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Genetic variability induced by chemical and physical mutagenic agents in oat genotypes

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ABSTRACT - *Objective of the present work was to compare the magnitude of genetic variability generated in hexaploid oat genotypes treated with two mutagenic agents: physical versus chemical. The physical agent was nearly always superior to the chemical agent, regardless of the evaluated cross or generation, providing a larger number of phenotypic classes of shorter plants. The mutant genotypes indicated a differentiated sensitivity to doses of the applied agents. This fact shows the efficiency of these agents in altering the genetic variability of this trait upwards (high height) as well as downwards (short height). In general, data pointed to a decrease in the trait plant height under increasing agent doses. The largest degree of genetic divergence and dominance for the new dwarfing genes was obtained by doses 100 Gy and 0.5%, respectively, of mutagenic physical and chemical agents applied to UFRGS 10 genotype in the M_2 generation.*

Key words: *Avena sativa* L., ^{60}Co, EMS - ethylmethanesulphonate.

INTRODUCTION

When introduced in Brazil, the oat crop was exposed to very distinct environmental conditions from its center of origin. As a consequence, the variability in traits of agronomic importance such as plant height was considerably reduced. The narrowing of the genetic basis among cultivated oat varieties (A*vena sativa* L.) drastically decreases the chances of successfully selecting new genotypes in segregating generations (Carvalho and Federizzi 1989). Induced mutations are defined as inheritable changes in DNA of qualitative or quantitative order, not derived from genetic segregation or recombination. Since spontaneous mutation rates are very low, induced mutations are being used to increase its rate and frequencies. Mutations can be induced either by chemical, i.e., alkylating (ethylmethanesulphonate) or physical agents like ionic radiation (Predieri 2001). Alterations in the DNA of the nucleus or organelles give rise to gene, genome, or chromosome mutations that generate variability, whose limitation in breeding is the lack of specificity of the mutated trait (Tulmann Neto et al. 1998).

The direct use of mutations is helpful, especially when the improvement of one or two easily selectable traits in a well adapted variety is aimed at. Main advantage is that the basic genotype is only slighty altered, by contrast to procedures involving crossing of two distinct varieties (Donini and Sonnino 1998).

The technical aspects of mutation breeding proceed with the choice of the mutagenic agent, dose, and mainly the ratio between mutation frequency and dose. However, the population size, the handling of the treated tissue or organ, and the appropriate selection methods require ongoing investigation.

In this context, our study had the objective to compare chemical and physical mutagenic agents that generate genetic variability for the trait plant height, aiming at the selection of oat plant ideotypes for the Southern Region of Brazil.

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MATERIAL AND METHODS

The study was conducted in 1997 and 1998, in the field and greenhouse, at the Federal University of Pelotas, in Capão do Leão, State of Rio Grande do Sul, Brazil. Nearly 1200 genetic seeds from four fixed hexaploid oat populations (CTC 3, UFRGS 10, UFRGS 14, and UPF 16) were gamma ray-treated under a ^{60}Co source and subjected to ethylmethanesulphonate (EMS), thus originating the M_1 generation. The total doses of irradiation and absorption were 100; 200 and 400 Gy and 0.5; 1.5 and 3.0% per treatment of physical and chemical agents, respectively, coded as doses 1, 2 and 3.

Seeds of the M_1 generation were sown in a greenhouse in winter 1997. Each treatment corresponded to one plot, composed of 10 seeds from each genotype. The panicles originating from M_1 seeds from each treatment were selected randomly to compose the M_2 generation, constituting distinct genotypes. To advance to M_3 generation, ten seeds taken from each M_2 panicle, totally over 85 seeds, were sown in plastic pots (height 23 cm x diameter 22 cm), at a depth of 2.0 cm in sieved soil (Summer 1998). Seeds from all populations were sown on the field in winter 1998.

Phenotypic observations were performed individually for each plant in all treatments and generations. In all different populations and generations the parameters mean (μ), variance (σ^2), skweness (s) and kurtosis (k) were obtained. It was still possible to make a qualitative description of interaction in generations M_2 and M_3. Under these circumstances, the analysis proceeded to the study of the variation ascribed to the quantitative factor (dose) separately for each of the qualitative specific (genotype) factor. Estimates of means and variances were compared among the mutagenic agents tested in both generations by test t and F, respectively.

All applied analyses were described by Steel and Torrie (1980). The procedures PROC GLM, REG, and TTEST of the software SAS were used to evaluate the estimates. PROC REG was used to estimate and evaluate the linear regression models that best fitted the data (Schlotzhauer and Littell 1987).

RESULTS

Table 1 shows that both physical (gamma ray) and chemical (EMS) mutagens were efficient at changing the magnitude of genetic variability, quantified and evaluated through estimates for the studied genetic-statistical parameters, mainly the estimatives of skewness (s) and kurtosis (k). These estimates provide, respectively, valuable information regarding the dominance of trait and degree of the genetic divergence involved in each tested population, independently of the mean and variance estimates; comparisons were always related to the tested mutagenic agents. These, comparatively, did not induce significant changes on the mean by the t test for all the evaluated populations, except for CTC 3 genotype under extreme doses. On the other hand, the variance estimates for all studied genotypes revealed statistical significance by the F test between the variances of mutagenic treatments, except for UPF 16 under intermediate doses and UFRGS 10 and UFRGS 14 populations under the highest dose.

The physical mutagen engendered superior estimated values of variance in most populations, independently of the tested dose (Table 1). In general, compared to macromutations, a notable predominance of micromutations was observed.

The average behavior (Table 2) of each population in generation M_3 subjected to treatment with physical and chemical mutagens

Table 1. Mean (μ), variance (σ^2), skewness (s), and kurtosis (k) from evaluated plants (n) for plant height (cm) in M_2 generations originating from different doses of physical (gamma rays - ^{60}Co) and chemical EMS mutagens applied to four hexaploid oat genotypes

Genotypes	Doses	Mutagens									
		Gamma rays - ^{60}Co					EMS				
		M_2 generation					M_2 generation				
		n	μ	σ^2	s	k	n	μ	σ^2	s	k
CTC 3	1	245	105*	129*	-1.08	4.43	398	137	217	-1.43	5.91
UFRGS 10	1	332	138	158*	-2.56	17.49	376	136	204	-2.21	14.40
UFRGS 14	1	298	108	119*	-0.12	1.95	358	103	98	-0.39	0.24
UPF 16	1	335	107	126*	-2.28	14.95	191	104	102	-0.46	1.02
CTC 3	2	248	129	112*	-0.81	1.51	344	133	212	-0.43	0.28
UFRGS 10	2	355	138	143*	-0.16	0.61	390	145	100	-1.11	2.33
UFRGS 14	2	377	112	120*	-1.42	6.79	299	104	162	1.24	12.87
UPF 16	2	331	119	86	-0.28	0.29	206	113	107	-1.44	2.80
CTC 3	3	295	126*	268*	-1.21	3.31	278	99	125	0.30	1.94
UFRGS 10	3	177	127	125	-0.86	1.17	75	123	156	-0.93	1.74
UFRGS 14	3	95	96	103	-0.79	-0.23	202	103	98	-0.44	0.63
UPF 16	3	258	125	145*	0.04	0.51	221	111	80	-1.00	4.46

* Significant at 0.05 probability by the t test for means and by the F test for variances.

under the distinct doses was divergent. This fact indicates that these mutagenic agents can create the genetic variability in oat crop required for successful breeding. They were efficient at altering the variance estimate in M_3, which is consistent with the obtained results in generation M_2 (micromutation in the great majority of genotypes). On the other hand, no genotype tested in M_3 showed significant changes in the mean by the t test, except for genotype UFRGS 14 under the lowest dose. Besides, in the comparison of the treatments, data show homogeneity among variances by the F test, with exception of four tested mutant genotypes: UFRGS 14 under doses 1 and 2, CTC 3, and UPF 16 under the highest dose. Genotype UPF 16 under dose 1 attained the lowest estimate for variance and the highest for kurtosis. In general, micromutations were once more predominant, compared to macromutations.

The mutagens were efficient at changing direction and magnitude of the genetic variability for the trait plant height, with reductions (negative skewness) or increases (positive skewness) and, clearly with a strong predominance of dwarfing genes, regardless of the tested dose or mutagen.

The four regression equations were adjusted, one for each genotype in each evaluated segregating generation. The analysis of variance was significant by the F test ($P < 0.01$) for the component models linear and quadratic, indicating the need to adjust distinct curves for the different doses of the mutagenic agents tested in this study (Figures 1 and 2).

Results of the regression analysis for the dependent variable plant height (cm) in generations M_2 and M_3 for the significance tests of the linear and quadratic components of the variation due to dose effects of physical and chemical mutagens are presented in Figures 1 and 2. In generation M_2 the significant variations due to the dose of the mutagens are mainly of the quadratic type. The linear regression coefficient (b) was significant ($P < 0.01$) in generation M_2 for all evaluated populations (Figure 1). In other words, there was a regression coefficient of -7.68 for the mutant population CTC 3 treated with chemical mutagen. This indicates that as the dose of mutagen (EMS) increases, the plant height is reduced to a certain point in the studied period. Likewise, the regression model for all evaluated M_3 populations was significant ($P < 0.01$) by the F test for the plant height. Only for populations UPF 16 and UFRGS 10 subjected to the chemical mutagen the significant variations attributed to the dose are of the linear type. Biologically, the regression equation for population UFRGS 10 can be interpreted as follows: mutagenically untreated populations have an average plant height of 140.8 cm, which is reduced by 10.6 cm at each 1% of increment of the chemical agent. The behavior of the studied trait can be observed in Figure 2, where the regression curves are presented adjusted to generation M_3 for both evaluated mutagens.

DISCUSSION

The use of mutagens in plant breeding has generated striking results. According to Ahloowalia and Maluszynski (2001), more than 1,800 cultivars were obtained direct or indirectly through induced mutants in seed producing plants such as wheat, rice, barley, peanut, oat, and beans, among others. In vegetatively propagated plants, 465 mutants, mostly

Table 2. Mean (μ), variance (σ^2), skewness (s), and kurtosis (k) of the evaluated plants (n) for the plant height (cm) of M_3 generations originating from different doses of physical (gamma rays - ^{60}Co) and chemical EMS mutagens, applied to four hexaploid oat genotypes

Genotypes	Doses	Mutagens									
		M_2 generation									
		Gamma rays - ^{60}Co					EMS				
		n	μ	σ^2	s	k	n	μ	σ^2	s	k
CTC 3	1	329	129	96	-0.02	5.81	93	126	79	-0.34	-0.20
UFRGS 10	1	102	119	223*	-0.27	-0.56	138	135	99	-1.35	5.66
UFRGS 14	1	277	134*	90	-0.72	0.52	142	100	77	-0.75	0.70
UPF 16	1	137	120	195*	-0.40	0.64	176	115	63	-2.51	14.55
CTC 3	2	307	137	147*	-0.54	0.55	209	102	67	-0.95	4.01
UFRGS 10	2	258	105	91*	-0.61	0.41	223	125	269	-0.70	4.88
UFRGS 14	2	193	106	79	-0.95	1.60	83	106	60	-0.23	2.01
UPF 16	2	60	128	142*	0.17	4.18	230	109	65	-0.63	0.71
CTC 3	3	254	140	151	-0.62	1.40	193	121	164	0.33	4.77
UFRGS 10	3	256	124	69*	-1.14	2.81	194	109	247	0.63	6.21
UFRGS 14	3	331	137	103*	-0.40	0.68	382	128	167	0.04	-0.07
UPF 16	3	327	132	165	0.27	1.26	53	101	142	-0.98	1.70

* Significant at 0.05 probability by the t test for means and the F test for variances.

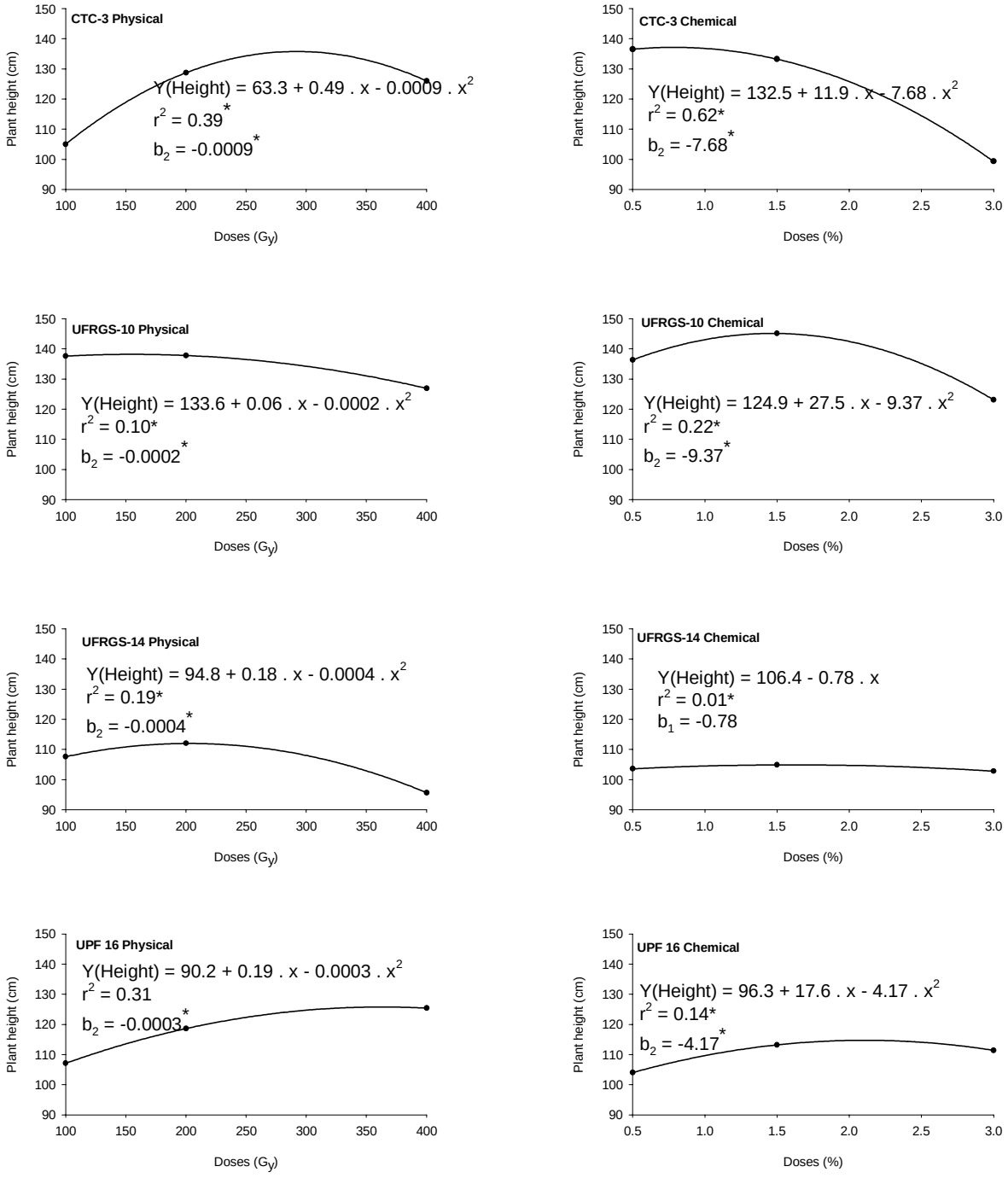

Figure 1. Adjusted regression for the plant height (cm) of four fixed hexaploid oat genotypes in generation M_2 subjected to physical (left graphs) and chemical (right graphs) mutagens, under different doses.

ornamental but a small part also fruit plants were released. Mutation inducing is a tool that could increase the genetic variability in crops where the cultivated germplasm has a narrow genetic basis. The analysis of 70 oat cultivars (*A. sativa* and *A. bizantina*) based on 13 morphological traits over two experimental years revealed four large clusters with overall correspondence to the center of origin or adaptation region (Souza and Sorrells 1991). When the parents used for artificial crosses are genetically very close, progenies present a reduced genetic variability within populations.

Results obtained in this work revealed that the physical mutagen was more efficient at changing the genetic variability of the great majority of the studied populations. However, these agents could increase as well as reduce the genetic variability of the character plant height, confirming other results obtained with oat subjected to mutagen treatment

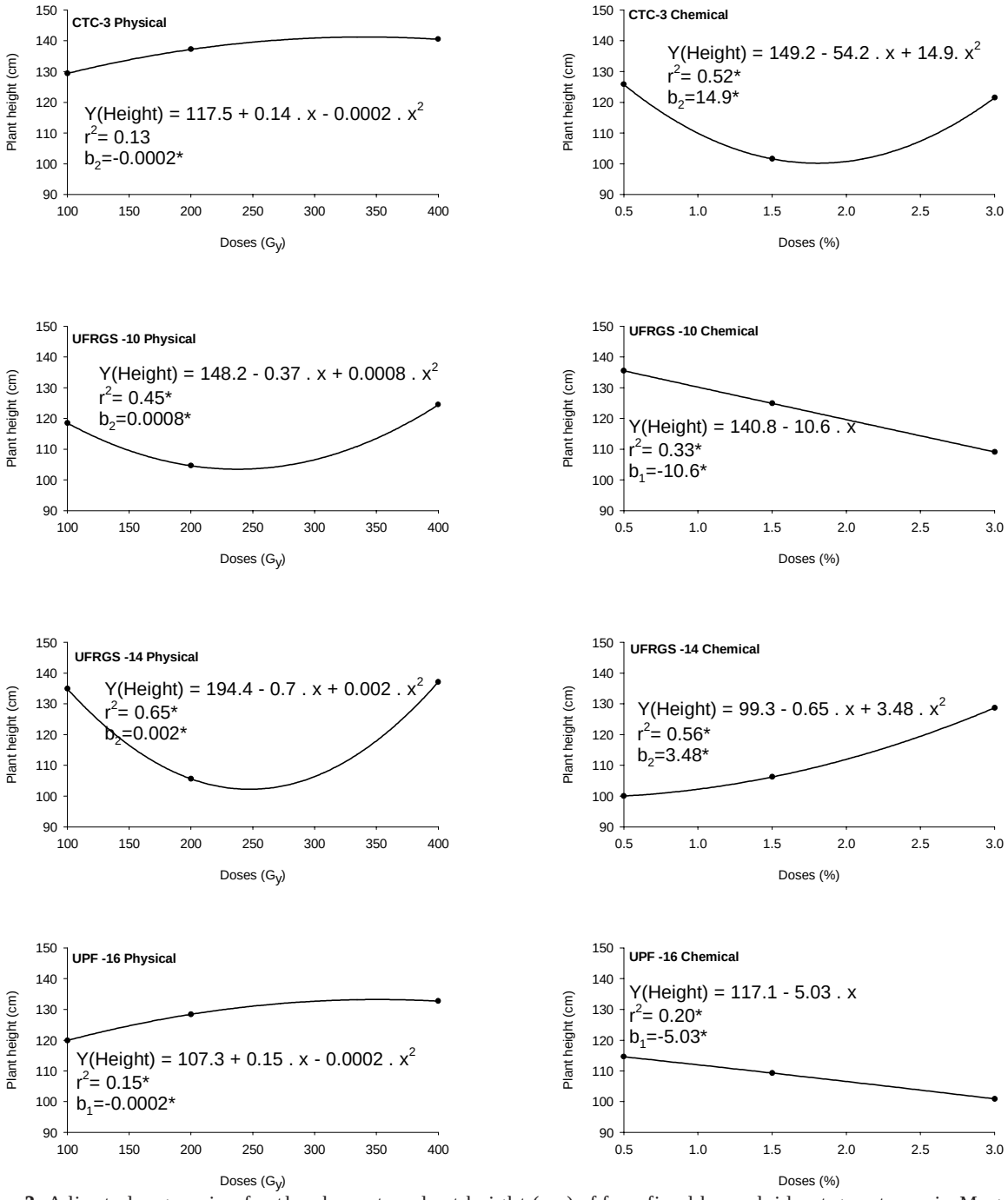

Figure 2. Adjusted regression for the character plant height (cm) of four fixed hexaploid oat genotypes in M_3 generation by physical (left graphs) and chemical (right graphs) mutagens in different doses.

(Abrams and Frey 1964, Nascimento Júnior et al. 1990). The authors pointed out that these techniques are effective at altering the frequency of agronomically important traits, for example, plant height. Thus, the use of physical mutagens can induce the occurrence of new genes through allelic and/or chromosomal changes.

Alternatively, according to Briggs and Knowles (1967) and Chandhanamutta and Frey (1974) the phenotypic variation can be studied through genetic-statistic parameters fit to a detailed evaluation of its origin. Therefore, the parameter estimates like mean variance, skewness, and kurtosis can efficiently detect the genetic variability among the evaluated populations. Gregory (1967) and Chandhanamutta and Frey (1974) considered that the change in the mean of populations treated with mutagens indicates the occurrence of alterations in few genes of strong effect on the trait called macromutation;

however, the effect on a large number of genes of small effect on the phenotype results in variance change, originating micromutations. In our study, it can easily be observed that micromutations were detected in most evaluated populations regardless of the dose, mutagen, genetic constitution, or the segregating generation.

The distribution of individuals in generations M_2 and M_3 of the different mutagens for the character plant height is presented in Figures 3 and 4. Genotype UPF 16 subjected to the physical agent with dose 3, revealed a very close to zero (0.04) skewness estimate; this fact indicates absence of dominance for this character since the distribution is

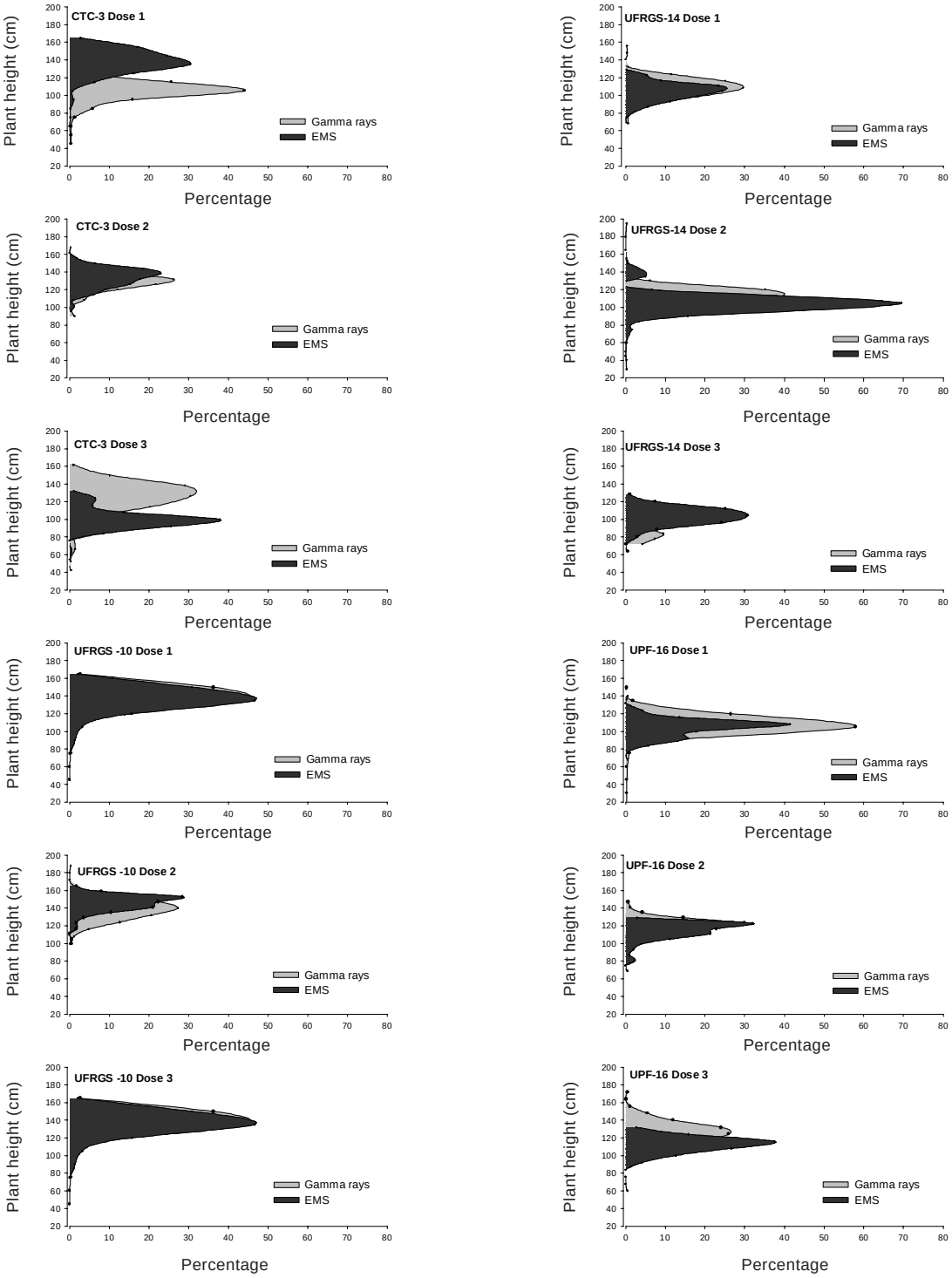

Figure 3. Distribution curve of the percentage of individuals evaluated for the plant height for four populations in the segregating M_2 generation subjected to different mutagens, in three different doses.

symmetrical (Allard 1964). All other populations evaluated in this study for this same mutagen in generation M_2 showed unidirectional dominance, i.e., dominance always occurred in one direction since skewness estimates presented negative values for the remaining studied populations. This fact discloses the real possibility a breeder has to select plants with shorter height by segregating populations subjected to the treatment with physical mutagen. On the other hand, in the same segregating generation the chemical agent produced three populations with positive skewness estimates. This fact gives wind to the idea of successful breeding - specifically for this trait - with the study mutagens, mainly the physical agent.

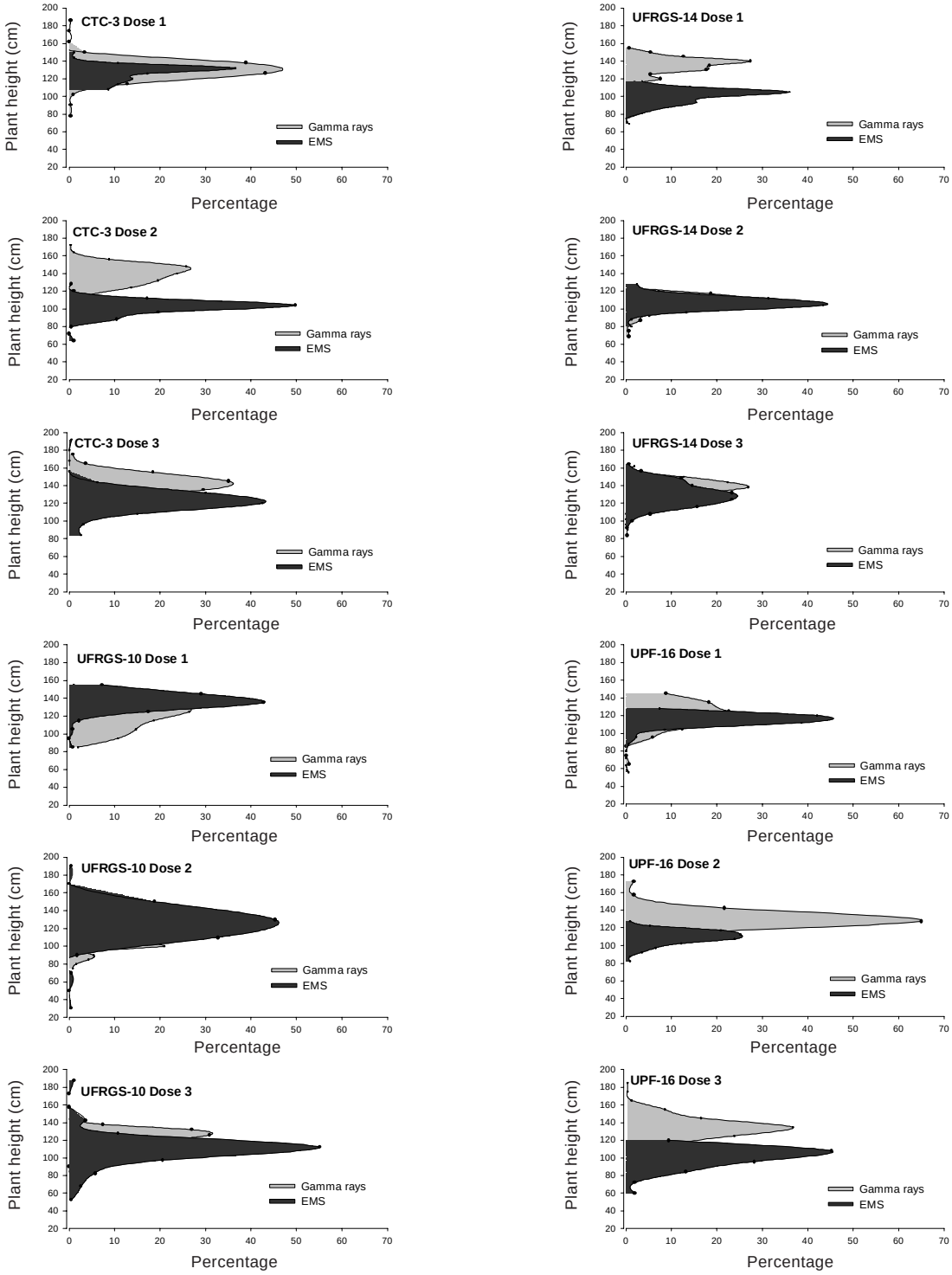

Figure 4. Distribution curve of the percentage of individuals evaluated for the plant height for four populations in the segregating M_3 generation subjected to different mutagens, in three different doses.

There are clear situations where the use of mutagenesis is recommended, as in the case of, for instance, lack or narrow natural genetic variability for the desired character (Tulmann Neto et al. 1998).

This fact is of remarkable importance, considering that according to Milach et al. (1997), three genes (DW6, DW7, and DW8) that grant the plant height in oat a considerable reduction, analyzed by RFLP (Restriction Fragment Lenght Polymorphism), revealed very close linkage groups. For Rasmusson (1991), in this situation, the possibility of creating new genes through artificial crosses can be slow, arduous, and onerous. Regarding the kurtosis degree, population UFRGS 10 presented a wide distribution interval and the highest degree of kurtosis (17.49) under physical mutagen dose 1 and, consequently, a larger degree of genetic divergence among all studied populations. On the other hand, population UFRGS 14 treated with the highest EMS dose revealed the smallest degree of genetic divergence among all tested populations, independently of the segregating generation. If this variation is obtained and analyzed separately from other estimates, problems may surge at interpreting the experimental results which may lead the investigator to wrong conclusions. Therefore, these estimates, which are greatly influenced by the real sample size, could best be interpreted by their association for analysis, mainly with kurtosis, which reveals the degree of flatness of the curve, thus giving a real idea of genetic divergence. Information on the genetic distance of superior adapted genotypes is fundamental to increase the chance of success in plant breeding programs, as well as to permit time and cost reductions (Coimbra et al. 1998).

Variance analyses for the tests of significance of the linear and quadratic components of the variance due to mutagen dose for each evaluated genotype in the segregating generations was significant by the F test. This indicates that within the studied interval, the character plant height decreases under increased doses up to a certain point in the curve, regardless of the mutagen in use. Except for the mutant populations UFRGS 14 (M_2), UFRGS 10 (M_3), and UPF 16 (M_3), results of the regression analysis of variance for the dependent variable plant height (Figures 1 and 2) indicate that the variation due to the mutagen doses is obviously of quadratic type, regardless of generation and genetic constitution.

Usually, the number of mutants increases as doses rise, in absence of lethal effects (Scossiroli 1977). A decrease in the mean of the character in relation to the tested radiation doses, as well as the high number of individuals present in classes of shorter height, mainly for the physical mutagen, supports the idea that the alleles responsible for the reduction of the character were also attained (Figures 3 and 4).

The physical mutagen was efficient at creating new genes that confer shorter plant height, showing the real ability of gamma rays to induce mutations and mainly to cause consistent changes in allelic and genotypic frequencies of the character.

Plant height data (Figures 1 and 2) indicate that the response of white oat to mutagens is mostly quadratic, showing that for lower doses there was a linear increase. As the doses increased, the gain tended to sink, but to stabilize at highest doses, exclusively for this character. The behavior of genotypes subjected to increasing mutagen doses is consistent with the results of Carneiro et al. (1987) in common bean, Pandini et al. (1997) in triticale and Coimbra et al. (1999) in white oat. In general, once the physical mutagen dose is increased there is a reduction in plant height up to a certain point in generation M_3 (Figure 2), with exception of genotypes UFRGS 10 and UFRGS 14, which expressed a distinct behavior. Figures 1 and 2 show that all populations responded differently to the physical mutagen dose. For example, the character plant height in populations UPF 16 and UFRGS 10 in generation M_3, at the maximum and minimum points under application of one 370 Gy and 230 Gy dose, obtained an estimate value of about 133 and 105 cm, respectively. Physical and chemical mutagens increased the character under lower doses in the M_2 generation, opposite to when higher doses were employed.

Variabilidade genética em populações de aveia induzida por agentes mutagênicos químicos e físicos

RESUMO - *O presente trabalho teve como objetivo comparar a magnitude da variabilidade genética gerada em populações de aveia hexaplóide tratadas com dois agentes mutagênicos: físico versus químico. O agente físico foi quase sempre superior ao químico, independentemente da geração segregante e do cruzamento avaliados, proporcionando maior incremento no número de classes fenotípicas de plantas de porte baixo. Os genótipos mutantes apontaram uma sensibilidade diferenciada em relação à dose dos mutagênicos aplicados. Este fato ratifica a eficiência destes agentes mutagênicos em alterar a variabilidade genética do caráter porte da planta, tanto para incrementos quanto para decréscimos. Os dados indicaram redução do porte de plantas sob incremento das doses dos mutagênicos. A população UFRGS 10, geração M_2, submetida ao tratamento com os agentes mutagênicos físico e químico nas doses de 100 Gy e 0,5%, respectivamente, revelou maior grau de divergência genética e de dominância para os novos genes redutores de porte de plantas.*

Palavras-chave: *Avena sativa* L., ^{60}Co, EMS - etil-metanossulfonato.

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