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RESEARCH ARTICLE

ETHYL METHANE SULFONATE AND ITS EFFECTS ON MORPHOLOGICAL TRAITS OF DUAL-PURPOSE WHEAT

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ABSTRACT

The aim of this study was to evaluate different doses of *Ethyl Methane Sulfonate* in dual-purpose wheat seeds, and their answers on morphological traits, when measured in different evaluation periods. The experiment was conducted at 2013 crop year, in the experimental field of the Federal University of Santa Maria Campus Frederico Westphalen – RS, Brazil. The experimental design was a randomized block in a factorial design, two dual-purpose wheat genotypes x five doses of *Ethyl Methane Sulfonate* x four periods of evaluation, with four replications. Analysis of variance revealed a significant interaction among dual-purpose wheat genotypes x doses of *Ethyl Methane Sulfonate* x evaluation periods for leaf area and root diameter. It was observed interaction among dual-purpose wheat genotypes x evaluation periods of traits number of fertile tillers, plant height, tillers diameter and chlorophyll content. There was no interaction for root length. The dual-purpose wheat genotypes have differential behavior among themselves and evaluation periods, when the seeds subjected to the application of *Ethyl Methane Sulfonate* mutagen agent. Increasing doses of *Ethyl Methane Sulfonate* agent reveals variability for leaf area, root diameter, number of fertile tillers, tillers diameter and chlorophyll content; however, it has a negative effect on the length of the main root.

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INTRODUCTION

The dual-purpose wheat (*Triticum aestivum* L.) is a cereal characterized by providing forage during the vegetative growth, and also enable the production of grain (Martin*et al*., 2010). It is an excellent alternative to overcome periods of forage shortage and economically optimize farms of Brazilian South (Meinerz *et al*., 2011). Its importance is due to the use of traditional pastures, pre-dried, hay and silage (Fontaneli *et al*., 2009). The diversity of dual-purpose wheat genotypes commercially available indicating to the south of Brazil is limited. Among the genotypes can be highlighted the BRS Figueira, BRS Umbu, BRSGuatambu, BRS Tarumã and BRS 227 (Cairão *et al*., 2014).

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The genotypes with dual aptitude must have as characteristics the speedy field establishment, heavy tillering, high dry matter production, the grazing or cutting tolerance, good bromatologic quality and great grain yield (Martin *et al*., 2010; Carvalho*et al*., 2015). Because of the variability of edaphoclimatic conditions of the country, genotype environment interaction provides different responses of genotypes, when subjected to different environmental conditions, changing its performance, causing reduction in the relationship between genotype and phenotype (Yan and Holland, 2010). Given this context aims to increase the genetic variability for agronomic important traits, that can make the most adapted and stable genotypes to adverse conditions. The *Ethyl Methane Sulfonate* chemical agent when applied to seeds can assist in obtaining genetic modifications, morphological and physiological (Borém andMiranda, 2009; Moturi and Charya, 2010). This technique is used in the wheat crop in order to assess damage caused by different concentrations on the physiology of hexaploid plants (Silva, 1998). In oat, to analyze genetic variability of changes in the plant height trait, thus creating genetic variability for vegetative cycle (Coimbra, 2004, 2005). As well as to compare the efficiency of artificial crosses versus chemical mutagen in plant stature oat (Oliveira, 2012). In barley, can be used for analyzing cytological effects of pyrethroid insecticides (Karnopp, 1999). Recently, over 2500 genotypes have been launched with the use of this technique (Ahloowalia *et al*., 2004). Given the lack of research involving chemical agents in dual-purpose wheat crop, the aim of this study was to evaluate different doses of *Ethyl Methane Sulfonate*in dual-purpose wheat seeds, and their answers on morphological traits, when measured in different evaluation periods.

MATERIALS AND METHODS

The experiment was conducted at 2013 crop year, in the experimental field of the Federal University of Santa Maria Campus Frederico Westphalen – RS. The coordinates correspond to latitude 27º23'26"S and longitude 53º25'43"O, with an altitude of 490 meters. The soil is classified as ferric aluminum red Latosol (EMBRAPA, 2006), and the climate characterized by Köppen as Cfa subtropical (Bernardi *et al*., 2008). The experimental design was a randomized block in a factorial design, two dual-purpose wheat genotypes x five doses of *Ethyl Methane Sulfonate*x four periods of evaluation, with four replications. Seeding was in direct seeding system, with a population density three million seeds per hectare, being used to base fertilization with 250 kg ha^{-1} NPK formulation 05-20-20, coverage was applied 100 kg $ha^{-1}$of N in full tillering stage. The source of nitrogen was urea, 45% nitrogen, and the experimental units were composed of 12 rows of 2.0 m in length with a spacing of 0.17 m. Genotypes were: BRS Tarumã and BRS Umbu. The doses of *Ethyl Methane Sulfonate* corresponded to 0 mL kg^{-1}, 0.25 ml mL kg^{-1}; 0,50 mL kg^{-1}; 0,75 mL kg^{-1}; 1.0 mL $kg^{-1}$of seed and the evaluation periods corresponded to phenologic stages: I (Feeks 3 - tillering), II (Feeks 8 - booting); III (Feeks 10.5.1 - flowering) and IV (Feeks 11.1 - filling grain).

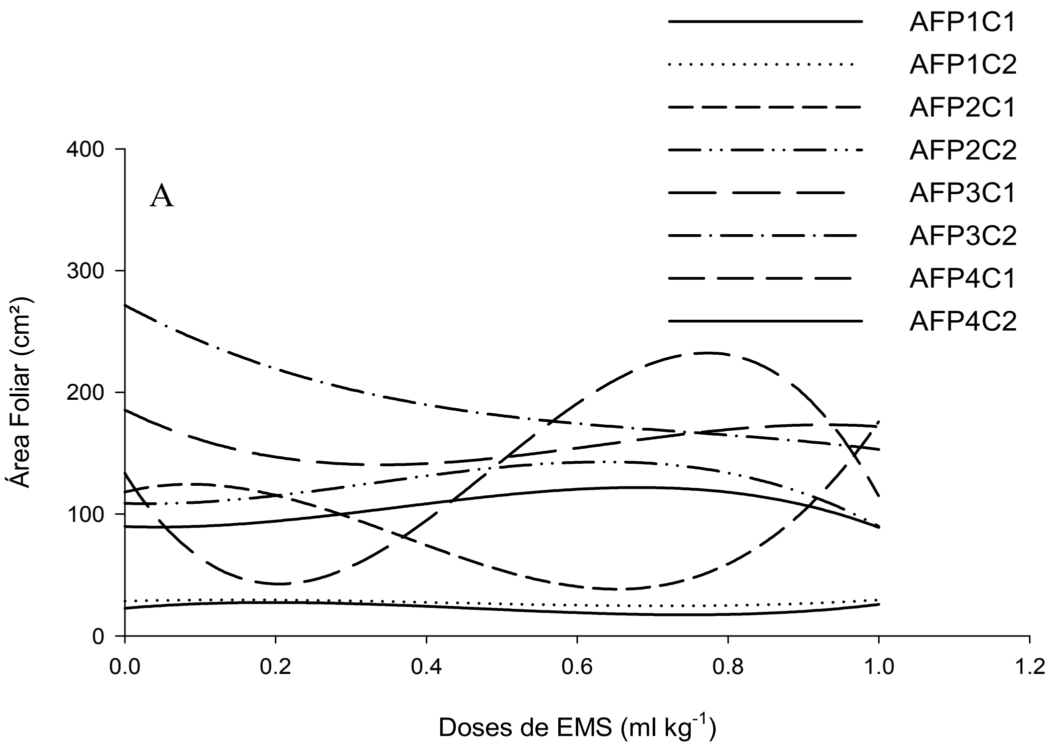
The seeds were allocated under a mesh type nylon where remained for two hours with the *Ethyl Methane Sulfonatex* treatment, under the established doses. After removed from the treatment, the seeds were washed in water for one hour. The traits were measured by ten sampling random plants in each experimental unit, leaf area was determined by meter LI-3000® model, the results being expressed in square centimeters (cm²). Root diameter was measured with digital caliper, and measured the average root diameter, results in millimeters (mm). Number of fertile tillers was determined by direct counting the number of tillers with ear emission in a linear meter, results in units.Plant height, measured from the ground level to the apex of the main ear and disregarding the edges, and the results expressed in centimeters (cm) of stem. Tillers diameter measured the average diameter of the stem of tillers through a caliper digital, and results expressed in millimeters (mm).The root length was determined by measurement of the lap plant to end roots, results in millimeters (mm). Chlorophyll content was measured by a chlorophilometer SPAD-502®, from measurements on 50 leaves per experimental unit, always at 14 hours, on the first leaf after flag leaf, fixing the chlorophyll in third-average leaf blade (results in mg m^{-2}). The data were submitted to analysis of variance at 5% probability by F test and subsequently, tested the interaction between genotype x doses x evaluation periods. The traits that showed significance for the interaction have been dismembered to simple effects, and the quantitative factor proceeded linear regression analysis and were tested significantly greater degree polynomial.

RESULTS AND DISCUSSION

Analysis of variance revealed a significant interaction among dual-purpose wheat genotypes x doses of *Ethyl Methane Sulfonate*x evaluation periods for leaf area and root diameter. It was observed interaction among dual-purpose wheat genotypes x evaluationperiods of traits number of fertile tillers, plant height, tillers diameter and chlorophyll content.There was no interaction for root length. The leaf area is directly related to maximizing interception of the incident solar radiation, which may reflect an increase in the growth rate of culture, increasedthousand kernel weight and grain yield (Okuyama *et al*., 2004). There was the period I (tillering) different doses of *Ethyl Methane Sulfonate*x howed low influence on trait increase for both genotypes. In the evaluation period II (booting), it was found that BRS Tarumã e presented greater increase with the dosage of 1.0 ml kg^{-1}, however, the BRS Umbu expressed positive response only up to a dose 0.75 ml kg^{-1}. In the period III (flowering) there was no increase for trait dependingdose of *Ethyl Methane Sulfonate*. For the period IV (filling grain), dose 0.75 ml kg^{-1} revealed increased for both genotypes (Figure 1-A).

Comparing evaluation periods (Table 1), it is observed that higher magnitude was revealed in period III (flowering).This way, leaf area has great importance as active photosynthetic tissue, providing greater partition of assimilated in the filling grain (Silva *et al*., 2003). Therefore, the increased availability of assimilates near anthesis can represent more fertile flowers, and consequently, great number and grain size (Rodrigues, 2000; Silva*et al*., 2003). For root diameter, depending on dose of*Ethyl Methane Sulfonate*agent, it was observed that the period I (tillering) does not result in an increased to genotypes. In the period II (booting) shows up, small reduction in magnitude for both genotypes. In period III (flowering) BRS Tarumã, reveled reducing diameterwith dose 1.0 ml kg^{-1}, however, BRS Umbu revealed reduction. In the period IV (filling grain) presenteddiscret increase observed for both genotypes (Figure 1-B).

Comparing the evaluation periods for root diameter (Table 2) showed an increase to BRS Tarumã in periods II (booting) and III (flowering), but BRS Umbu revealed greater magnitude in II periods (booting) and IV (filling grain). It was observed that the number of fertile tillers, depending doses of *Ethyl Methane Sulfonate*showed no significant increase (Figure 2, Graphic A).The highest values were checked in the period III (flowering) for both genotypes, and most expressive trait revealed in BRS Tarumã (Table 3). Number of fertile tillers contributes to the number of ears per unit area, and thus increases the grain yield. This trait is dependent on the intrinsic characteristics of the genotype, management applied, the cultivation and interaction of genotype x environment (Valério, 2008, 2013; Ozturk*et al*., 2006). The height of plant expressed high degree of importance, as genotypes with high size are more sensitive to lodging and consequently significant losses of productive potential. Lower-sized plants present higher solar



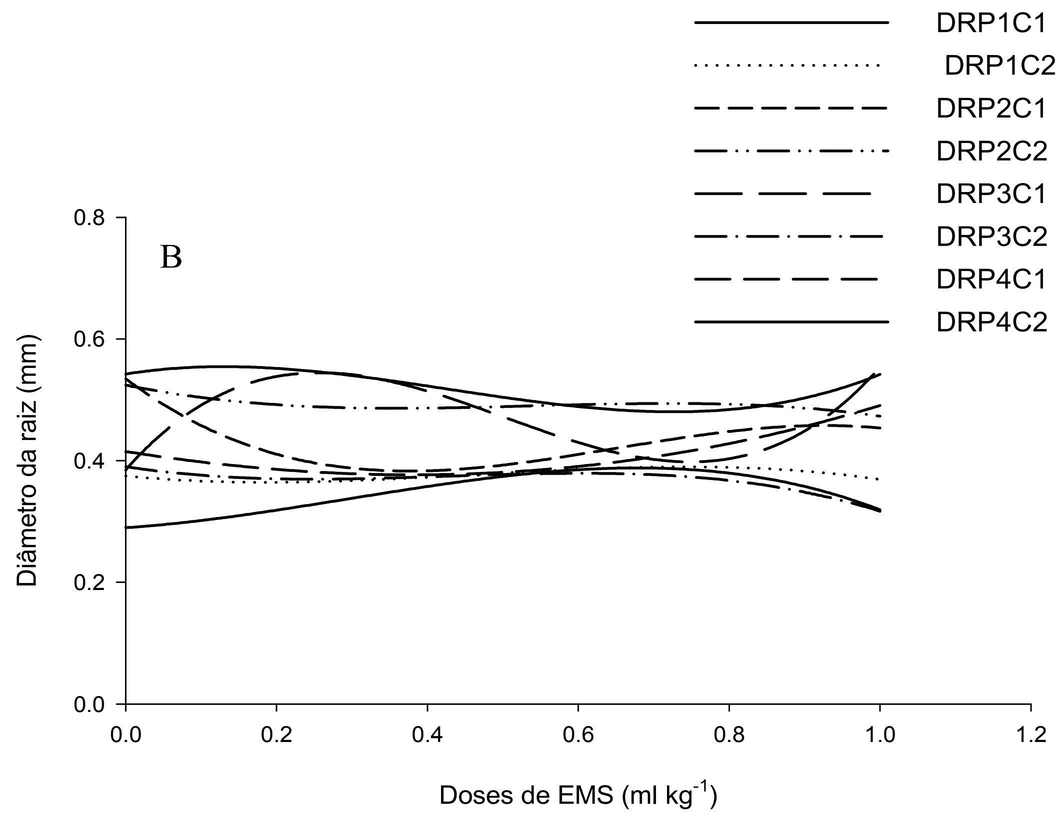


Figure 1: Regression equations for the trait leaf area (cm^2) (A) and the root diameter (b), of dual-purpose wheat genotypes under different doses of *Ethyl Methane Sulfonate* evaluated at different evaluation periods. (A) for trait leaf area. *LAP1C1* leaf area period one, genotype one ($Y=23.21$ R^2:0,00); *LAP1C2*leaf area period one, genotypedois ($Y=27.90$ $R^2=0,00$); *LAP2C1*leaf area period two, genotype one ($Y=130.58–329.72+356.23x^2$ $R^2=0.50$); *LAP2C2*leaf area period two, genotype two ($Y=104.9+146.79-160.24x^2$ $R^2=0.46$); *LAP3C1*leaf area period three, genotype one ($Y=168.84$ $R^2=0.01$); *LAP3C2*leaf area period three, genotype two ($Y=278.72-2104.28x+9965.36x^2-15688.24x^3+7713.86x^4$ $R^2=0.60$); *LAP4C1*leaf area period four, genotype one ($Y=128.12+430.09x-4475.32x^2+9977.28x^3-5954.66x^4$ $R^2=0.84$); *LAP4C2*leaf area period four, genotype two ($Y=83.37+1460.30x-7755.46x^2+12741.24x^3-6451.41x^4$ $R^2=0.66$). (B) For trait root diameter. RDP1C1 root diameter period one,genotype one ($Y=0.30-2.06x+12.36x^2-20.24x^3+9.96x^4$ $R^2=0.53$); RDP1C2 root diameter period one, genotype two ($Y=0.37$ $R^2=0.00$); RDP2C1 root diameter period two, genotype one ($Y=0.46$ $R^2=0.01$); RDP2C2 root diameter period two, genotype two ($Y=0.51$ $R^2=0.02$); RDP3C1 root diameter period three, genotype one ($Y=0.38+1.37x-3.61x^2+2.40x^3$ $R^2=0.50$); RDP3C2 root diameter period three, genotype two ($Y=0.39$ $R^2=0.06$); RDP4C1 root diameter period four, genotype one ($Y=0.38$ $R^2=0.11$); RDP4C2 root diameter period four, genotype two ($Y=0.56-3.26x+16.86x^2-27.68x^3+14.10x^4$ $R^2=0.44$).

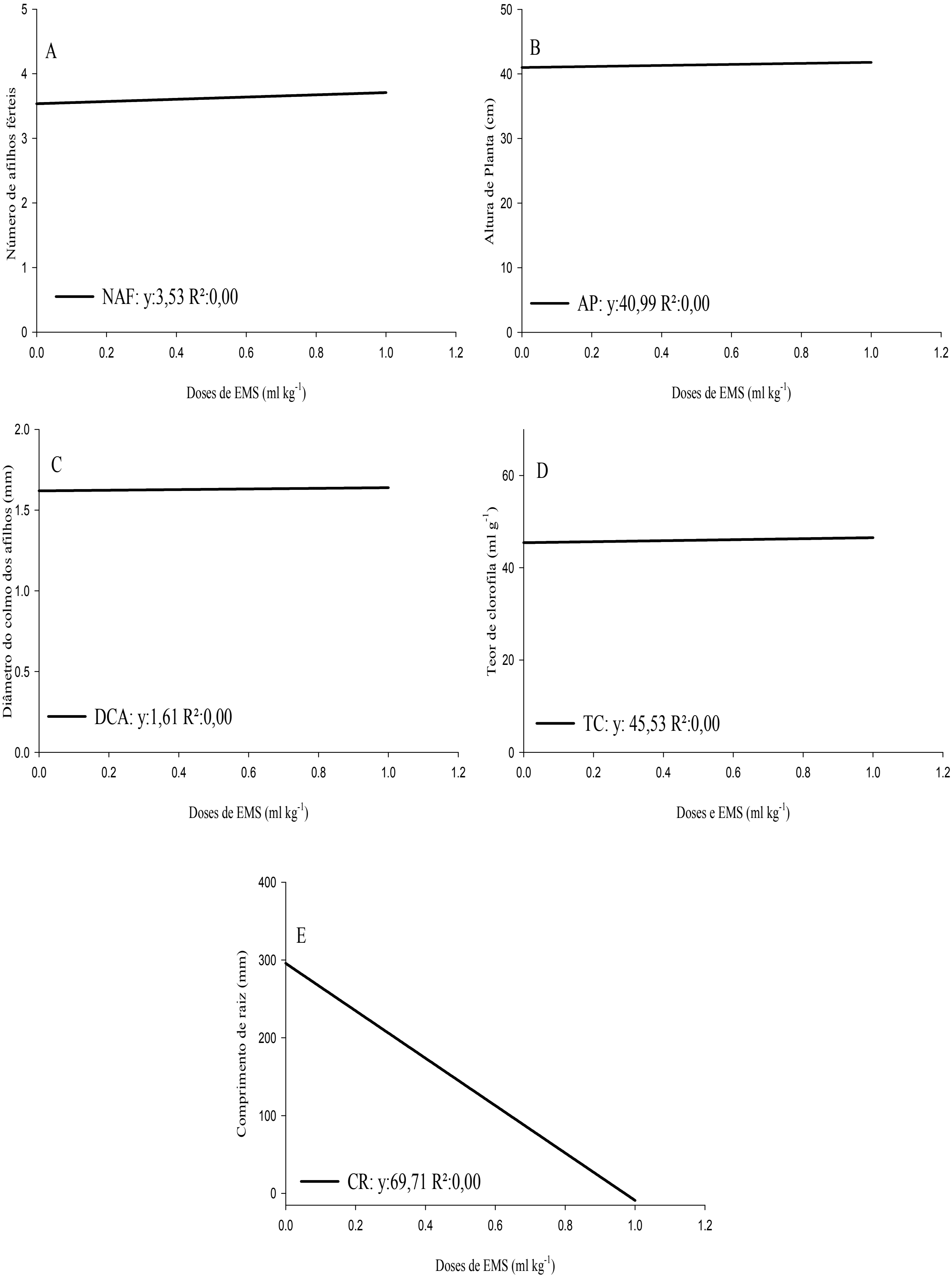


Figure 2. **Regression equations for number of fertile tillers (NFT) graphic (A); plant height (PH) graphic (B); tillers diameter (TD) graphic (C); chlorophyll content (CC) graphic (D) and root length (RD) graphic (E)**

Table 1. Average for interaction wheat genotypes dual purpose x doses of*Ethyl Methane Sulfonate*x evaluation periods for leaf area

Doses ofEMS (mLKg-1)	Evaluation periods							
	I		II		III		IV	
	Dual-purpose wheat genotypes							
	BRS Tarumã	BRS Umbu	BRS Tarumã	BRS Umbu	BRS Tarumã	BRS Umbu	BRS Tarumã	BRS Umbu
0.00	22.9αC	28.5αD	118.8αB	106.1αB	178.8βA	277.4αA	126.8αAB	83.4αC
0.25	25.2αA	28.5αA	92.3αAB	130.5αA	167.5αB	160.5αA	87.3αC	137.6αB
0.50	23.3αC	26.5αC	59.7βBC	128.2αB	147.2βA	239.0αA	99.3αAB	64.1αC
0.75	16.5αC	24.8αB	55.2βC	138.9αA	155.5αB	128.3αA	257.1αA	150.4βA
1.00	26.2αC	29.5αC	178.2αA	87.5βB	172.4αA	165.4αB	104.2αB	79.0αA
CV(%)	31.9							

* Means followed by the same uppercase letter in line, for periods, and the same Greek letter for genotypes did not differ statistically Tukey at 5% probability.

Table 2. Average forinteractiondual-purpose wheat genotypes xdoses of*Ethyl Methane Sulfonate*x evaluation periodsfor root diameter

Doses of EMS (mL Kg-1)	Evaluationperiods							
	I		II		III		IV	
	Dual-purpose wheat genotypes							
	BRS Tarumã	BRSUmbu	BRS Tarumã	BRSUmbu	BRS Tarumã	BRSUmbu	BRS Tarumã	BRSUmbu
0,00	0.30αB	0.37αB	0.54αA	0.53αA	0.38αB	0.40αB	0.41βAB	0.56αA
0.25	0.28αB	0.37αAB	0.35αB	0.48αA	0.58αA	0.33βB	0.41αB	0.42αAB
0.50	0.46αA	0.37αB	0.45αA	0.50αAB	0.41αA	0.43αAB	0.33αA	0.57βA
0.75	0.33αA	0.39αAB	0.41αA	0.48αA	0.44αA	0.33αB	0.46αA	0.38αAB
1,00	0.34αB	0.37αBC	0.46αAB	0.48αAB	0.54αA	0.33βC	0.48αA	0.58αA
CV(%)	19.95							

*Means followed by the same uppercase letter in line, for periods, and the same Greek letter for genotypes did not differ statistically Tukey at 5% probability.

Table 3. Average for the traits, number of fertile tillers (NFT), plant height (PH), tillers diameter (TD) and chlorophyll content (CC), in two dual-purpose wheatgenotypes, depending on evaluation periods

Evaluation periods	NFT		PH		SDT		CC	
	Dual-purposewheatgenotypes							
	BRS Tarumã	BRS Umbu	BRS Tarumã	BRS Umbu	BRS Tarumã	BRS Umbu	BRS Tarumã	BRSUmbu
I	2.24cA	1.64cA	19.56dB	23.74dA	1.19cA	1.04cA	38.03cA	38.43cA
II	5.02aA	3.09bB	24.63cB	35.15cA	1.48bA	1.63bA	48.03bB	50.23aA
III	5.42aA	4.76aB	40.78bB	63.73bA	1.4 bB	1.68bA	50.91aA	46.50bB
IV	3.78bA	3.02bB	51.07aB	72.49aA	2.12aB	2.47aA	47.45bA	47.43bA
CV(%)	24.1		7.7		13.6		6.5	

*Means followed by the same lowercase letter in line, for periods, and the same uppercaseletter for genotypes did not differ statistically Tukey at 5% probability.

Table 4. Average for root length (RL)for dual-purpose wheat genotypes (BRS Tarumã and BRS Umbu) and evaluation periods (I tillering, II booting, III flowering and IV filling grain), results expressed in millimeters

Evaluation periods	RL
I	55.1 c
II	77.6 a
III	75.8 ab
IV	63.4 bc
Dual-purposewheatgenotypes	
BRS Tarumã	63.3 b
BRS Umbu	72.7 a
CV(%)	27.6

*Means followed by the same letter did not differ statistically Tukey at 5% probability.

radiation interception capacity during critic periodsofdefinition production, as well as the carbon direction not used in growth in height (Chavarria*et al*., 2015). Increased doses of *Ethyl Methane Sulfonate* did not give interference in trait in evidence (Figure 2-B). The evaluation period IV (filling grain) reveled increase both genotypes (Table 3). There was no increase in stem diameter of fertile tillers due to increased doses of *Ethyl Methane Sulfonate* (Figure 2-C).

The evaluation period IV (filling grain) gave superior to both genotypes, with the largest magnitude were observed on BRS Umbu (Table 3). Studies Martin *et al*. (2010) showed that yield components dual-purpose wheat are largely influenced by tillering, water availability, nutrition, quantity and quality of light, temperature, and number of cuts. According Simmons *et al*. (1982), genotype expressing greater tillering potential, show a reduction in tillers diameter, according to what was shown in

Table 3. For chlorophyll content compared to the doses of *Ethyl Methane Sulfonate*, there were not increasing for trait with increasing doses (Figure 2-D). Superiority was given to the chlorophyll content in the period III (flowering) to BRS Tarumã and period II (booting) to BRS Umbu that among genotypes being observed decreased in magnitude variation (Table 3). In wheat crop chlorophyll content is widely used to predict the need for nitrogen fertilization, because the amount of this pigment is positively correlated with the nitrogen content in the plant (SenaJúnior*et al*., 2008). The similarity between the genotypes for trait may be related to the fact that same cultivation. Regarding the root length due doses of*Ethyl Methane Sulfonate*, there was a reduction oftrait with increasing dose (Figure 2-E), reveals phytotoxic effect of the mutagen agent on root growth dual-purpose wheat. The size or volume of roots allows better soil occupation, favoring the absorption of water and minerals for plant growth and development (Barber, 1988). The evaluation period II (booting) gave the highest root length, did not differ evaluation period III (flowering). As for the genotypes, BRSUmbu expressed superiority to root length, regarding the BRS Tarumã (Table 4). The trait in evidence is influenced by soil pH, exchangeable aluminum content, density, water storage and hydraulic conductivity. As can also be compromised by management, soil compaction, toxicity of chemicals and excess water (Müller *et al*., 2001; Valadão *et al*., 2015).

Conclusion

The dual-purpose wheat genotypes have differential behavior among themselves and evaluation periods, when the seeds subjected to the application of *Ethyl Methane Sulfonate* mutagen agent. Increasing doses of *Ethyl Methane Sulfonate* agent reveals variability for leaf area, root diameter, number of fertile tillers, tillersdiameter and chlorophyll content, however, it has a negative effect on the length of the main root.

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