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Plant height reduction in populations of triticale (*X triticosecale* Wittmack) by induced mutations and artificial crosses

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ABSTRACT

Induced mutations by gamma radiation (0, 5, 10, 20 and 40 kR doses) and reciprocal crosses were tested as mechanisms of enhancing genetic variability for plant height in two triticale cultivars, BR4 and EMBRAPA18. The reciprocal crosses and all doses of radiation showed similar increase in genetic amplitude for this trait, being suitable for increasing variability in breeding programs. Genotypes showed different responses as the gamma ray doses were increased, expressing shorter plant height. The decision of using induced mutations or artificial crosses depends on the resources available and the selection method to be used.

INTRODUCTION

Triticale is an intergeneric cross between wheat and rye, joining in the same genetic constitution wheat high quality and yield and rye resistance (Baier, 1990). Utilization of induced mutations in this species may have application in breeding programs; mutations are useful when the objective is modification of some agronomic characters in cultivars. It makes possible new genetic combinations through allelic alterations or chromosomal changes. Artificial crosses permit recombination of parents that have different alleles to the characters of interest, creating a wide range of recombinants. These techniques have presented positive results in the improvement of genetic variability, enlarging genotypic classes and facilitating the practices of selection. These techniques permit one to obtain genotypes with short height that minimize losses due to lodging and make possible the use of higher levels of nitrogen fertilization.

Occurrence of spontaneous mutations in nature is relatively rare and difficult to identify because they are mainly recessives and deleterious. Controlling mutation rate and using techniques that increase the number of mutant individuals may be an important aspect in breeding programs.

Mutagenic agents are not employed only in obtention of new cultivars, but also to create new genetic combinations that can be used in artificial hybridization programs (Micke and Donini, 1993). Hybridization has made possible the development of an intense variability to most of the qualitative and quantitative characters, with direct and indirect implications in grain yield. Larger genetic diversity also may be obtained by hybridizing superior lines of wheat and rye to bring better genetic combinations in triticale that can be used in the breeding programs (Carvalho *et al.*, 1977). However, artificial crosses in triticale have contributed less to the increase of genetic variability when compared to other species, because triticale is a new species with narrow genetic basis.

The use of high radiation doses caused lethal effects in triticale seeds and low doses had no mutagenic effects in most research, justifying the use of intermediate doses (Wolski and Pojmaj, 1991). Increment of genetic variability in triticale with decrease in mean plant height in M_2 generation was obtained utilizing 25

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kR (Rajput, 1982). High fertility mutants with superior grain quality were also obtained by gamma radiation (Shakoor *et al.*, 1980). Low height mutants were isolated in triticale populations in M_3 and M_4 generations after treatement with gamma rays at 10, 20 and 40 kR (Reddy, 1988).

Induced mutations and artificial crosses have been described separately in the literature to promote genetic variability in different cultivated species. The objective of the present study was to evaluate the effect of gamma rays to induce mutations in two triticale genotypes, using different radiation doses, as well as to determine usefulness of hybridization to promote recombination between the same parents, and characterize the efficiency of these methods to improve genetic variability to plant height.

MATERIAL AND METHODS

Seeds at 13% of moisture of the BR4 and EMBRAPA18 genotypes were irradiated at the Departamento de Engenharia Nuclear of Universidade Federal do Rio Grande do Sul with a 0.25 Gy/min (25 R/min) rate using Cobalt-60. Doses of 5, 10, 20 and 40 kR were used to treat 1000 seeds of each cultivar. Reciprocal crosses were made between the two parental genotypes.

Randomized blocks with two replications were used. Individual plants were the experimental unit. M_1 seeds were sown at field in the winter of 1994 after receiving the application of the mutagenic agent. Plots of 20 rows 3 m in length, spaced 0.2 m from each other, and 0.1 m between plants were used for each treatment. Only the main tiller was collected from the M_1 plants. The M_2 generation was composed of 100 spikes randomly selected from M_1 plants of each treatment, making 100 families. Seeds of M_3 generation were obtained in the summer of 1994/1995 from seeds of the M_2 generation. The remaining M_2 seeds (not used in the summer of 1994/1995) were sown in the winter of 1995. Individual evaluation was made for M_2 and M_3 plants grown in the winter of 1995.

The evaluation method for populations obtained from artificial crosses was similar to the one for the irradiation treatments but in this treatment only the F_2 generation was evaluated. Generations M_2 and M_3 of the mutagenic treatments and F_2 of the artificial crosses were evaluated in the winter of 1995. Plant height was measured from soil surface to the top of main tiller, excluding the awns, during maturity.

The distribution of frequency, means (X) and variances (s^2) were obtained for all the treatments and evaluated generations. All treatments were compared with the control in both generations. Also, the M_2 treatments were compared to F_2 of the artificial crosses. Means were compared by *t*-test, and variances by F-test, as described by Steel and Torrie (1980). To verify the amplitude difference between mutagenic treatments statistical regressions were used. The SAS software was used for statistical analysis (SAS Institute, 1988).

RESULTS

Frequency distribution data for each mutagenic treatment (Figures 1 and 2) and artificial crosses (Figure 3) revealed an increase in the number of classes and range of variation of plant height compared to the control. In M_2 generation of BR4 genotype a range from 70 to 120 cm was detected (Figure 1A). In M_3 generation

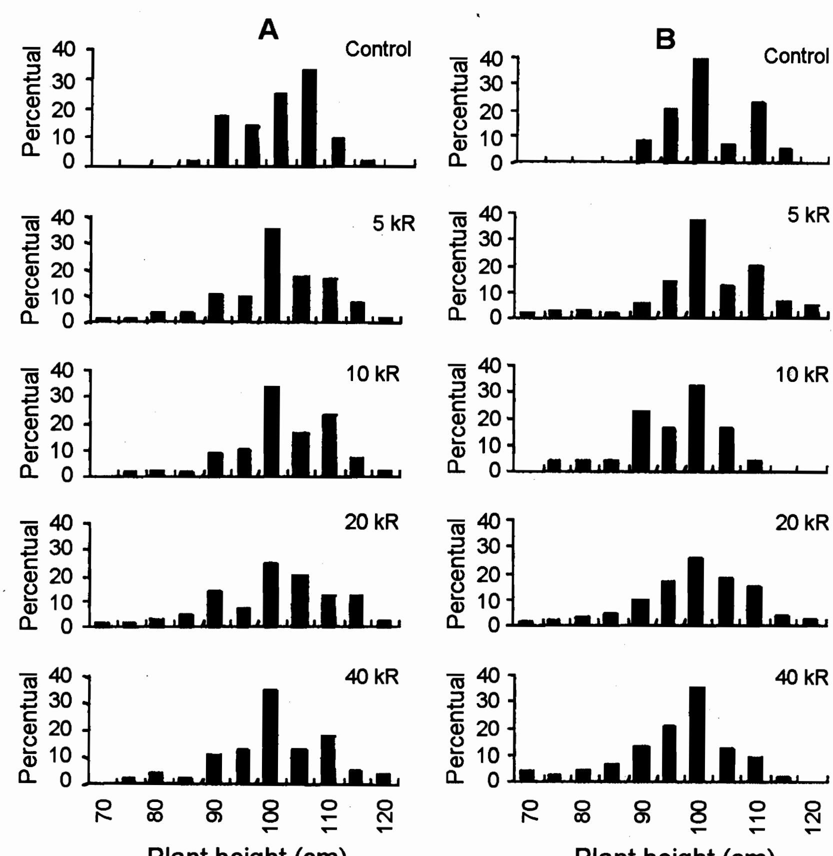

Figure 1 - Frequency distribution of plant height (cm) for BR4 genotype, generations M_2 (A) and M_3 (B), treated with different gamma ray doses from radioisotope Cobalt-60.

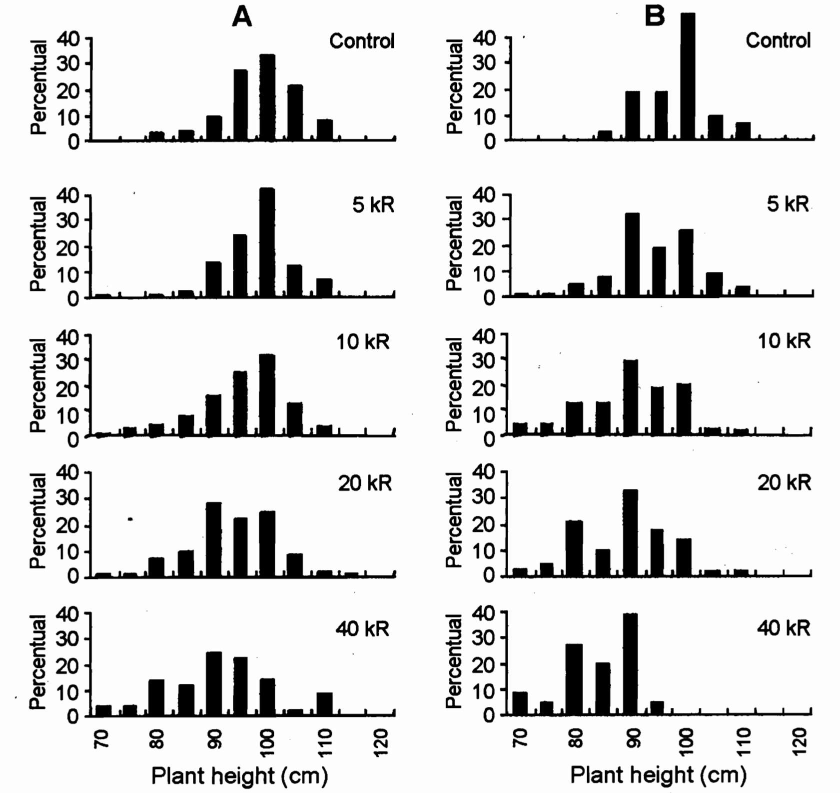

Figure 2 - Frequency distribution of plant height (cm) for EMBRAPA18 genotype, generations M_2 (A) and M_3 (B), treated with different gamma ray doses from radioisotope Cobalt-60.

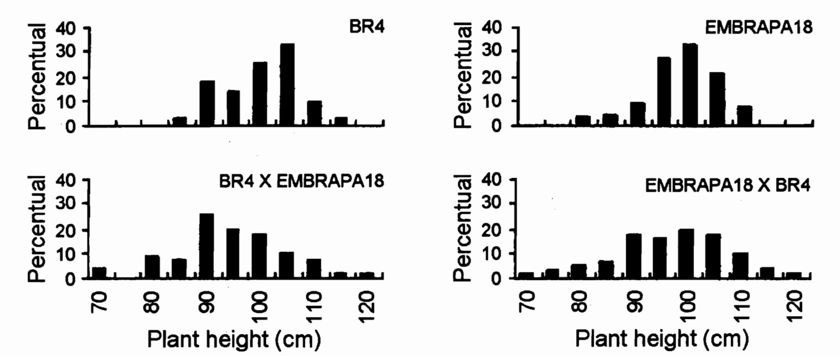

Figure 3 - Frequency distribution of plant height (cm) for generation F_2 from reciprocal artificial crosses between genotypes BR4 and EMBRAPA18.

amplitude and number of classes were similar to M_2 (Figure 1B). Evaluated plants of the genotype EMBRAPA18 presented smaller height amplitude, varying from 70 to 110 cm, and a smaller number of classes (Figure 2A). Drastic effects on stature distribution were observed in the 40 kR treatment when compared with the control, determining the largest changes, mainly in M_3 generation (Figure 2B).

In artificial crosses plant height frequency was more uniformly distributed among the different classes, compared with the irradiation treatments. Range of variation and class number showed similar behavior to the irradiated populations (Figure 3).

For generation M_2 of BR4, there were no significative differences between means of mutagenic treatments and that of the control (Table I). Significative differences occurred in the mean plant height for the treatments 10 and 40 kR. The largest alteration in plant height was observed in the 10 kR-treated population, which had the number of individuals reduced (Table II). More drastic effects were found for the genotype EMBRAPA18, showing a decrease in plant height with the increase of radiation doses in both generations (Tables I and II).

Significative differences were found between means of the mutagenic-treated BR4 populations and the cross BR4 x EMBRAPA18 that has presented greater reduction in plant height. However, the reciprocal cross EMBRAPA18 x BR4, when compared with the irradiated populations, had less reduction in plant height than 20 and 40 kR (Table I).

Mutagenic treatments induced an increase in variances of BR4 in both the generations studied. These variances were significantly higher than the control, except 10 kR in M_2 and M_3 (Tables I and II). Significative increases of the variance were similar for the different doses.

Genotype EMBRAPA18 showed a different performance from the BR4 genotype. Although most of the treatments were effective in changing the variances, in most cases these changes were smaller. In M_2 generation, it was observed that the variance increased from the small to the high doses used. The variance did not show any difference from control only for the smallest doses. In M_3 generation the 40 kR treatment showed the smallest variance of the mutagenic treatments, and was not statistically different from the control (Table II).

Artificial crosses were also efficient in promoting changes in variances in relation to control for both genotypes. The BR4 x EMBRAPA18 cross showed greater variance in relation to the 5 and 10 kR treatments. Higher doses promoted similar increases in variances, and the reciprocal cross showed similar results (Table I).

Table I - Means ($\overline{X}$) and variances (s^2) of plant height (cm) for generation F_2 from reciprocal artificial crosses and generation M_2 from gamma ray treatments (Co^{60}) of triticale genotypes BR4 and EMBRAPA18, Eldorado do Sul (RS), 1995.

Treatment	BR4			EMBRAPA18		
	N	$\overline{X}$	s^2	N	$\overline{X}$	s^2
Control	53	100.15	45.63	84	98.64	41.26
BR4xEMB18	128	94.55*	96.64*	-	-	-
EMB18xBR4	-	-	-	144	96.85	105.02*
5 kR	252	101.17**	69.78* **	278	98.18	35.31**
10 kR	243	102.62**	59.27**	216	95.62*	58.83* **
20 kR	199	100.62**	105.51*	235	93.79* **	54.72* **
40 kR	200	100.66**	76.38*	62	91.34* **	88.65*

*Significance at 5% according to *t*-test for means and F-test for variances in relation to control.
**Significance at 5% according to *t*-test for means and F-test for variances in relation to hybridization.
N = Number of plants evaluated.

The regression analyses between height and radiation doses for the BR4 and EMBRAPA18 genotypes, in both M_2 and M_3 generations, are shown in Figure 4. Most of the analyses followed linear regression, although the determination coefficients were small.

DISCUSSION

The use of means and variances has been appointed as a potential technique for detecting the occurrence of variation when using mutagenic treatments (Scossiroli, 1977). Frequency distributions have also been used to characterize the presence of genotypic variability and to show the variation occurred when using mutagenical products, as to evaluate the performance in relation to the control genotype (Borojevic and Borojevic, 1972).

Gamma rays and artificial crosses used for the BR4 and EMBRAPA18 triticale genotypes showed the possibility of identifying the changes that occurred in plant height. This was shown by the frequency distributions and by the changes in means and variances of treated populations. Our results in this work showed that both methods contributed significantly to obtain increase in variability of plant height. Similar results were obtained when using gamma rays in oats (Nascimento Junior *et al.*, 1990) and beans (Tulmann Neto and Sabino, 1994). The genetic changes caused by recombination in artificial crosses and also by induced mutations of gamma rays generally resulted in reduction of plant height. The effectiveness of the mutagenic agent in modifying the distribution of frequency for plant height compared to the control was verified for the different doses of radiation. These results corroborate the capacity of the gamma rays to induce mutations and make changes in genotypic frequencies that lead to changes in the performance of the character. Similar results were obtained for plant height in triticale, with increase in variance and decrease in means compared to the control in M_3 generation (Sinha and Joshi, 1986). Changes of small effect which were verified by alterations in variance occurred for the BR4 genotype. The mutagenic-treated populations of EMBRAPA18 genotype showed alterations in means, suggesting that more genes or genes of

Table II - Means ($\overline{X}$) and variances (s^2) of plant height (cm) for generation M_3 from gamma ray treatments (Co^{60}) of triticale genotypes BR4 and EMBRAPA18, Eldorado do Sul (RS), 1995.

Treatment	BR4			EMBRAPA18		
	N	$\overline{X}$	s^2	N	$\overline{X}$	s^2
Control	62	101.43	45.76	69	97.86	32.65
5 kR	190	102.08	77.45*	215	94.25*	51.83*
10 kR	31	96.13*	63.05	100	90.02*	71.94*
20 kR	142	99.79	86.20*	99	88.96*	62.00*
40 kR	161	96.77*	71.76*	26	84.50*	40.74

*Significance at 5% according to *t*-test for means and F-test for variances in relation to control.

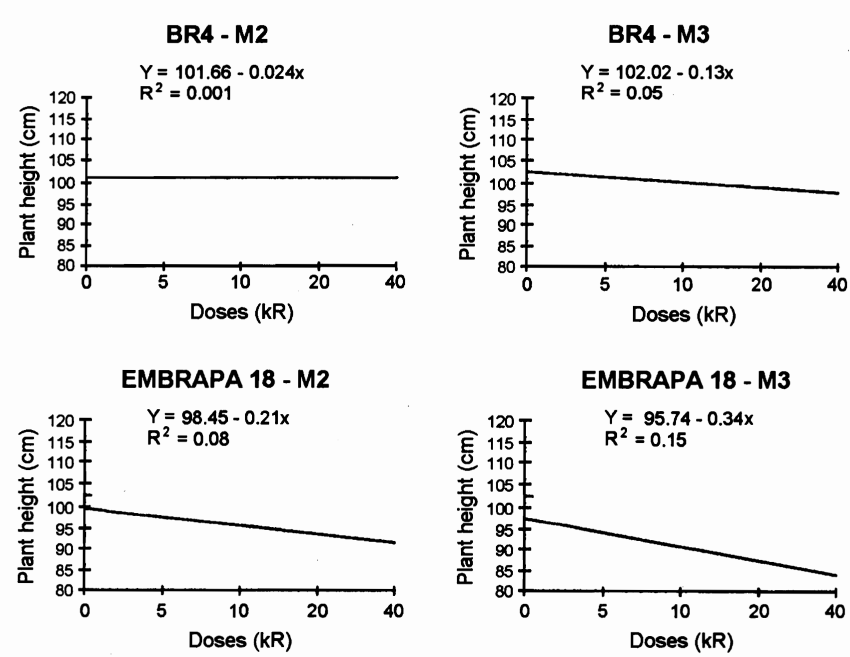

Figure 4 - Adjusted regressions between plant height and radiation doses for BR4 and EMBRAPA18 genotypes treated with different gamma ray doses.

higher expression for the character were affected. This can also be better seen in M_3 generation where there was a decrease of 13.36 cm (13.5%) in the highest doses in relation to control. All the doses of radiation were efficient in promoting increase in variance compared to control, which can be confirmed by the amplitudes shown in frequency distributions. This suggests that the number of affected genes for the character may be fixed and that all doses were efficient in affecting these genes to greater or lesser extent, resulting in similar variances.

The linear reduction of means with increasing doses was evidenced in M_2 generation for the EMBRAPA18 genotype and confirmed in generation M_3. Such linear reduction was shown only in generation M_3 for the BR4 genotype. The linear performance was significative for most of cases. This indicates that increasing the doses contributed to the presence of mutants with more alleles for height reduction. Furthermore, a larger number of genotypes that represent these classes were produced.

The results showed that it is possible to use any tested doses. Although more lethal effects were seen in 40 kR treatment, it was more effective in reducing plant height for both studied genotypes. Small changes in plant height may contribute more effectively for selection practices, once the morphological hazards and deleterious effects would be smaller (Gaul *et al.*, 1972). Therefore, low effect modifications would be desired by plant breeders.

The two genotypes had different behaviors. The BR4 genotype showed the largest amplitude and the EMBRAPA18 genotype showed the largest effects in reduction of plant height. The high radiation sensitivity that occurred for the EMBRAPA18 genotype is demonstrated by the larger effects produced on means. The differences in genetic constitution promote differences between genotypes (Abrams and Frey, 1964; Gregory, 1967). In addition, internal mechanisms that repair the DNA structure may have influenced the frequency of mutations (Swaminathan, 1977).

The variation amplitudes that are shown in the frequency distributions were maintained from M_2 to M_3 generation. The largest differences in plant height compared to the control occurred in generation M_3 as is verified by the larger effects in means for both genotypes. This occurred probably because of segregation that has changed genotypic frequencies. This was more expressive for the 40 kR doses that promoted larger effects in reduction of means in generation M_3.

Because of the possibility of manifestation of recessive alleles and the eradication of large effect deleterious mutations in M_2 generation, the identification of mutations is better accomplished in generation M_3 (Sigurbjörnsson, 1983). The same is described by Swaminathan (1977) who registered that poliploid species may show mutation buffering since they contain duplicated genes. That is why M_3 generation is recommended as the best one for making evaluations.

The occurrence of recombination for low height genes is demonstrated by the effectiveness of artificial crosses in increasing frequency distributions, promote large increasing in variances and to determine smaller means in relation to control. The possibility of selecting and obtaining genetic gains is demonstrated by the capacity of getting wide genetic variability which is possible because of the recombination of genes as is shown on pronounced decrease in means.

The analysis of mutagenic treatments compared to artificial crosses makes possible the establishment of relations between generations M_2 and F_2. It helps with some evidence on the most effective mechanism for promoting genetic changes. The increase in variance promoted by the larger doses of radiation was similar to what was obtained from artificial crosses for both of the genotypes. This characterizes the effect of the mutagenic and the artificial crosses in increasing variances and suggests the increase in genetic variability by both methods.

Similar comparisons were made by other authors who noted the capacity in obtaining similar variance amplitudes, when they used irradiation and hybridization techniques (Krull and Frey, 1960). Different results were produced for the genotypes as can be seen on means. The mutagenic was more effective in reducing the plant height for the EMBRAPA18 genotype, while the artificial crosses promoted larger changes in plant height for the BR4 genotype. It may have occurred because of the sensibility to radiation of the EMBRAPA18 genotype.

CONCLUSIONS

Mutagenic agents and artificial crosses between the triticale genotypes showed variability different than the original cultivar. Both methods were efficient and promoted similar levels of genetic variability for plant height. It made possible the identification of superior and inferior individuals for the character. The mutagenic treatments showed tendency to reduce plant height, and increasing in radiation doses produced larger effects. The artificial crosses and mutagenic treatments were efficient in increasing genetic variability for the evaluated genotypes and they may help obtaining superior genotypes.

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RESUMO

Mutações induzidas pelo uso das doses de radiação gama 0, 5, 10, 20 e 40 kR e cruzamentos artificiais recíprocos foram testados como mecanismos para aumentar a variabilidade genética em duas cultivares de triticale, BR4 e EMBRAPA18. Os tratamentos empregados provocaram amplitudes similares de variabilidade genética, com alterações nas médias e variâncias, podendo favorecer o emprego da seleção na obtenção de genótipos superiores. As respostas dos genótipos foram diferenciadas, sendo mais acentuadas quando submetidos a doses superiores do tratamento mutagênico, determinando uma redução na estatura. Em relação ao nível de variabilidade genética obtida, os tratamentos mutagênicos foram tão eficientes quanto as hibridações artificiais. A decisão do método a ser utilizado deve ser adequada aos recursos disponíveis, assim como o mecanismo mais eficiente de seleção para os caracteres de interesse.

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