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Induction of genetic variability in oat

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ABSTRACT – *Genetic variability in plants can be maximized through techniques of induction to make selection of genotypes with improved adaptation to cultivation conditions possible. For oat, these techniques are important for a sustainable development through plant breeding programs in southern Brazil. The effects of mutagens (one physical: ^{60}Co gamma rays and two chemical agents: ethyl - methanesulfonate and methyl-methanesulfonate) were compared in the segregating M_2 and M_3 generations derived from artificial hybridization and induced mutation to compare mechanisms of widening the genetic variability of oat. The methodologies increased the genetic variability in the trait vegetative cycle effectively, by either increasing or reducing the number of days from emergence to full heading; both can be applied in oat breeding programs.*

Key words: *Avena sativa*, mutagens, kurtosis, skewness.

INTRODUCTION

Over the last seven decades, more than 2250 varieties commercial mutants, used directly or through controlled crosses, have been created (Ahloowalia et al. 2004). Oat (*Avena sativa* L.) was introduced into Brazil, where the environmental conditions are completely different from the center of origin of the species. The existing genetic variability for traits of agronomic importance, such as plant vegetative cycle, is considered restricted. The narrowing of the genetic base in cultivated oat varieties can be a constraint on the efficacy of genotype selection in segregating generations (Carvalho and Federizzi 1989).

Genetic variability, indispensable for all effective natural and/or artificial selection, consists essentially of processes of evolution and plant improvement (Jennings et al. 1981). Aside from the predetermined genetic variability in the germplasm, variability can be added by means of artificial mutations, gene recombination, genetic transformation, and somaclonal mutations.

Low genetic variability in cultivated species hampers the selection of superior genotypes for breeding (Silva et al. 1998). Modifications in the genetic structure of living creatures occur naturally, though at low frequency, but can be increased through mutagens, according to Morishita et al. (2003). A greater genetic variability for traits of interest can thus be obtained based on induced mutations. In this sense, mutations have been induced by several types of mutagens, as for instance gamma rays (Co^{60}), into a wide range of species, such as rice, banana, grape, oat, among others,

Mutations can be defined as inheritable alterations of qualitative or quantitative nature, not derived from recombination (Bennetzen 2000). Mutations create genetic variability. They provide the raw material for the evolution process and are sometimes fundamental for improvement, whose success depends on the

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existence of variability. Mutations occur randomly and mostly make organisms less competitive. On the other hand, there is a chance of introducing new desirable characteristics through induced mutations, namely in plants. For instance, barley mutants with higher yields and protein contents, stronger resistance to fungal diseases, and hull-less seeds have been developed. Despite the costliness of mutagen techniques, laborious and of unpredictable outcome, the use is justified when no more variability is available in the germplasm.

Since spontaneous mutation rates are very low, artificially induced mutations have been used more frequently to raise the frequency of mutations and variations, which can be amplified through chemical mutagens as for instance alkylating agents (ethyl-methanesulfonate and methyl-methanesulfonate), as much as physical mutagens, such as ionizing radiations (Predieri 2001). The latter is the most commonly used mutagen to create mutant varieties (Waugh et al. 2006).

The determination of mainly the target population size, the mutagen type and dose rate, along with appropriate selection methods of mutants need more in-depth research (Scossiroli 1977, Coimbra et al. 2004).

The main strategy of improvement by mutation must be to alter one or two traits that restrict the yield or grain quality of a well adapted cultivar, e.g. vegetative cycle (Ahloowalia and Maluszynski 2001).

The objective of this study was to evaluate and compare different techniques that can expand genetic variability, exemplified in the trait vegetative cycle in oat.

MATERIAL AND METHODS

The study was conducted in a screenhouse and on the field, in the growing seasons of 1997 and 1998, at the Universidade Federal de Pelotas (UFPel), in the county of Capão do Leão, state of Rio Grande do Sul. The two cultivars used in this study, namely UPF 16, are grown on a considerable percentage of the cultivated area in the southern region of Brazil, despite the drawback of rust susceptibility.

To develop the mutant populations, plants of the hexaploid oat genotypes UFRGS 10 and UPF 16 were obtained from seeds treated previously with ^{60}Co gamma irradiation and ethyl-methanesulfonate (EMS) and methyl-methanesulfonate (MMS). The mutations were induced at the Centro Regional de Oncologia da Faculdade de Medicina of the UFPel. For the physiacal mutagen, a dose rate of 0.25 G_y min^{-1} was used. Each treatment absorbed 0, 100, 200 and 400 G_y (gray). For the chemical mutagens, EMS and MMS were used in the following concentrations: 0, 0,5, 1.5 and 3.0% (v/v) and 0.25, 0.50 and 1.0% (v/v) for two hours, (respectively). Next, all seeds were rinsed under tap water for one hour and left in standing water for another hour. Each treatment comprised 1200 seeds.

The M_2 generation consisted of the M_1 seeds of each treatment, thus representing distinct mutant populations. Of these, 10 seeds of each panicle were planted in screen-protected buckets, in the summer of 1997/98, to advance generations.

In the winter of 1998, all seeds of the mutant populations M_2 and M_3 were sown on the field. The experimental design was completely randomized and each plant considered a replication. The rows were 5.0 m long and spaced 0.2 m apart with approximately 25 seeds per row, totalizing a sowing density of 250 kg ha^{-1}. Each plant was evaluated individually for phenotypic traits. The vegetative cycle was evaluated by counting the number of days from sowing until growth of the first panicle.

In all generations, the parameters of skewness (s), kurtosis (k), mean (μ) and variance (s^2), were calculated for all study populations. In the M_2 and M_3 generations it was moreover possible to give a qualitative description of the interaction; on this account, the analysis was focused on the variation ascribed to the quantitative factor (dose rate), separately for each level of the specific qualitative factor (genotype). The t and F tests were used, respectively, for a comparison of the means and the variances.

For the t statistics the hypothesis was assumed that $\mu^1 = \mu^2$, where μ^1 and μ^2 are the means; n_1 and n_2 the number of observations; and s^2_1 and s^2_2 the variances of samples 1 and 2, respectively. The model of simple linear regression that best fits the data and the frequency distributions as well as the means and variances were obtained as described by Steel and Torrie (1980).

RESULTS AND DISCUSSION

In the populations originated by artificial crosses (UFRGS 10 x UFRGS 14 and UPF 16 x CTC 3) the

magnitude of variance for the adaptable trait vegetative cycle with negative as well as positive skewness values, respectively, was significantly modified (Table 1). This evidences the possibility of selecting plants with a contrasting vegetative cycle in relation to the parents. Estimates of the skewness coefficient can be useful in the evaluation of the dominance of the trait (Allard 1960). For instance, for population UFRGS 10 x UFRGS 14 which presented the highest value for phenotypic variance, the skewness coefficient was close to zero (-0.179) indicating the absence of dominance or partial dominance regarding the phenotype of the earlier parent. This means that the two tails of the distribution curve are practically symmetrical (Figure 1). Exclusive for this situation, the positive skewness indicated a favorable trend for selection of genotypes with longer vegetative cycles (UPF 16 x CTC 3).

The coefficient of kurtosis (k) is frequently used to describe the characteristic shape of a frequency distribution (Joanes and Gill 1998). The estimate of this coefficient gives a notion of the genetic dissimilarity degree of the parents. The kurtosis values of frequency distributions with a normal distribution are zero, independent of the variance. Leptocurtic kurtosis estimates (>0) indicate a greater weight of the tails, that is, a greater amplitude in the number of phenotypic classes, evidencing a higher degree of genetic divergence for the trait. Population UPF 16 x CTC 3 presented quite high kurtosis and skewness values (Table 1). These values can be explained by the high weight of the right tail of the distribution curve, favoring the selection of individuals of longer vegetative cycle (Figure 1). This information, along with the high kurtosis value, can give an idea of the degree of genetic divergence between the parents involved in the cross.

The performance of each M_2 population subjected to the mutagens ethyl-methanesulfonate (EMS) and methyl-methanesulfonate (MMS) and ^{60}Co gamma rays, under the different dose rates is described in Table 2. The genotypes presented differentiated response to the mutagen, suggesting that this mechanism of creation of genetic variability can be used in oat, fundamentally, in the absence of genetic variability for the trait under selection. At all times, ^{60}Co gamma rays were more effective than the chemical agents, which are little used in improvement programs (Ahloowalia et al. 2004). The efficacy for increment as well as diminution of the number of days from emergence to full heading (50% of the plot) is shown in Figures 1 (M_2 generation) and 2 (M_3 generation). The response of the mutant populations to the different mutagens was differentiated. For instance, mutagen MMS effectively altered the genetic variability in the mutant population UFRGS 10, in the M_3 generation, in the sense of earliness (Figure 2).

EMS induced significant modifications in the mean of the mutant UFRGS 10 populations, with exception of population UFRGS 10 subjected to the highest dose rate in the segregating M_2 generation (Table 2). Likewise, the homogeneity of variance evaluated by the F test demonstrated differences between the variances of the mutant populations UFRGS 10 at the lowest doses, independent of the generation evaluated. Waugh et al. (2006) reported that induced mutations can increase genetic variance, favoring the selection of genotypes well adapted to cultivation conditions.

In general, all mutant genotypes originated from UFRGS 10 subjected to chemical mutagens at the intermediate dose rate evidenced absence of dominance for the trait. This kind of information is an indispensable tool for plant improvement, since additive variance (absence of dominance) is the variance of the genetic values and is the most important component, as it is the main cause of similarity between relatives and, consequently, the principal determinant of the genetic

Table 1. Skewness (s), kurtosis (k), population mean (m) and variance (s^2) of the number of plants evaluated (n) for the trait vegetative cycle of plant (days) of the segregating F_2 generation derived from artificial reciprocal crosses of the two genotypes UFRGS 10 and UPF 16

Populations	Generation	n	m	s^2	s	k
UFRGS 10 x UFRGS 14	F_2	381	107	26.40†	-0.179	3.259
UPF 16 x CTC 3	F_2	190	105	14.13†	2.763	22.297
UFRGS 10	Standard	80	109	5.79	-0.407	-0.654
UPF 16	Standard	146	107	8.09	-0.344	-0.644

† Significant at 0.05 probability by the t test for the means and F for variances in relation to the standard

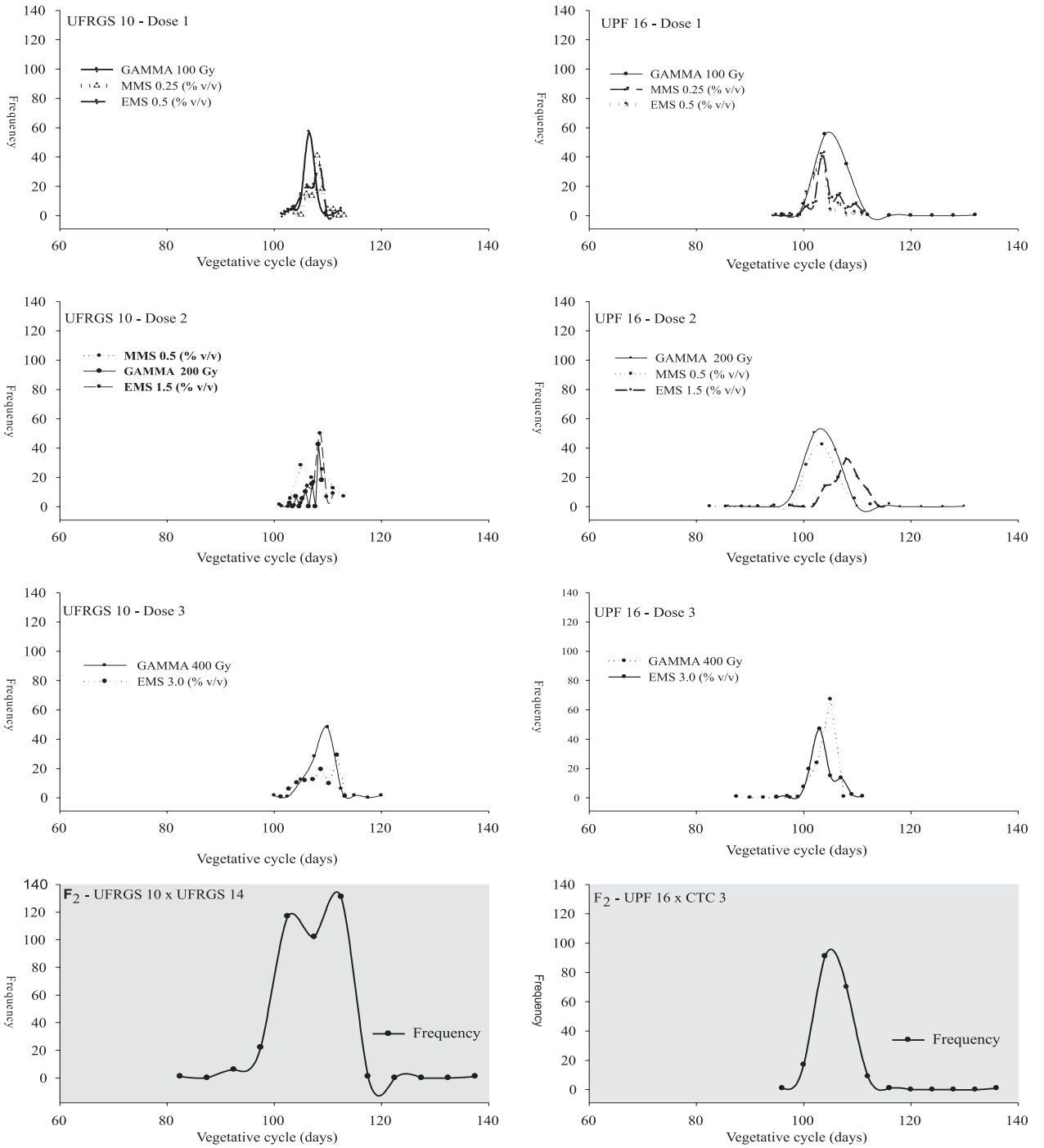

Figure 1. Frequency distribution of the total number of individuals evaluated for the trait plant vegetative cycle (days) for the genotypes UFRGS 10 and UPF 16, separately for each dose evaluated, in the segregating M_2 generation subjected to three mutagens: one physical (100; 200 and 400 G_y) and two chemical EMS (0.5; 1.5 and 3.0%) and MMS (0.25; 0.5 and 1.0%) in three different dose rates, in relation to two populations derived from artificial crosses

Table 2. Mean (m), variance (s^2), skewness (s) and kurtosis (k) of the number of plants evaluated (n) for the trait vegetative cycle of plant (cm) in the generations M_2 and M_3 derived from treatments with different dose rates (1, 2 and 3) of the mutagen physical: gamma rays (^{60}Co) and of the mutagen chemical: methyl-methanesulfonate (MMS) and ethyl-methanesulfonate (EMS) in two fixed genotypes of oat UFRGS 10 (U 10) and UPF 16 (U 16)

	Gamma rays ^{60}Co											
	M_2						**M_3**					
	100 G_y		**200 G_y**		**400 G_y**		**100 G_y**		**200 G_y**		**400 G_y**	
	U 10	**U16**	**U 10**	**U16**	**U 10**	**U16**	**U 10**	**U16**	**U 10**	**U16**	**U 10**	**U16**
n	95	265	189	309	131	122	103	118	259	62	252	307
m	107*	105*	107*	103*	109	104	109	107	107	109*	107	101*
s^2	3.38*	7.73	2.34*	9.92	6.67	4.77*	22.27	9.40	19.41	4.47*	3.06	41.81*
s	0.86	3.09	-1.10	1.59	0.62	-3.84	1.50	0.03	-2.51	-0.13	0.94	-0.63
k	3.24	34.29	0.46	18.44	4.29	26.21	0.99	-0.73	18.38	0.66	1.13	0.40
	Methyl-methanesulfonate - MMS											
	M_2						**M_3**					
	0.25% (v/v)		**0.50% (v/v)**		**1.00% (v/v)**		**0.25% (v/v)**		**0.50% (v/v)**		**1.00% (v/v)**	
	U 10	**U16**	**U 10**	**U16**	**U 10**	**U16**	**U 10**	**U16**	**U 10**	**U16**	**U 10**	**U16**
n	268	392	71	378	-	-	206	338	177	197	-	-
m	108*	105*	107*	102*	-	-	105*	107	111	107	-	-
s^2	2.01*	6.75	7.37	9.56	-	-	23.70*	9.90	2.84	7.44	-	-
s	-0.01	0.43	0.27	-0.58	-	-	0.15	-0.10	-0.37	0.35	-	-
k	0.92	0.26	-0.65	5.78	-	-	1.40	-0.33	-0.59	-0.12	-	-
	Ethyl-methanesulfonate - EMS											
	M_2						**M_3**					
	0.50% (v/v)		**1.50% (v/v)**		**3.00% (v/v)**		**0.50% (v/v)**		**1.50% (v/v)**		**3.00% (v/v)**	
	U 10	**U16**	**U 10**	**U16**	**U 10**	**U16**	**U 10**	**U16**	**U 10**	**U16**	**U 10**	**U16**
n	198	236	256	212	186	215	129	265	211	309	183	122
m	107*	103*	108*	108	108	103*	111*	105*	106*	103*	106*	104
s^2	2.78*	4.91*	2.40*	8.62	4.47	6.01*	2.28*	7.73	9.14*	9.92	6.70	4.77*
s	-0.64	0.97	0.01	0.07	-0.31	0.31	-1.26	3.09	-0.03	1.59	0.33	-3.84
k	0.70	2.19	1.24	0.30	-1.06	0.78	0.98	34.29	0.36	18.44	0.76	26.21

* = $P < 0.05$ probability by the t test for means and the F test for variances in relation to the standard

population properties and the response of a population to selection (Falconer and Mackay 1996). A complete characterization of the direction as well as of the magnitude of the variability of the data set involves skewness and kurtosis estimates, always associated to the mean and the variance (Coimbra et al. 2004).

Different data distributions can have the same variance, but if one of them presents a greater concentration of individuals around the mean (statistics of first order), the other will naturally have longer and heavier tails; this determines the higher or lower kurtosis degree (Chandhanamutta and Frey 1974). For instance, the mutant genotype UFRGS 10 presented variances of 3.38 and 22.27, respectively, at the doses 1 and 3 of the mutagen gamma rays. The treatment of lower variance therefore presented a higher degree of kurtosis, and consequently a higher degree of genetic variability for the trait late vegetative cycle (an undesirable characteristic in oat improvement). Furthermore, the chemical mutagen agent MMS was acutely lethal at 1%, for all populations (Table 2).

When the factor under study is quantitative (ex: mutagen or fertilizer rates), the adjustment of a regression equation is the most appropriate procedure (Gill 1978). It is therefore priority to determine the quantitative limits within which the factor is to be studied, whereas the levels used directly in the experiment are less important and are only used to

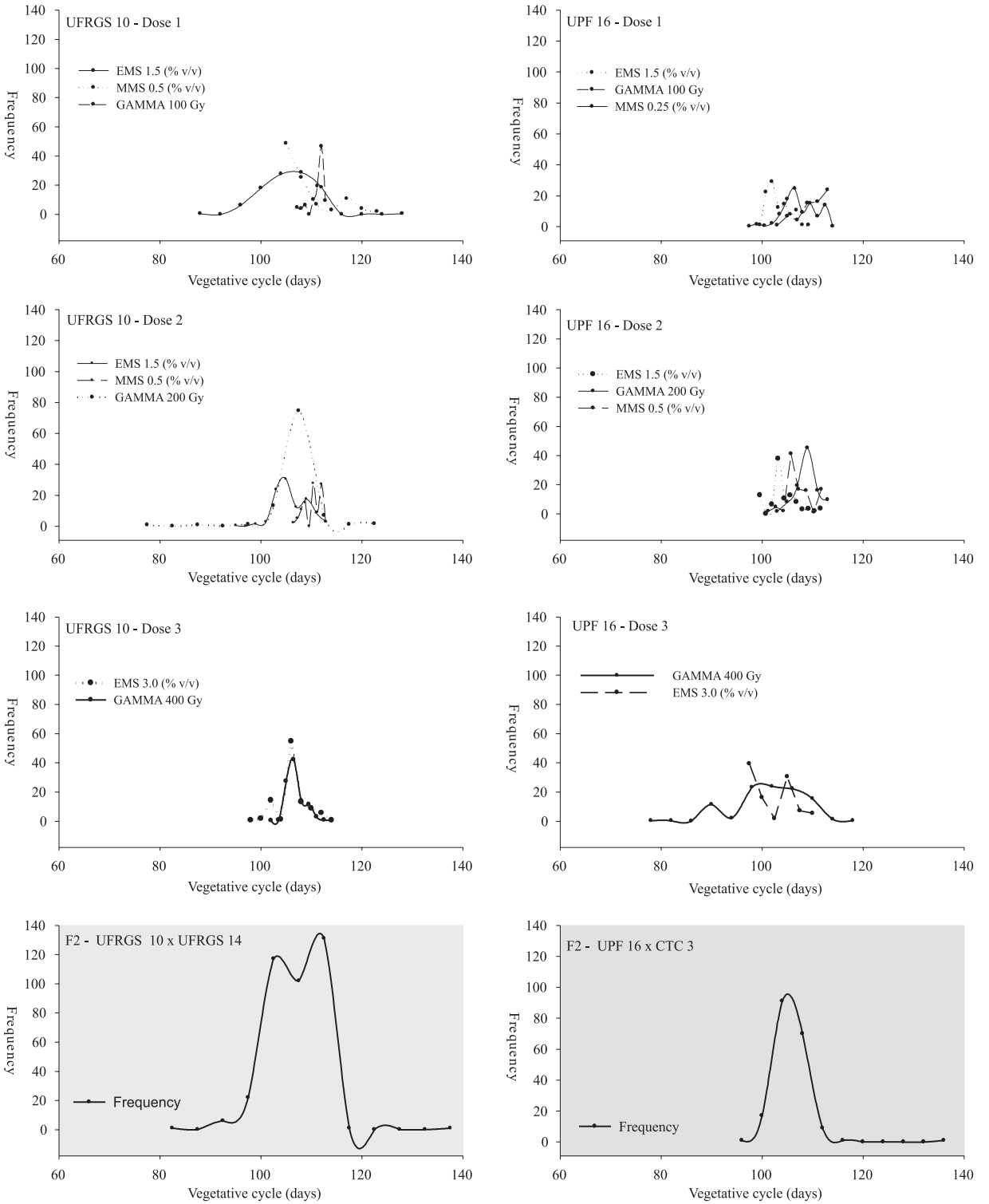

Figure 2. Frequency distribution of the total number of individuals evaluated for the trait vegetative cycle of plants in days for the genotypes UFRGS 10 and UPF 16, for each dose separately, in the segregating M_3 generation subjected to three mutagens: one physical (100; 200 and 400 G_y) and two chemical EMS (0.5; 1.5 and 3.0%) and MMS (0.25; 0.5 and 1.0%) in three different dose rates in relation to two populations derived from artificial crosses

estimate the regression equation (Cardellino and Siewerdt 1992).

No mutant population derived from genotype UFRGS 10 evidenced greater variability than the population derived from the artificial cross (UFRGS 10 x UFRGS 14) (Figures 1 and 2), indicating that the mutagens were not sufficiently effective to alter the genetic variability of this trait, except for the mutagen agent gamma rays (UFRGS 10 at dose 2 in the M_3 generation). On the other hand, the mutant population derived from cultivar UPF 16 at the highest gamma ray dose (UPF 16 – dose 3) seemed to be an incomparable opportunity of selection of earlier genotypes in the M_2 (Figure 1) and M_3 generation (Figure 2), in comparison to the population derived from the artificial cross (UPF 16 x CTC 3). Our results evidenced the existence of variability in segregating populations derived from artificial crosses and that the efficacy of mutagens to create variability in the widely cultivated genotypes of the south of Brazil, in agreement with Coimbra et al. (2004).

Results of the analysis of simple linear regression for vegetative cycle (Figure 3) indicated that the variation ascribed to the mutagen dose rate is eminently of the quadratic type in all cases, except for UFRGS 10 in the M_2 generation subjected to the physical mutagen. Normally, in the absence of lethal effects the number of mutants increases with rising doses (Chandhanamutta and Frey 1974, Scossiroli 1977).

A similar fact was reported for rice by Kim et al. (2003) who stated that an irradiation of gamma rays (^{60}Co) increased the mutation spectrum even in the callus stage. Data of the plant vegetative cycle (Figure 3) indicate that the response of oat to EMS application is quadratic demonstrating that at the lowest EMS doses the seeds presented linear increments; as the EMS dose rises, the increase gets smaller, tending to become stable at the highest dose rates, exclusively for this trait. The performance of the genotypes subjected to increasing mutagen doses is in line with observations of Ramirez-Calderón et al. (2003) on triticale, Coimbra et al. (2004) on oat and Mahar et al. (2003) on wheat. This indicates that, as the dose increases, the number of days from emergence to flowering increases to a certain point, with exception of UFGRS 10 which performed differently. Generally speaking, the same performance was observed by several of the above-mentioned authors. When subjected to MMS, independent of the segregating generation or genotype, the frequency of mutant genotypes increased at a dose rate of approximately 0.40 % (v/v), but at the highest dose all plants died (Figure 3).

Chemical and/or physical mutagens have been effectively used in plant improvement to induce biological diversity (Waugh et al. 2006). Besides, future research on induced mutations can become important in the area of functional genomics of many crops of agricultural importance.

CONCLUSIONS

Artificial crosses versus induced mutations are similarly effective at inducing alterations in the trait vegetative cycle, favoring increase in the number of phenotypic classes, for reduction as much as for the increment of the number of days from emergence to flowering. These procedures must be considered complementary and not excluding.

Indução de variabilidade genética em aveia

RESUMO - *Técnicas que maximizam a indução de variabilidade genética em plantas podem viabilizar a seleção de genótipos mais adaptados às condições de cultivo. No caso da aveia, estas técnicas são importantes para o desenvolvimento sustentável de programas de melhoramento genético para a região sul do Brasil. Para tanto, foram comparados os efeitos de três agentes mutagênicos (um físico: raios gama ^{60}Co e dois químicos: etilmetanossulfonato e metilmetanossulfonato) em duas gerações segregantes (M_2 e M_3), comparativamente com duas populações segregantes oriundas de cruzamentos artificiais, objetivando a comparação dos mecanismos de ampliação de variabilidade genética em aveia branca. Ambas metodologias ampliaram a variabilidade genética para o caráter ciclo vegetativo, sendo que o aumento na variabilidade foi observado tanto para o incremento quanto para redução no número de dias entre a emergência e o florescimento pleno, independente da técnica avaliada, podendo ser útil em programas de melhoramento genético na cultura da aveia branca.*

Palavras-Chave: *Avena sativa*, mutagênicos, curtose e assimetria.

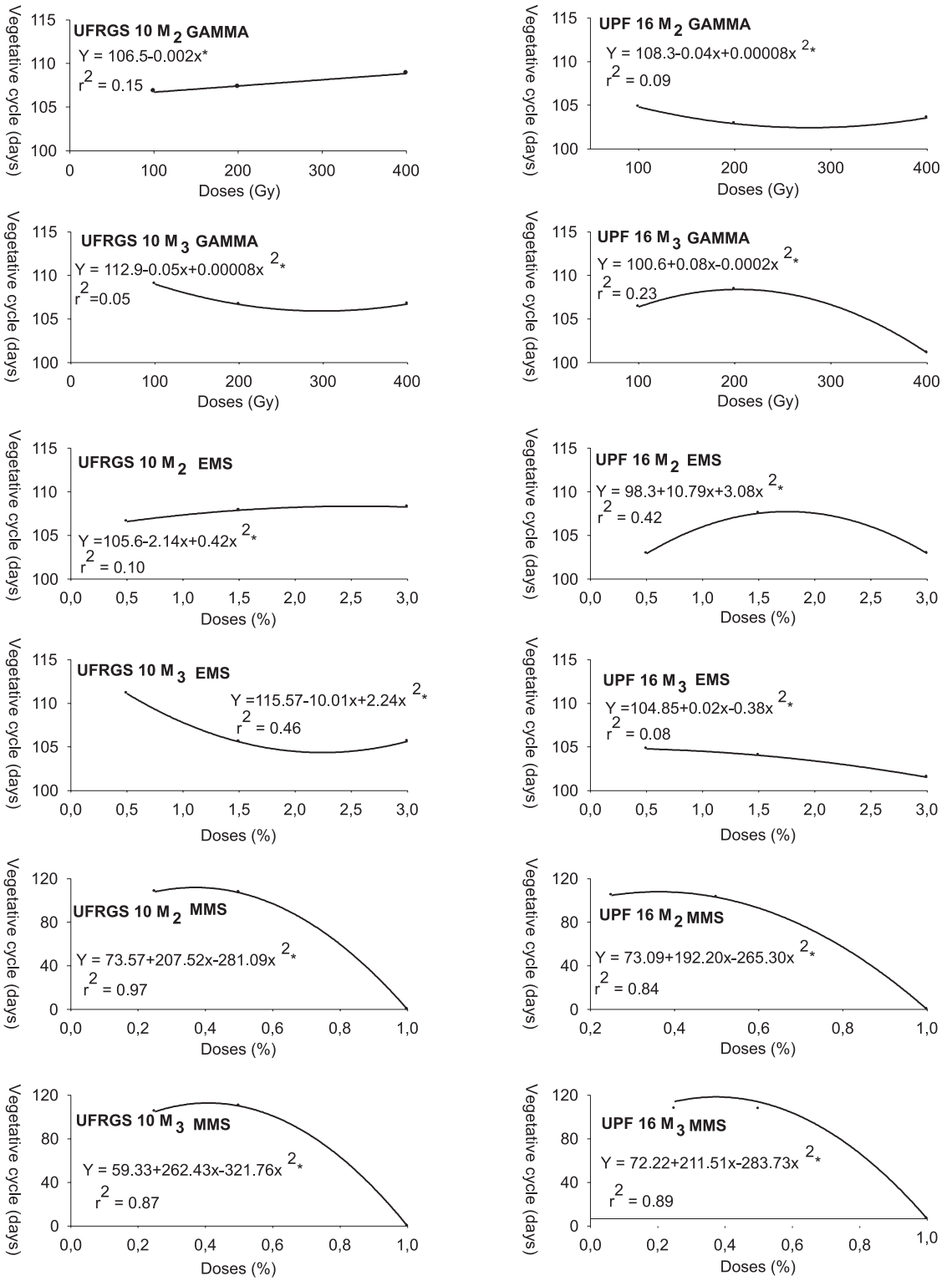

Figure 3. Equations of adjusted regression for the trait plant vegetative cycle in days for two distinct genetic constitutions of oat in the segregating M_2 and M_3 generations subjected to the physical (100. 200 and 400 G_y) and chemical mutagens EMS (0.5; 1.5 and 3.0%) and MMS (0.25; 0.5 and 1.0%) at three different dose rates

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