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PLANT BREEDING - Article

Identification of variability for agronomically important traits in rice mutant families

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ABSTRACT: The increase of yield potential in new rice (*Oryza sativa* L.) varieties has been a major challenge for genetic improvement. The generation of mutants, followed by their characterization, constitutes a great possibility to isolate and select genes and genotypes that present agronomic traits of interest. This study aimed to evaluate the effect of chemical mutagen ethyl methane sulphonate (EMS) on agronomic traits in 340 M_3 families of rice derived from BRS Querência cultivar. Seeds from each family and the original genotype BRS Querência were sown in the experimental field, and the characters main panicle length, main panicle weight, main panicle grain weight, flag leaf width and plant height were evaluated. Data were subjected to analysis of variance ($p \leq 0.05$), and a comparison of means was carried out by Dunnett's test at 5% significance. The results show that there is genetic variability among the mutant families, suggesting that the mutagen EMS at 1.5% is effective for generating mutants for all assessed traits. Among the characters, plant height was the most affected by the mutagen, which provided an increase in the character. For the main panicle length character, seven families showed means above the control; for main panicle weight and grain weight, four and six mutant families were observed, respectively, with a superior performance in comparison to the control.

Key words: *Oryza sativa*, ethyl methane sulphonate, mutagenic, variability.

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INTRODUCTION

Rice (*Oryza sativa* L.) is the second most-produced cereal in the world, representing a staple food source for more than half of the world's population. In 2012, Brazil was the largest rice producer (11.5 million tons) out of the Asian continent (FAOSTAT 2014), being a country with high export potential.

Rice consumption and cultivation in different regions, followed by selection of specific types of rice (variation in amylose content and/or aroma), generated a remarkable phenotypic diversity in the genus *Oryza* (Harland 1975). The genetic variability constitutes the principle of evolutionary processes and plant breeding, enabling its creation through mutations or expansion through hybridization, followed by selection of genotypes (Jennings et al. 1981; Jalata et al. 2011).

Mutation induction assists breeding programs by increasing variability and therefore obtaining genotypes with agronomically important traits, more adapted to different environmental conditions (Serrat et al. 2014). The spontaneous mutation rate in higher plants is very low, ranging from about 10^{-5} to 10^{-8} (Jiang and Ramachandran 2010). Accordingly, mutation induction is an important strategy to increase the frequency of mutations.

The use of physical and chemical mutagens may be of utmost importance for the development and incorporation of new genes and/or alleles of agronomic interest and may result in more stable genotypes, better adapted to climatic and soil conditions in Brazil. Numerous studies have shown positive results obtained with the use of mutagens, thus generating a genotype that may have agronomically desirable traits (Maluszynski et al. 1998; Martins et al. 2007). In Brazil, there are already commercial rice varieties obtained by Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (EPAGRI) through mutation induction: the first, called SCS 114 Andosan, and the second, SCS 118 Marques (Ishiy et al. 2006; Schiocchet et al. 2014).

Mutation can be induced by treatment with either physical mutagens, such as ionizing radiation, or chemical mutagens, including alkylating substances, for example, ethyl methane sulphonate (EMS) (Predieri 2001; Wu et al. 2005; Talebi et al. 2012). Among chemical mutagens, EMS is the most widely used due to its ease of use and potential for producing high frequency of nucleotide substitution, generating a relatively high density of irreversible mutations (Henikoff and Comai 2003; Talebi et al. 2012).

Thus, this study evaluated the effect of the chemical mutagen EMS on agronomic traits in 340 M_3 families of rice derived from BRS Querência.

MATERIAL AND METHODS

Obtaining the mutant families

Mutant genotypes were obtained by treating seeds of the rice variety BRS Querência with the chemical mutagen EMS at 1.5% (v/v) (0.15 M). Previous studies indicate that concentrations of 1.5 and 1.6% EMS were efficient in generating mutant populations of rice (Wu et al. 2005; Till et al. 2007). The variety BRS Querência, of early cycle, consists of modern American-type plants, with smooth leaves and grains. It has high tillering capacity, strong stems and stands out for presenting long panicle with many fertile spikelets (SOSBAI 2012).

The treatment consisted of soaking a sample of 40,000 seeds in distilled water. Seeds remained submerged for 6 h and then were subjected to the mutagen for 2 h. This soak time in distilled water was set in a preliminary test, in which seeds of BRS Querência were soaked in distilled water for 24 h and weighed every hour until constant weight. This procedure aimed to standardize the water content of seeds and activate their metabolism to favor the mutagenic action of the product. Soon after the application of the mutagenic agent, seeds remained for 1 h in running water and 1 h in still water (Coimbra et al. 1999, 2005). Afterwards, these seeds were sown in experimental field for generation advance.

Generation advance

Seeds of the M_1 generation were sown in 2009 for generation advance in an experimental field. Plants harvested in bulk gave rise to the M_2 population.

In the following year, in experimental field, 20,000 M_2 seeds were sown, aiming at the generation advance and obtaining a population of 1,500 plants. Each plant was harvested individually, giving rise to M_3 families.

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Field trial

In an experimental field, ten seeds of BRS Querência (control) were sown as well as a sample of 340 families randomly chosen, following a completely randomized design. Of these, we evaluated four plants of each family and the control, at random, where each plant was considered an experimental unit, totaling four replications.

For the field trial, the minimum cultivation system was employed according to the technical recommendations for irrigated rice (SOSBAI 2012).

Plants were evaluated for the traits: main panicle length (PL), in cm; main panicle weight (PW), in g; main panicle grain weight (PGW), in g; flag leaf width (FLW), in cm; and plant height (PH), in cm. Data were subjected to analysis of variance ($p \leq 0.05$), and the comparison between the means was performed by Dunnett's test at a 5% level of significance. Subsequently, multivariate analysis was run for the separation of families using cluster analysis by the Tocher's method (Rao 1952), based on the generalized Mahalanobis distance D^2, as well as the canonical variable analysis (Cruz and Regazzi 1997). The relative contribution of each variable in the determination of genetic dissimilarity was detected by the method proposed by Singh (1981). Statistical analyses were performed using the Genes software (Cruz 2001).

RESULTS AND DISCUSSION

The results presented by analysis of variance (Table 1) evidenced significant differences at 5% significance by F-test between mean values of mutant families for all traits, demonstrating the efficiency of mutation induction with EMS at 1.5%.

Results of Dunnett's test for the five traits studied are listed in Tables 2 to 6. These data indicated genetic variability among the families studied, i.e. for all the traits there were families with mean values significantly different from the control BRS Querência.

Considering the main panicle length (Table 2), seven families presented mean values higher than that of the control (24.72 cm), and only the M_3 213 family showed smaller main panicle length, while the others remained in the same length class of the control.

The panicle length is positively related to the number of grains per panicle and consequently is associated with grain yield (Ashfaq et al. 2012; Aditya and Bhartiya 2013; Ranawake et al. 2013). In this way, the mutagenic agent used promoted a significant increase in the main panicle length of some families (M_3 331, M_3 335, M_3 332, M_3 277, M_3 217, M_3 336 and M_3 218), which may be correlated with an increased grain yield (Table 2).

As for the main panicle weight (Table 3), four families (M_3 36, M_3 34, M_3 28 and M_3 2) had mean values higher than the control (3.19 g) and only one family (M_3 277) showed a lower mean. The other families exhibited the main panicle weight significantly similar to the control.

The main panicle weight directly and positively affects grain yield and is correlated with the number and weight of grains per panicle (Senapati et al. 2009; Abd Allah et al. 2010; Li et al. 2014), representing a desirable trait in rice breeding programs.

Families showing increased main panicle weight also presented a higher value of main panicle grain weigh than that of the control, suggesting that mutation interfered with the accumulation of photoassimilates (Tables 3, 4).

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Table 1. Summary of the analysis of variance for the traits main panicle length, main panicle weight, main panicle grain weight, flag leaf width and plant height of 340 M_3 mutant families and control BRS Querência. Federal University of Pelotas, Pelotas (RS), Brazil, 2015.

Source of variation	DF	Mean square				
		PL	PW	PGW	FLW	PH
Genotypes	340	12.45*	1.41*	1.22*	0.07*	70.39*
Residual	1,023	3.97	0.54	0.45	0.03	26.62
Overall mean		24.97	3.20	2.89	1.73	85.16
CV (%)		7.98	22.98	23.33	9.62	6.05

*Significant values at 5% error probability by F-test. DF = Degrees of freedom; PL = Main panicle length (cm); PW = Main panicle weight (g); PGW = Main panicle grain weight (g); FLW = Flag leaf width (cm); PH = Plant height (cm); CV (%) = coefficient of variation.

With regard to main panicle grains (Table 4), EMS caused an increase in the trait, with six mutant families exhibiting a better performance than the control. Whereas the control had a mean grain weight of 2.89 g, superior mutant families presented a mean value of 4.40 g. This result observed in these mutants can be related to a greater yield, since the grain mass is a major component of yield (Jeng et al. 2006; Song et al. 2007; Hasan et al. 2013; Karim et al. 2014). It is worth emphasizing that this increase in the main panicle grain weight will be relevant provided that there is no reduction in other components of grain yield.

For the trait flag leaf width (Table 5), the chemical agent used had a negative influence, since three families had lower mean values than that of the control, and the other families had mean values significantly similar to that of the control (1.67 cm). Morphological characteristics of leaves along with chlorophyll levels affect the leaf photosynthetic efficiency and directly influence the yield of rice grains (Zhang et al. 2009). The flag leaf width is correlated with grain yield in rice (Agahi et al. 2007; Aditya and Bhartiya 2013).

In relation to plant height (Table 6), 177 families had mean height higher than that of the control BRS Querência

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Table 2. Distribution of 340 M3 mutant families in relation to the control BRS Querência for the main panicle length. Federal University of Pelotas, Pelotas (RS), Brazil, 2015.

Main panicle length
M$_3$ families with mean values higher than that of the control BRS Querência
331, 335, 332, 277, 217, 336 and 218
M$_3$ families with mean values similar to that of the control BRS Querência (24.72 cm)
196, 36, 242, 297, 33, 236, 88, 18, 30, 132, 339, 31, 17, 204, 176, 227, 34, 284, 317, 2, 75, 52, 185, 178, 189, 265, 3, 10, 55, 184, 149, 177, 243, 125, 37, 171, 206, 40, 271, 35, 179, 190, 47, 19, 43, 197, 209, 320, 289, 172, 153, 188, 258, 337, 65, 80, 15, 91, 49, 148, 173, 238, 298, 5, 95, 24, 195, 253, 9, 46, 262, 276, 302, 72, 180, 245, 257, 268, 270, 105, 175, 39, 45, 131, 205, 26, 29, 83, 186, 300, 25, 32, 340, 84, 230, 288, 76, 79, 27, 111, 237, 273, 13, 234, 12, 170, 48, 78, 203, 69, 110, 116, 121, 246, 81, 140, 161, 187, 194, 210, 308, 67, 202, 286, 303, 92, 255, 283, 324, 4, 1, 135, 280, 50, 53, 89, 99, 107, 146, 207, 259, 87, 137, 232, 315, 240, 290, 193, 23, 28, 183, 314, 38, 254, 313, 115, 130, 323, 102, 106, 154, 200, 291, 127, 198, 157, 159, 318, 223, 261, 11, 208, 156, 133, 338, 225, 98, 118, 147, 275, 7, 241, 136, 166, 287, 152, 163, 214, 70, 108, 22, 112, 215, 272, 212, 231, 249, 267, 295, 139, 244, 327, 16, 211, 305, 63, 150, 224, 256, 142, 333, 8, 20, 167, 292, 322, 328, 239, 278, 73, 174, 248, 122, 145, 134, 141, 155, 182, 307, 21, 126, 251, 334, 94, 269, 124, 168, 221, 281, 151, 57, 86, 120, 299, 316, 129, 164, 274, 226, 306, 310, 60, 143, 199, 93, 85, 90, 109, 144, 219, 233, 247, 266, 51, 128, 228, 279, 309, 62, 114, 330, 119, 162, 264, 165, 169, 77, 101, 263, 41, 64, 117, 220, 66, 235, 301, 68, 325, 54, 42, 191, 103, 104, 97, 181, 14, 285, 96, 282, 58, 319, 6, 293, 312, 229, 192, 294, 59, 71, 252, 158, 304, 296, 329, 123, 222, 74, 260, 326, 321, 216, 56, 201, 113, 44, 311, 138, 250, 160, 100, 61 and 82
M$_3$ family with mean values lower than that of the control BRS Querência
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Table 3. Distribution of 340 M$_3$ mutant families in relation to the control BRS Querência for the main panicle weight. Federal University of Pelotas, Pelotas (RS), Brazil, 2015.

Main panicle weight
M$_3$ families with mean values higher than that of the control BRS Querência
36, 34, 28 and 2
M$_3$ families with mean values similar to that of the control BRS Querência (3.19 g)
17, 16, 30, 1, 236, 29, 33, 3, 219, 40, 206, 339, 18, 38, 15, 19, 9, 31, 176, 227, 22, 27, 37, 132, 25, 43, 52, 48, 197, 12, 35, 203, 214, 205, 196, 13, 223, 26, 55, 239, 177, 189, 149, 4, 187, 64, 230, 204, 21, 11, 237, 50, 179, 262, 45, 286, 147, 51, 148, 340, 209, 153, 75, 46, 232, 10, 7, 23, 280, 215, 111, 265, 140, 89, 8, 62, 253, 300, 24, 212, 298, 150, 255, 163, 240, 185, 194, 172, 173, 313, 107, 238, 268, 207, 125, 188, 210, 32, 234, 102, 131, 41, 66, 5, 47, 99, 159, 155, 166, 195, 291, 54, 167, 92, 108, 135, 49, 137, 76, 157, 20, 175, 290, 251, 283, 338, 88, 220, 243, 199, 228, 245, 126, 161, 164, 152, 94, 226, 14, 193, 323, 67, 136, 42, 320, 6, 184, 57, 257, 273, 242, 105, 73, 330, 146, 198, 247, 289, 337, 178, 224, 254, 292, 304, 241, 65, 270, 144, 83, 112, 77, 314, 312, 264, 208, 39, 70, 284, 235, 181, 287, 288, 336, 174, 170, 72, 271, 295, 310, 333, 143, 127, 130, 53, 261, 95, 186, 315, 221, 103, 154, 118, 334, 180, 211, 256, 183, 246, 171, 302, 128, 139, 59, 202, 114, 63, 82, 200, 307, 267, 58, 133, 165, 116, 134, 158, 263, 104, 156, 106, 324, 101, 233, 80, 142, 282, 145, 168, 169, 269, 331, 301, 97, 91, 121, 109, 325, 192, 141, 303, 276, 87, 299, 297, 151, 317, 318, 68, 190, 119, 138, 258, 319, 93, 69, 309, 249, 296, 162, 90, 182, 306, 160, 275, 244, 122, 329, 98, 252, 124, 84, 279, 293, 308, 110, 294, 225, 229, 327, 129, 222, 248, 113, 60, 120, 85, 79, 328, 217, 335, 71, 115, 322, 201, 316, 231, 266, 272, 326, 332, 216, 218, 321, 56, 285, 250, 305, 100, 78, 44, 281, 191, 123, 74, 278, 274, 117, 96, 86, 61, 311, 81, 259, 260 and 213
M$_3$ family with mean values lower than that of the control BRS Querência
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(76.25 cm), considered a semidwarf genotype. No family showed a lower mean value, and the other families had mean height significantly equal to that presented by the control.

Our results suggest that possibly the mutagen promoted changes in alleles that confer short height, causing this increase. The *SD1-Semidwarf1* gene, isolated from semidwarf rice, has shown to be related to gibberellin biosynthesis (Monna et al. 2002; Sasaki et al. 2002), which is a key hormone for controlling plant height. It should be noted that the mutation *sd1* is considered recessive (Ashikari et al. 2002).

Comparing the results for the five traits evaluated in the study, plant height was the most affected by the mutagen EMS, with a higher number of families (52.05%) differing from the control BRS Querência (Table 6).

According to results obtained by Martins et al. (2005), the induction of ionizing mutation was effective in reducing plant height in rice genotypes. The use of EMS, at a dose of 0.04 M, in different rice mutant generations leads to an increment of the trait plant height (Siddiqui and Singh 2010).

In general, cereal breeding programs tend to select short plants, because tall plants without thick stems usually have low yield potential and are more susceptible to lodging (Ni et al. 2000). However, families with increased height should not be immediately discarded,

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Table 4. Distribution of 340 M_3 mutant families in relation to the control BRS Querência for the main panicle grain weight. Federal University of Pelotas, Pelotas (RS), Brazil, 2015.

Main panicle grain weight
M_3 families with mean values higher than that of the control BRS Querência
36, 34, 2, 28, 30 and 17
M_3 families with mean values similar to that of the control BRS Querência (2.89 g)
1, 16, 236, 29, 206, 33, 339, 3, 18, 40, 38, 9, 15, 19, 197, 22, 176, 31, 205, 25, 43, 214, 37, 27, 232, 227, 132, 48, 223, 52, 203, 12, 289, 13, 237, 26, 196, 64, 51, 177, 239, 230, 21, 189, 55, 340, 45, 4, 11, 148, 179, 35, 187, 286, 147, 204, 209, 50, 153, 46, 215, 262, 265, 149, 10, 280, 253, 140, 75, 111, 212, 23, 300, 62, 240, 210, 8, 298, 207, 163, 185, 172, 194, 255, 313, 107, 188, 150, 234, 24, 173, 66, 152, 242, 245, 32, 268, 125, 5, 159, 291, 54, 41, 243, 167, 102, 238, 166, 251, 338, 99, 126, 7, 47, 199, 290, 226, 157, 135, 220, 175, 228, 155, 20, 42, 137, 164, 108, 161, 195, 76, 283, 219, 49, 67, 14, 323, 136, 184, 92, 94, 57, 73, 247, 105, 88, 198, 337, 273, 304, 292, 146, 257, 330, 193, 224, 320, 6, 241, 131, 178, 181, 144, 235, 211, 208, 65, 336, 312, 112, 77, 83, 264, 270, 89, 70, 288, 174, 287, 72, 271, 333, 53, 63, 127, 170, 284, 314, 334, 130, 103, 310, 246, 221, 118, 295, 143, 154, 261, 39, 128, 95, 183, 186, 315, 114, 180, 256, 58, 202, 59, 158, 139, 200, 307, 324, 82, 233, 267, 254, 106, 101, 142, 133, 331, 165, 303, 134, 263, 282, 116, 145, 301, 171, 156, 266, 302, 98, 168, 309, 80, 109, 192, 269, 151, 121, 252, 141, 325, 169, 249, 319, 68, 138, 87, 318, 299, 276, 69, 119, 162, 91, 296, 90, 244, 306, 182, 104, 93, 122, 329, 258, 275, 248, 190, 160, 225, 113, 293, 297, 229, 317, 308, 222, 217, 124, 129, 84, 85, 294, 327, 335, 120, 97, 328, 279, 201, 332, 110, 115, 79, 218, 71, 272, 316, 60, 321, 322, 231, 250, 56, 216, 326, 285, 100, 305, 281, 123, 44, 191, 74, 78, 117, 278, 96, 274, 61, 86, 311, 213, 259, 260, 277 and 81
M_3 family with mean values lower than that of the control BRS Querência
No family had a mean value lower than the control

Table 5. Distribution of 340 M_3 mutant families in relation to the control BRS Querência for the flag leaf width. Federal University of Pelotas, Pelotas (RS), Brazil, 2015.

Flag leaf width
No family had a mean value higher than the control
M_3 families with mean values similar to that of the control BRS Querência (1.67 cm)
46, 86, 93, 112, 9, 89, 139, 183, 129, 130, 196, 280, 17, 30, 47, 52, 79, 88, 102, 133, 178, 190, 236, 45, 48, 53, 58, 80, 91, 92, 105, 115, 122, 125, 137, 174, 177, 184, 185, 187, 336, 15, 29, 36, 64, 83, 87, 101, 103, 109, 111, 119, 168, 189, 206, 221, 227, 238, 245, 254, 313, 10, 25, 34, 39, 54, 66, 114, 124, 127, 132, 172, 180, 186, 195, 224, 260, 271, 16, 27, 28, 40, 43, 55, 82, 85, 94, 95, 98, 107, 108, 120, 141, 148, 149, 166, 203, 230, 243, 297, 1, 18, 22, 31, 35, 37, 42, 49, 50, 65, 69, 77, 99, 104, 126, 134, 146, 173, 179, 182, 202, 255, 262, 263, 298, 2, 12, 21, 26, 62, 68, 78, 84, 121, 123, 135, 152, 163, 171, 175, 228, 265, 270, 286, 287, 290, 295, 268, 3, 5, 8, 19, 23, 24, 41, 97, 110, 117, 118, 142, 144, 145, 161, 169, 170, 176, 194, 208, 209, 223, 226, 233, 246, 251, 256, 267, 274, 281, 315, 317, 7, 11, 32, 33, 38, 57, 59, 71, 72, 106, 131, 136, 138, 153, 165, 188, 214, 215, 237, 249, 258, 261, 273, 276, 288, 300, 305, 308, 314, 4, 20, 56, 113, 116, 156, 159, 200, 204, 205, 219, 235, 275, 277, 291, 306, 67, 70, 90, 96, 100, 150, 157, 167, 232, 244, 252, 283, 284, 285, 296, 302, 304, 310, 318, 324, 326, 73, 81, 147, 154, 162, 211, 241, 250, 259, 301, 307, 312, 338, 76, 128, 151, 155, 191, 197, 198, 199, 201, 218, 239, 240, 247, 266, 269, 279, 292, 293, 320, 322, 325, 60, 63, 207, 220, 222, 229, 231, 248, 257, 303, 311, 319, 330, 337, 6, 13, 61, 140, 158, 289, 294, 299, 323, 340, 14, 75, 164, 192, 193, 212, 282, 335, 216, 217, 234, 242, 316, 328, 332, 339, 210, 264, 331, 253, 272, 278, 333, 334, 321, 327, 309, 51, 181, 329, 74, 160 and 225
M_3 family with mean values lower than that of the control BRS Querência
213, 44 and 143

and other traits should also be considered, such as stem thickness. It should be emphasized that taller plants have the benefit of reducing the amount and quality of weeds (Balbinot Junior et al. 2003).

Many factors influence the induced mutation rate, such as concentration of mutagen, mutagen exposure time, washing time, types of organs treated (seed or pollen) and species or genotype chosen. Seed structure may vary from species to species, which can influence the efficacy of the mutagenic agent. For example, in paddy rice, the chemical product used must penetrate the husk first and then interact with the embryo, inducing mutation (Xin et al. 2008).

The cluster analysis by Tocher's method (Table 7) revealed the formation of seven groups. The first one grouped most families of mutants together with the control BRS Querência; the second group was composed of five families (M_3 331, M_3 332, M_3 335, M_3 217 and M_3 277); the third one grouped three families (M_3 44, M_3 143 and M_3 160); the fourth (M_3 218 and M_3 336) and fifth (M_3 89 and M_3 219) groups were formed by two families each, while the sixth and seventh groups had one family each (M_3 213 and M_3 289, respectively).

Regarding the relative contribution of each trait to the genetic dissimilarity among mutant families (Table 8), the 340 families evaluated as well as the control presented, in descending order of contribution, the traits: main panicle length, main panicle grain weight, flag leaf width, plant height and main panicle weight. Among them, the traits main panicle length, main panicle grain weight and flag leaf width contributed with 69.49% to genetic dissimilarity, though the traits plant height and main panicle weight contributed with only 30.51%.

The trait that contributed most to the genetic dissimilarity was the main panicle length (31.07%), with values ranging from 36.07 cm (M_3 331 family) to 18.62 cm (M_3 213 family). The second most important variable for genetic dissimilarity was the main panicle grain weight (19.31%), with values ranging from 4.58 g (M_3 36 family) to 1.67 g (M_3 81 family).

The variances (eigenvalues), percentage variances and cumulative variances of canonical variables are presented in Table 9. As the estimates of eigenvalues, corresponding to the first three canonical variables, obtained 80.41% of the total variance of the analyzed traits, it was possible to explain satisfactorily the variability among the evaluated mutant families and represent them in a three-dimensional scatter plot (Figure 1).

The graphic dispersion indicated the distinction of mutant families into groups and can be used as a strategy to select different families to be used in artificial crosses in breeding programs. The groups formed by the graphic dispersion of scores (Figure 1) followed the same trend of the groups obtained by the Tocher's optimization method (Table 7), with the exception of group III (Tocher), which was not separated by the canonical variable analysis, appearing clustered with genotypes from group I.

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Table 6. Distribution of 340 M_3 mutant families in relation to the control BRS Querência for the plant height. Federal University of Pelotas, Pelotas (RS), Brazil, 2015.

Plant height
M_3 families with mean values higher than that of the control BRS Querência
33, 9, 36, 43, 26, 39, 47, 25, 48, 23, 17, 19, 46, 230, 11, 15, 29, 20, 37, 31, 40, 22, 45, 2, 5, 16, 52, 212, 223, 3, 24, 42, 84, 196, 12, 21, 34, 53, 66, 6, 7, 28, 262, 1, 8, 145, 18, 115, 41, 13, 178, 179, 4, 162, 204, 207, 222, 236, 60, 118, 205, 92, 202, 291, 259, 56, 215, 30, 287, 298, 283, 35, 38, 243, 177, 313, 10, 14, 51, 234, 255, 256, 307, 129, 188, 227, 297, 320, 143, 182, 140, 147, 271, 295, 104, 149, 172, 221, 245, 246, 263, 267, 272, 150, 315, 27, 32, 50, 132, 168, 218, 268, 301, 304, 314, 161 and 192
M_3 families with mean values similar to that of the control BRS Querência (76.25 cm)
49, 185, 276, 299, 206, 261, 77, 187, 233, 316, 336, 281, 112, 241, 332, 70, 197, 122, 79, 183, 186, 214, 251, 266, 289, 300, 308, 311, 75, 91, 144, 148, 101, 105, 146, 253, 254, 280, 98, 158, 173, 238, 265, 194, 99, 114, 166, 176, 198, 136, 171, 330, 108, 124, 189, 235, 55, 191, 200, 226, 237, 264, 328, 152, 102, 107, 155, 224, 78, 103, 121, 228, 65, 81, 89, 100, 126, 248, 324, 193, 270, 305, 338, 151, 184, 275, 303, 310, 317, 63, 180, 76, 110, 113, 117, 244, 257, 258, 292, 294, 82, 83, 74, 95, 109, 142, 163, 286, 64, 116, 203, 240, 260, 273, 93, 111, 153, 54, 170, 174, 285, 309, 339, 125, 190, 239, 44, 130, 208, 269, 71, 169, 86, 73, 302, 326, 331, 229, 128, 225, 319, 325, 164, 159, 219, 157, 199, 220, 85, 139, 209, 247, 249, 284, 321, 131, 175, 61, 68, 282, 323, 327, 58, 127, 57, 217, 288, 87, 80, 154, 242, 277, 290, 306, 329, 201, 232, 322, 120, 278, 69, 279, 141, 312, 340, 106, 137, 94, 134, 59, 135, 318, 333, 97, 119, 165, 334, 195, 293, 72, 138, 216, 211, 252, 296, 337, 210, 62, 90, 167, 133, 96, 67, 156, 250, 274, 231, 335, 88, 181, 160, 123 and 213
M_3 family with mean values lower than that of the control BRS Querência
No family had a mean value lower than the control

The similar result obtained by the canonical variable analysis strengthens the reliability of the data obtained by Tocher's clustering technique (Benitezet al. 2011).

Table 7. Grouping of 340 M_3 mutant families and the variety BRS Querência by Tocher's method based on the Mahalanobis distance, considering traits evaluated in M_3 families. Federal University of Pelotas, Pelotas (RS), Brazil, 2015.

Groups	Families														
1	333	334	323	164	282	247	73	292	128	199	220	240	157	338	159
	198	76	155	330	310	325	319	241	269	136	70	151	229	154	57
	312	273	208	194	235	300	63	32	257	193	153	161	200	150	144
	163	99	268	226	251	283	142	261	170	173	188	146	314	244	324
	291	169	306	107	228	290	175	166	288	249	126	108	286	255	233
	270	49	301	315	295	267	116	275	256	307	246	298	287	224	148
	65	68	77	165	172	118	265	248	106	294	71	293	279	285	121
	238	135	59	152	134	127	209	50	263	114	318	111	158	179	10
	95	83	55	303	299	186	105	313	237	141	180	90	94	296	174
	189	109	85	113	201	202	182	221	326	102	101	103	124	145	24
	168	185	243	262	149	125	271	177	98	308	120	245	320	5	215
	92	187	304	162	276	191	8	4	252	322	72	184	305	69	302
	54	137	280	122	130	87	132	204	110	117	258	171	281	192	211
	119	195	227	147	207	176	41	104	203	140	58	53	27	328	35
	183	167	264	12	45	20	18	52	178	112	156	131	139	222	42
	31	230	37	91	316	66	138	284	234	56	100	21	60	97	216
	14	205	3	206	67	80	40	11	214	239	197	337	23	38	13
	75	223	62	6	15	236	19	340	250	321	254	96	309	129	311
	7	84	212	327	61	64	253	78	93	272	26	47	46	79	115
	133	25	48	22	190	29	329	30	231	317	266	260	43	196	39
	210	278	274	74	82	17	33	2	232	1	339	225	9	81	34
	123	297	259	86	242	51	181	36	16	28	88	341 (BRS Querência)			
2	331	332	335	217	277										
3	44	143	160												
4	218	336													
5	89	219													
6	213														
7	289														

Table 8. Estimates of the relative contribution of each trait for genetic divergence between rice mutant families, based on the partitioning of total D^2. Federal University of Pelotas, Pelotas (RS), Brazil, 2015.

Variable	S.j	S.j value (%)
Main panicle length	129,199.84	31.07
Main panicle grain weight	80,291.68	19.31
Flag leaf width	79,454.55	19.11
Plant heigth	72,708.77	17.49
Main panicle weight	54,141.17	13.02

S.j = Estimates of the relative contribution of each trait.

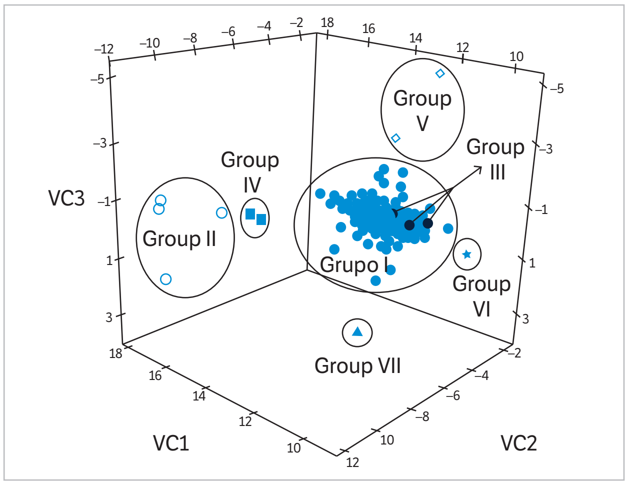

Figure 1. Scatter plot of scores of 340 M_3 mutant families and the control BRS Querência along the first three canonical axes (CV1, CV2 and CV3). Federal University of Pelotas, Pelotas (RS), Brazil, 2015.

CONCLUSION

The mutation induction by EMS, at 1.5%, is capable of generating mutants with superior traits. Of a total of 340 families, for the trait main panicle length, seven families (2.06%) presented mean values higher than that of the control, as to the traits main panicle weight and main panicle grain weight, there were observed four (1.18 %) and six (1.76%) mutant families, respectively, with better performance than that of the control.

Table 9. Variances (eigenvalues), percentage variances and cumulative variances of canonical variables, aiming to estimate the dissimilarity among rice mutant families. Federal University of Pelotas, Pelotas (RS), Brazil, 2015.

Canonical variables	Variances (eigenvalues)	Percentage variances	Cumulative variances
1	1.51	42.11	42.11
2	0.73	20.32	62.43
3	0.64	17.97	80.41
4	0.42	11.78	92.19
5	0.28	7.81	100.00

Among the traits, plant height was the most affected by the mutagenic agent, which provided the increase in the trait.

There is genetic dissimilarity among rice families, and the traits contributing most to it are the main panicle length, main panicle grain weight and flag leaf width.

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REFERENCES

Abd Allah, A. A., Ammar, M. H. and Badawi, A. T. (2010). Screening rice genotypes for drought resistance in Egypt. Journal of Plant Breeding and Crop Science, 2, 205-215.

Aditya, J. P. and Bhartiya, A. (2013). Genetic variability, correlation and path analysis for quantitative characters in rainfed upland rice of Uttarakhand Hills. Journal of Rice Research, 6, 24-34.

Agahi, K., Fotokian, M. and Farshadfar, E. (2007). Correlation and path coefficient analysis for some yield-related traits in rice genotypes (*Oryza sativa* L.). Asian Journal of Plant Sciences, 6, 513-517. http://dx.doi.org/10.3923/ajps.2007.513.517.

Ashfaq, M., Khan, A. S., Khan, S. H. U. and Ahmad, R. (2012). Association of various morphological traits with yield and genetic divergence in rice (*Oryza sativa*). International Journal of Agriculture & Biology, 14, 55-62.

Ashikari, M., Sasaki, A., Ueguchi-Tanaka, M., Itoh, H., Nishimura, A., Swapan, D., Ishiyama, K., Saito, T., Kobayashi, M., Khush, G. S., Kitano, H. and Matsuoka, M. (2002). Loss-of-function of a rice gibberellin biosynthetic gene, GA20 oxidase (GA20ox-2), led to the rice "Green Revolution". Breeding Science, 52, 143-150. http://dx.doi.org/10.1270/jsbbs.52.143.

Balbinot Junior, A. A., Fleck, N. G., Menezes, V. G. and Agostinetto, D. (2003). Competitividade de cultivares de arroz irrigado com cultivar simuladora de arroz-vermelho. Pesquisa Agropecuária Brasileira, 38, 53-59. http://dx.doi.org/10.1590/S0100-204X2003000100007.

Benitez, L. C., Rodrigues, I. C. S., Arge, L. W. P., Ribeiro, M. V. and Braga, E. J. B. (2011). Análise multivariada da divergência genética de genótipos de arroz sob estresse salino durante a fase vegetativa.

Revista Ciência Agronômica, 42, 409-416. http://dx.doi.org/10.1590/S1806-66902011000200021.

Coimbra, J. L. M., Carvalho, F. I. F., Oliveira, A. C., Silva, J. A. G. and Lorencetti, C. (2005). Comparação entre mutagênicos químico e físico em populações de aveia. Ciência Rural, 35, 12-18. http://dx.doi.org/10.1590/S0103-84782005000100008.

Coimbra, J. L. M., Marchioro, V. S., Lorencetti, C., Azevedo, R., Carvalho, F. I. F. and Oliveira, A. C. (1999). Comparação dos efeitos de agentes mutagênicos na geração M1 em genótipos fixos de aveia (*Avena sativa* L.). Revista Brasileira de Agrociência, 5, 12-18.

Cruz, C. D. (2001). Programa genes: aplicativo computacional em genética e estatística. Viçosa: UFV.

Cruz, C. D. and Regazzi, A. J. (1997). Divergência genética. In C. D. Cruz e A. J. Regazzi. Métodos biométricos aplicados ao melhoramento genético (p. 287-324). Viçosa: Imprensa Universitária.

Harland, J. R. (1975). Crops and man. Madison: American Society of Agronomy.

Hasan, M. J., Kulsum, M. U., Akter, A., Masuduzzaman, A. S. M. and Ramesha, M. S. (2013). Genetic variability and character association for agronomic traits in hybrid rice (*Oryza sativa* L.). Bangladesh Journal of Plant Breeding and Genetics, 24, 45-51. http://dx.doi.org/10.3329/bjpbg.v24i1.16999.

Henikoff, S. and Comai, L. (2003). Single-nucleotide mutations for plant functional genomics. Annual Review of Plant Biology, 54, 375-401.

Ishiy, T., Schiocchet, M. S., Bacha, R. E., Alfonso-Morel, D., Tulman, A. N. and Knoblauch, R. (2006). Rice mutant cultivar SCS114 andosan. Plant Mutation Reports, 2, 25.

Jalata, Z., Ayana, A. and Zeleke, H. (2011). Variability, heritability and genetic advance for some yield and yield related traits in Ethiopian barley (*Hordeum vulgare* L.) landraces and crosses. International Journal of Plant Breeding and Genetics, 5, 44-52. http://dx.doi.org/10.3923/ijpbg.2011.44.52.

Jeng, T. L., Tseng, T. H., Wang, C. S., Chen, C. L. and Sung, J. M. (2006). Yield and grain uniformity in contrasting rice genotypes suitable for different growth environments. Field Crops Research, 99, 59-66. http://dx.doi.org/10.1016/j.fcr.2006.03.005.

Jennings, P. R., Coffman, W. R. and Kauffman, H. E. (1981). Mejoramiento de arroz. Cali: Centro Internacional de Agricultura Tropical.

Jiang, S. Y. and Ramachandran, S. (2010). Natural and artificial mutants as valuable resources for functional genomics and molecular breeding. International Journal of Biological Sciences, 6, 228-251. http://dx.doi.org/10.7150/ijbs.6.228.

Karim, D., Siddique, N. E. A., Sarkar, U., Hasnat, Z. and Sultana, J. (2014). Phenotypic and genotypic correlation co-efficient of quantitative characters and character association of aromatic rice. Journal of Bioscience and Agriculture Research, 1, 34-46.

Li, G., Zhang, J., Yang, C., Song, Y., Zheng, C., Wang, S., Liu, Z. and Ding, Y. (2014). Optimal yield-related attributes of irrigated rice for high yield potential based on path analysis and stability analysis. The Crop Journal, 2, 235-243. http://dx.doi.org/10.1016/j.cj.2014.03.006.

Maluszynski, M., Ahoowalia, B., Ashri, A., Nichterlein, K. and Van Zanten, L. (1998). Induced mutations in rice breeding and germplasm enhancement. Proceedings of the 19th Session of the International Rice Commission; Cairo, Egypt.

Martins, A. F., Vieira, E. A., Kopp, M. M., Luz, V. K., Carvalho, M. F., Branco, J. S. C., Cruz, R. P., Carvalho, F. I. F. and Oliveira, A. C. (2007). Caracterização de famílias mutantes de arroz para tolerância ao frio nos períodos vegetativo e reprodutivo. Bragantia, 66, 227-233. http://dx.doi.org/10.1590/S0006-87052007000200006.

Martins, A. F., Zimmer, P. D., Oliveira, A. C., Carvalho, F. I. F., Vieira, E. A., Carvalho, M. F., Martins, L. F. and Fonseca, F. S. (2005). Variabilidade para caracteres morfológicos em mutantes de arroz. Ciência e Agrotecnologia, 29, 1215-1223. http://dx.doi.org/10.1590/S1413-70542005000600016.

Monna, L., Kitazawa, N., Yoshino, R., Suzuki, J., Masuda, H., Maehara, Y., Tanji, M., Sato, M., Nasu, S. and Minobe, Y. (2002). Positional cloning of rice semidwarfing gene. *sd-1*: rice "green revolution gene" encodes a mutant enzyme involved in gibberellin synthesis. DNA Research, 9, 11-17.

Ni, H., Moody, K., Robles, R. P., Paller, E. C. and Lales, J. S. (2000). *Oryza sativa* plant traits conferring competitive ability against weeds. Weed Science, 48, 200-204.

Predieri, S. (2001). Mutation induction and tissue culture in improving fruits. Plant Cell, Tissue and Organ Culture, 64, 185-210.

Ranawake, A. L., Amarasingha, U. G. S. and Dahanayake, N. (2013). Agronomic characters of some traditional rice (*Oryza sativa* L.) cultivars in Sri Lanka. Journal of the University of Ruhuna, 1, 3-9. http://dx.doi.org/10.4038/jur.v1i1.6150.

Rao, R. C. (1952). Advanced statistical methods in biometric research. New York: John Wiley and Sons.

Sasaki, A., Ashikari, M., Ueguchi-Tanaka, M., Itoh, H., Nishimura, A., Swapan, D., Ishiyama, K., Saito, T., Kobayashi, M., Khush, G. S., Kitano, H. and Matsuoka, M. (2002). Green revolution: a mutant gibberellin-synthesis gene in rice – new insight into the rice variant that helped to avert famine over thirty years ago. Nature, 416, 701-702. http://dx.doi.org/10.1038/416701a.

Schiocchet, M. A., Noldin, J. A., Raimondi, J. V., Neto, A. T., Marschalek, R., Wickert, E., Martins, G. N., Hicke, E., Knoblauch, R., Scheuermann, K. K., Eberhardt, D. S. and Andrade, A. (2014). SCS118 Marques - new rice cultivar obtained through induced mutation. Crop Breeding and Applied Biotechnology, 14, 68-70. http://dx.doi.org/10.1590/S1984-70332014000100012.

Senapati, B. K., Roy, S., De D. K. and Pal, S. (2009). Selection criteria for high yield in early segregating generation of rice (*Oryza sativa* L.) crosses. Journal of Crop and Weed, 5, 12-14.

Serrat, X., Esteban, R., Guibourt, N., Moysset, L., Nogués, S. and Lalanne, E. (2014). EMS mutagenesis in mature seed-derived rice *calli* as a new method for rapidly obtaining TILLING mutant populations. Plant Methods, 10, 1-14. http://dx.doi.org/10.1186/1746-4811-10-5.

Siddiqui, S. A. and Singh, S. (2010). Induced genetic variability for yield and yield traits in basmati rice. World Journal of Agricultural Sciences, 6, 331-337.

Singh, D. (1981). The relative importance of characters affecting genetic divergence. Indian Journal of Genetic Plant Breeding, 41, 237-245.

Sociedade Sul Brasileira de Arroz Irrigado (2012). Arroz irrigado: recomendações técnicas da pesquisa para o Sul do Brasil. Itajaí: SOSBAI.

Song, X. J., Huang, W., Shi, M., Zhu, M. Z. and Lin, H. X. (2007). A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. Nature Genetics, 39, 623-630. http://dx.doi.org/10.1038/ng2014.

Talebi, A. B., Talebi, A. B. and Shahrokhifar, B. (2012). Ethyl methane sulphonate (EMS) induced mutagenesis in malaysian rice (cv. MR219) for lethal dose determination. American Journal of Plant Sciences, 3, 1661-1665. http://dx.doi.org/10.4236/ajps.2012.312202.

The Statistics Division of the Food and Agriculture Organization of the United Nations (2014); [accessed 2014 Jun 1]. http://faostat.fao.org/#

Till, B. J., Cooper, J., Tai, T. H., Colowit, P., Greene, E. A., Henikoff, S. and Comai, L. (2007). Discovery of chemically induced mutations in rice by TILLING. BMC Plant Biology, 7, 19. http://dx.doi.org/10.1186/1471-2229-7-19.

Wu, J. L., Wu, C., Lei, C., Baraoidan, M., Bordeos, A., Madamba, M. R. S., Ramos-Pamplona, M., Mauleon, R., Portugal, A., Ulat, V. J., Bruskiewich, R., Wang, G., Leach, J., Khush, G. and Leung, H. (2005). Chemical- and irradiation-induced mutants of indica rice IR64 for forward and reverse genetics. Plant Molecular Biology, 59, 85-97. http://dx.doi.org/10.1007/s11103-004-5112-0.

Xin, Z., Wang, M. L., Barkley, N. A., Burow, G., Franks, C., Pederson, G. and Burke, J. (2008). Applying genotyping (TILLING) and phenotyping analyses to elucidate gene function in a chemically induced sorghum mutant population. BMC Plant Biology, 8, 1-14. http://dx.doi.org/10.1186/1471-2229-8-103.

Zhang, G. H., Xu, Q., Zhu, X. D., Qian, Q. and Xue, H. W. (2009). Shallot-Like1 is a Kanadi transcription factor that modulates rice leaf rolling by regulating leaf abaxial cell development. Plant Cell, 21, 719-735. http://dx.doi.org/10.1105/tpc.108.061457.