *durum* wheat var. HD 4502.

Mutation breeding has also been used for the improvement of grain quality. Higher percentages of protein without affecting grain yield have been achieved in some mutant lines of Lal Bahadur [6]. Induced variability for protein content in bread wheat was also reported by Singh et al. [7], where the protein range for mutant lines was 10.2 to 13.9 percent as compared to 11.1 percent in control. Some mutant lines with higher protein content and reduced grain yield were reported by Desai [8]. In the present study, some of the mutant lines of tetraploid var. HD 4502 having higher protein percentage also gave higher grain yield over the control (Table 1).

Yield and seed weight are negatively correlated, however, mutant lines 276, 314 and 104-122 showed not only substantial increase in yield but also gave higher kernel weight. It is interesting to note that in mutant line 104-122 an increase in grain yield (control 26.9 q/ha, mutant 32.3 q/ha) and higher kernel weight (control 4.13 g, mutant 5.25 g per 100 kernels) are coupled with higher protein percentage (Table 1).

Table 1. Comparative yield, 100 seed weight, protein percentage and rust reaction of control and selected mutant lines of wheat var. HD 4502 and NP 880

Entry	Treatment	Yield (q/h)	100 kernel wt. (g)	Protein (%)	Seedling reaction*	Rust reaction** bl@	br	y
		Tetraploid wheat var. HD 4502						
HD 4502	Control	26.91	4.13	13.3	R0-1	5s	80s	-
104-122	NMU 0.1%	32.31	5.25	14.1	R0-1	Ts	5s	-
214	EMS 0.05%	34.98	4.08	13.1	R0-1	5s	5s	5s
314	EMS 0.05%	33.39	4.67	12.8	-	-	-	-
246	MMS 0.1%	40.34	2.78	15.4	R2	Ts	5s	-
268	EMS 0.05%-MMS 0.05%	35.0	2.46	13.7	-	Ts	Ts	10s
276	EMS 0.05%-MMS 0.05%	37.7	5.65	12.5	-	Ts	10s	10s
286	MMS 0.05%-EMS 0.05%	29.6	4.15	12.0	R2	20s	5s	-
291	MMS 0.05%-EMS 0.05%	29.6	4.48	13.5	mixed	5s	5s	60s
		Hexaploid wheat var. NP 880						
NP 880	Control	28.15	5.52	14.3	R2	5s	40s	5s
13	Metepa 0.4	26.61	4.47	11.7	-	10s	20s	5s
15	Thio-Tepa 0.1%	37.11	5.16	12.1	-	20s	5s	Ts
7	MNG 0.05%	36.20	4.23	10.9	-	60s	5s	Ts
5-1	EMS 0.1%	26.87	3.68	13.9	-	5s	20s	-
3-11	EMS 0.1%	29.43	4.90	11.6	R	5s	5s	Ts
3-15	EMS 0.1%	34.6	4.04	10.9	R2	10s	5s	-
3-16	EMS 0.1%	35.90	4.80	12.3	R	5s	20s	10s
3-17	EMS 0.1%	27.86	4.07	11.0	R	20s	5s	-
CD at P=0.05		5.12	1.30	2.0				

*R-Resistant; S-Susceptible; **Ts-Trace severity; 5s, 10s, 20s, 60s and 80s severity on 1-100 scale; @bl = black rust; br = brown rust; y = yellow rust

In case of hexaploid var. NP 880 there was decrease in protein percentage in dwarf mutant lines viz. selection 3-15 and 3-16 from EMS treatments, but there was significant improvement for yield (Table 1). In protein percentage the selection 5-1 from EMS treatment showed improvement which could be due to a compensation in 100 kernel weight. Another set of promising mutant selections of hexaploid var.

NP 880, which showed better yield potentials along with reduction in plant height and variable maturity were subjected to requisite *chapati* making quality tests. The Pelshenke values and sedimentation values are of practical importance as there is a lot of variability for sedimentation value among the mutant selections in comparision to control (Table 2). It is evident from the *chapati* making score, that the mutant lines have maintained the excellent *chapati* making properties of the parental lines and the selection 15-2 has revealed the best quality.

Table 2. Quality characters of selected mutant strains of hexaploid wheat var. NP 880

Entry	Treatment	Pelshenke value (min.)	Sedimentation value (ml.)	Chapati making score
Control	-	11.1	30.0	8 very good
15-2	Thio-Tepa 0.1%	12.0	11.5	9 very good
3-10	EMS 01%	11.6	42.0	8 very good
3-11	EMS 01%	11.6	49.0	8 very good
3-12	EMS 01%	11.7	53.0	8 very good
3-14	EMS 01%	11.1	48.0	8 very good
3-15	EMS 01%	11.4	10.5	8 very good
3-16	EMS 01%	12.5	37.0	8 very good
3-17	EMS 01%	11.8	48.0	8 very good

The scope for simultaneous improvement in yield and protein through effective selection in mutation breeding is more rewarding in tetraploid than in hexaploid wheats. It could probably be due to the complex genetic architecture and compensating mechanism by triplicate loci in hexaploid wheat. Most of the desiarable mutants have been recovered with EMS or with the combination of EMS treatments in tetraploid wheats, whereas in hexaploid wheats Metepa and Thio-Tepa treatments have also induced some desirable mutants. Thus, the response of tetraploid and hexaploid wheats to chemical tretments is variable in terms of recovery of desirable mutants pertaining to disease resistance, yield and quality parameters. The inference can thus be drawn that there is ample scope for the induction, alteration and rectification in some negatively associated characters and that the improvement of quality aspects in conjunction with higher yield through appropriate mutagenic treatments in different wheat genotypes is possible.

Reaction to stem and leaf rusts : The selected mutant lines of both var. HD 4562 and var. NP 880 which were tested in three replications against mixture of stem rust races in seedling stage in the glass house under controlled temperature and humidity conditions showed variable range of resistance (Table 1). In tetraploid wheat var. HD 4502 (control) the rsistance observed was 0-1 type on pustule basis whereas mutant 104-122 exhibited O type reaction indicating better resistance for stem rust and was completely free of any infection. The material when tested for adult plant resistance (field resistance) at Wellington showed Ts infection of stem rust and 5s for leaf rust confirming its better resistance for stem and leaf rusts in comparison to control var. HD 4502, (80 S). Similarly tetraploid var. HD 4502 mutant line 246 which showed resistance to stem rust at seedling stage was highest yielder with high protein percentage. In adult-plant stage it showed only trace infection of stem rust and complete resistance to leaf rust. However, line 268 had maintained resistance to leaf rust whereas parental check showed suceptibility reaction to leaf rust. Mutant lines conferring resistance to stem rust in seedling stage and adult plant stage were isolated after NMU treatments in bread wheat variety Kharchia local and Lal Bahadur [6].

Hexaploid wheat var. NP 880 (control) showed moderate resistance for stem rust (0-2 type rust reaction), however, mutant line 3-25(3) and 3-26(1) showed immune reaction, indicating better rust resistance (Table 1). Interestingly all the mutant lines which exhibited better (O type) resistance in seedling stage originated from EMS treatments. This type of specificity of EMS treatments for manifestation of mutants with better resistance could be of great practical importance.

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