Acta Scientiarum

http://periodicos.uem.br/ojs
ISSN on-line: 1807-8621
Doi: 10.4025/actasciagron.v43i1.44974

Morphology and enzymatic activity of seedlings from wheat desiccated in pre-harvest

Glauber Monçon Fipke[1*], Evandro Ademir Deak[2], Jessica Deolinda Leivas Stecca[2], Daniele Bernardy[3], Mirian Berger[3], Luciane Almeri Tabaldi[3] and Thomas Newton Martin[2]

[1]Universidade Federal do Pampa, Rua Luiz Joaquim de Sá Brito, s/n, 97650-000, Itaqui, Rio Grande do Sul, Brazil. [2]Departamento de Fitotecnia, Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul, Brazil. [3]Departamento de Biologia, Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul, Brazil. *Author for correspondence. E-mail: gm.fipke@hotmail.com

ABSTRACT. Desiccation practiced during the preharvest period contributes to mechanized seed harvesting. This work aimed to verify whether wheat preharvest desiccation influences the morphological and physiological characteristics of the seedlings produced from the seeds of desiccated plants. The preharvest treatments included a combination of herbicides (glufosinate-ammonium, glyphosate, and paraquat) and phenological application stages (Zadoks: 83, 85, 87, and 92), as well as a control treatment (without application). Two wheat cultivars were used (BRS Parrudo and TBIO Sinuelo). Herbicide applications were observed to decrease the length and projected area of the shoots by 52 and 46%, respectively, as well as reduce the length, surface area and root volume when compared to the control treatment without application. The hydrogen peroxide concentration, as well as the enzymatic activity of guaiacol peroxidase, was observed to rise only in the treatments where the herbicides were applied. Physiologically, an increased hydrogen peroxide output was revealed, while the guaiacol peroxidase enzymatic activity increased in both cultivars, but with no effect noted in the superoxide dismutase enzymatic activity. The shoot and root morphology were negatively influenced, showing a rise in the enzymatic activity and hydrogen peroxide concentration in the seedlings from the desiccated plants.

Keywords: glufosinate-ammonium; glyphosate, paraquat; *Triticum aestivum* (L).

Received on October 17, 2018.
Accepted on September 30, 2019.

Introduction

To control late-appearing weeds and to speed up the drying process of agricultural crops to ensure quick and efficient harvesting, nonselective herbicides are applied prior to harvesting the plants (Bresnahan, Manthey, Howatt, & Chakraborty, 2003). Once the seeds reach physiological maturity, they are prone to physiological damage if they experience exposure to weather conditions. Thus, crop desiccation during the preharvest period indirectly reduces seed deterioration (Bellé et al., 2014). While this is a common practice in many countries, it must be emphasized that the current legislation must be complied with, in terms of the addition of the active ingredients and the grace period permitted until commercialization of the yield.

Among the action mechanisms of the various preharvest agrochemicals available, the most popular is the use of total action herbicides or desiccants. Glutamine synthetase inhibitors (glufosinate-ammonium) are molecules that restrict enzyme activity by boosting ammonia levels and impeding nitrogen metabolism. The 5-enolpyruvylshikimate-3-phosphate synthase (glyphosate) enzyme inhibitors are molecules that encourage the blockade of this enzyme, raise shikimate levels in the vacuole, and suppress aromatic amino acid synthesis. Photosystem I (paraquat) inhibitors are molecules that behave similar to electron acceptors by becoming free radicals that result in cell membrane disruption (Oliveira Jr., 2011).

In Brazil, preharvest desiccation is a common practice implemented for commercial agricultural crops, although no conclusive experimental data are available regarding its practical effectiveness, specifically on seed quality. From recent research, it is evident that the seed quality, both physically and physiologically, is affected by the kind of action mechanism employed, and the phenological stage at which the particular herbicide is utilized (Bellé et al., 2014; Krenchinski et al., 2017; Fipke et al., 2018). This implies that the morphological and physiological characteristics of these seedlings have been impacted because of the production of high percentages of abnormal seedlings (Brasil, 2009).

When glyphosate was applied between stages 85-87 (Zadoks, Chang, & Konzak, 1974), it was observed to exert a negative impact on the germination speed and the length and dry mass of the wheat seedlings, with the root system depth showing a marked decrease (Jaskulski & Jaskulska, 2014). However, when glufosinate-ammonium was applied at stage 92, the length and mass of the seedlings were unaffected (Fipke et al., 2018). When paraquat was applied at stage 80, the seed mass and total seedling length showed a reduction (Krenchinski et al., 2017). It is noteworthy that all the data cited were obtained from the seedlings that emerged under controlled conditions. In the research mentioned, measurements were taken of the corresponding length between the leaf tip and seed, as well as of the seed to the main root tip, ignoring the thickness. All the other morphological constituents that represent the leaf and secondary root emission, however, need to be measured.

Issues concerning seedling growth could arise due to biochemical and physiological disruptions. Aerobic metabolism, as well as biotic or abiotic stressors, could induce the generation of reactive oxygen species (ROS) in the cells, triggering oxidative stress (Del-Buono, Ioli, Nasini, & Proietti, 2011). Therefore, ROS affects the signaling pathways for gene expression in response to stressful conditions. When ROS accumulates in cells, they can react with biological molecules and irreversibly damage them to the extent of precipitating cell death (Barbosa, Silva, Willadino, Ulisses, & Camara, 2014). Hydrogen peroxide (H_2O_2) is a type of ROS that is produced when electrons are added to O_2 or through the reduction of hydroxyl radicals and acts as a substrate for antioxidant enzymes (Bhattacharjee, 2010). Therefore, to minimize ROS production and prevent oxidative damage, plants utilize their antioxidant defense system, which is either enzymatic or nonenzymatic in nature (Gill & Tuteja, 2010). In this study, among the enzymes generated in the chloroplasts, mitochondria and peroxisomes, we emphasize the roles of superoxide dismutase, catalase, peroxidases and peroxiredoxins (Mittler, 2002).

Some herbicides employed in the process of wheat desiccation obviously influence its productive yield and the physiological caliber of the seeds. Therefore, an investigation is mandatory to identify the causes for the anomalies observed in the seedlings, which can govern the recommendation of this common agronomic practice. From the information given thus far, this work aimed to verify whether wheat preharvest desiccation influences the morphological and physiological characteristics of the seedlings produced from the seeds of desiccated plants.

Material and methods

Description of the field experiment

The experiment was performed in the field during the growing season of 2016 in Santa Maria, RS, Brazil (29°42 'S, 53°42' W, at an altitude of 116 m). Two wheat cultivars (BRS Parrudo and TBIO Sinuelo) were managed, adhering to the technical instructions for the cultivated region (EMBRAPA, 2017). During the preharvest phase of the crop, nonselective herbicides were utilized (treatments). For post-harvesting the wheat cultivars in the field experiment, the seeds were dried until 13% moisture was achieved. They were later stored in paper packages and maintained under controlled temperature and humidity conditions for a three-month period.

Description of the treatments

The treatments included the use of the herbicides, as listed: glufosinate-ammonium (350 g active ingredient (a. i.) ha^{-1}), glyphosate (1440 g a. i. ha^{-1}) and paraquat (400 g a. i. ha^{-1}). Applications were performed during the following phenological stages: 83 (the seed initiating mass texture, green in color, with the sticky contents being kneaded using thumb pressure), 85 (soft mass seed, light green in color, and when the thumbnail is used to make a mark it quickly disappears), 87 (seed mass is hard and light red in color, and when the thumbnail is used to press it, the mark is retained), and 92 (very hard seed mass, red in color, and no kneading required with thumb pressure); an additional control treatment (with no application) was used. A pressurized backpack sprayer was used to apply the herbicides (200 kPa) to CO2 and was mounted on a bar provided with four flat jet tips (Teejet® XR 100.02), with 0.5 m spacing and a 150 L ha-1 volume.

Obtaining seedlings

To obtain normal seedlings, the germination pattern was employed. First, 800 seeds were seeded in germitest paper rolls and moistened to 2.5 times their mass content. They were then transferred to an incubator (Biochemical Oxygen Demand EL202, Eletrolab, São Paulo, São Paulo State, Brazil) set at 20ºC (Brasil, 2009). The normal seedlings revealed completely formed coleoptiles and a minimum of 2 mm of root system. Sectioning of the seedlings was performed with the leaves, distinguishing the aerial portion from the roots for the assessments.

Seedling morphology

First, 4-day-old seedlings were selected, as they represent the result of the vigor exhibited by the first count of the germination test (Brasil, 2009). Imaging was performed using a professional scanner (XL 10000®, Epson America, Inc., Long Beach, CA, USA) provided with an additional light unit (TPU). Specific software (WinRHIZO Pro 2007a®, Regent Instruments Inc., Ville de Québec, QC, Canada) enabled the seedling morphology to be identified: length (mm) and projected area of the shoot (mm^2), and length (mm), surface area (mm^2), average diameter (mm) and volume (mm^3) of the root.

Hydrogen peroxide content

The 8-day-old seedlings were utilized as they represent the result of the standard germination test, corresponding to the final period during which the seedlings obtain their nourishment from the seed reserves (Brasil, 2009). The aerial portions of the seedlings were first macerated with liquid nitrogen and then homogenized in trichloroacetic acid (0.1%, w/v) in sequence and centrifuged (12,000 x g, 15 min., 4ºC). A small supernatant fraction was drawn after adding a potassium phosphate buffer (10 mM, pH 7) plus potassium iodide (1 M). The hydrogen peroxide concentration (H_2O_2, μmol g^{-1} dry matter) of the supernatant was determined by comparing the absorbance in the length of 390 nm with a standard calibration curve (Loreto & Velikova, 2001).

Antioxidant enzymes

The aerial portions and roots of the 8-day-old seedlings were individually macerated with liquid nitrogen. The enzyme superoxide dismutase activity [SOD, units of enzyme (U) mg^{-1} protein] was estimated using the spectrophotometric method (Giannopolitis & Ries, 1977). A solution of potassium phosphate (50 mM, pH 7.8), methionine (13 mM), riboflavin (2 μM), nitroblue tetrazolium (75 μM), ethylene diamine tetra-acid (0.1 mM) and the enzyme extract (100 μL) was used. Formosan blue photochemically produced from the nitroblue tetrazolium was monitored by the rise in the absorbance of 560 nm. One SOD unit is defined as the quantity of enzyme needed to inhibit the photoreduction of nitroblue-tetrazolium by 50% (Beauchamp & Fridovich, 1971). To evaluate the enzyme guaiacol peroxidase activity (POD, U mg^{-1} protein), guaiacol was obtained as the substrate (Zeraik, Souza, Fatibello-Filho, & Leite, 2008). A solution of potassium phosphate (100 mM, pH 6.5), guaiacol (15 mM) and hydrogen peroxide (3 mM) was used. The enzyme activity was measured by the oxidation of guaiacol to tetraguaiacol and recording the absorbance at 470 nm. To calculate the enzyme activity, the molar extinction coefficient of 26.6 mM^{-1} cm^{-1} was used.

Data analysis

Adopting the completely randomized design for the experiment, the data were verified in terms of the assumptions of the mathematical model. Analysis of variance was performed [F-test ($p < 0.05$)] by unfolding the degrees of freedom of the herbicide treatments *versus* the non-application (control) treatment [Scheffé ($p < 0.05$)]. The mean clustering test [Scott-Knott ($p < 0.05$)] was used for the herbicide treatments. The software Action® (Estatcamp, São Carlos, São Paulo State, Brazil) and Genes (Cruz, 2013) were used.

Results

Characters linked to seedling morphology failed to meet the assumption of the normality of the errors when the Shapiro-Wilk test ($p < 0.05$) was used. The F-test involved the use of the Box-Cox transformation to estimate the transformation parameter λ by the maximum likelihood method. Interactions were noted between the factors, as well as significant differences for the contrasts in the treatments using herbicides during the preharvest phase and the treatment without application (Table 1).

Table 1. Summary of variance analysis represented by the mean squares and contrasts between treatments with nonselective herbicide application in preharvest *versus* treatment without application on the characteristics related to the morphology and physiology of wheat seedlings.

VR[1]	DF[2]	---------- Shoot ----------					---------- Root ----------					
		SL	PA	H_2O_2	SOD	POD	RL	SA	AD	VO	SOD	POD
		---------- BRS Parrudo ----------										
Treatments	12	171.71*	1.19*	0.0039*	913.09*	852.27*	2763.42*	64.60*	0.0003*	0.01083*	90.29^{ns}	60791.61*
Factor	11	163.64*	1.11*	0.00382*	992.03*	855.46*	2936.40*	69.59*	0.00032*	0.01176*	97.94^{ns}	60967.16*
Contrast	1	260.52*	2.09*	0.00482^{ns}	44.74^{ns}	817.25*	860.66^{ns}	9.66^{ns}	0.00003^{ns}	0.0006^{ns}	6.17^{ns}	58860.58*
Error	39/26	12.59	0.19	0.00146	213.13	35.58	557.91	8.94	0.00008	0.00124	123.24	2275.99
Phenological stage	3	41.78	0.54	0.00237	355.11	84.96	4540.01	83.03	0.0002	0.01049	18.64	39833.37
Herbicide	2	389.10	1.84	0.00866	1037.83	2736.62	2356.09	108.65	0.0008	0.02479	368.86	169429.19
Phen. Stage. x Herb.	6	149.41	1.15	0.00293	1295.23	613.65	2328.03	49.85	0.00022	0.00805	47.28	35380.05
Λ		2.05	1.84	-	-	-	1.29	1.99	2.50	1.79	-	-
Average		28.44	2.47	0.07	75.97	67.33	112.43	16.51	0.16	0.19	43.85	507.48
CV (%)		12.5	17.7	55.1	19.2	8.9	21.0	18.1	5.8	18.2	25.3	9.4
		---------- TBIO Sinuelo ----------										
Treatments	12	183.00*	2.80*	0.00318*	1264.10*	4408.68*	6478.14*	136.69*	0.00105*	0.01874*	919.56*	41444.23*
Factor	11	143.44*	2.68*	0.00249^{ns}	1353.60*	4598.43*	5384.19*	122.09*	0.00113*	0.01772*	1003.08*	45004.85*
Contrast	1	618.27*	4.10*	0.01079**	279.61^{ns}	2321.48*	18511.63*	297.40*	0.00011^{ns}	0.03*	0.89^{ns}	2277.46^{ns}
Error	39/26	10.88	0.16	0.00122	175.45	124.79	137.74	4.03	0.00015	0.00088	260.65	7646.17
Phenological stage	3	300.63	6.21	0.00023	515.09	429.68	9929.55	255.91	0.00259	0.04011	392.06	317.78
Herbicide	2	268.58	4.03	0.0043	4021.14	12409.87	10876.87	181.83	0.00064	0.01907	3824.14	37774.49
Phen. Stage. x Herb.	6	23.12	0.47	0.00302	883.67	4078.99	1280.61	35.26	0.00057	0.00607	368.23	69758.50
λ		-	-	-	-	-	-	-	2.50	0.88	-	-
Average		24.52	2.25	0.11	99.79	95.32	75.59	10.95	0.15	0.13	62.31	446.28
CV (%)		13.5	18.0	31.3	13.3	11.7	15.5	18.3	8.2	23.3	25.9	19.6

[1]Variation range (VR), coefficient of variation (CV), 2 degrees of freedom (DF), DF of experimental error for morphological (39) and enzymatic characters (26); [3]λ transformation estimate for those characters that did not meet the assumption of error normality. Characters: shoot length (SL, mm), projected area (PA, mm^2), hydrogen peroxide (H_2O_2, μmol g^{-1} fresh matter), superoxide dismutase [SOD, units of enzyme (U) mg^{-1} protein], guaiacol peroxidase (POD, U mg^{-1} protein), root length (RL, mm), surface area (SA, mm^2), average diameter (AD, mm) and volume (VO, mm 3) of wheat seedlings; not significant and 5% significant differences by the F-test (^{ns} e *, respectively).

Shoot morphology

The herbicide treatments reduced the parameters evaluated in the aerial portions of the seedlings (Figure 1). For the cultivar TBIO Sinuelo, the herbicides applied showed a decrease of 52% and 46% in the length and projected area of the aerial portions of the seedlings, respectively. The herbicides applied in phenological stage 83 caused a reduction in length (8.4 mm) and projected area (1.3 mm2) when compared with the other stages. The cultivar BRS Parrudo showed a decline of 29% in length and 30% in the surface area. When the herbicide paraquat was added at stage 83, a drop in the length (5.7 mm) and projected area (0.7 mm2) was induced when compared with the other stages of application. Upon estimating the phenological stages when the herbicide was applied, a definite decrease was noted in the yield, as the application had been performed well in advance for the cultivar TBIO Sinuelo. This behavior was reported only in the cultivar BRS Parrudo in response to the application of the herbicide paraquat.

Root morphology

The root system of the cultivars showed differences in the morphological characteristics in response to herbicide applications, with the exception of the variable average root diameter (Figure 2). This particular variable was completely unaffected by the application of the herbicides during the preharvest for both cultivars. In the control treatment, in which no herbicides were applied, an average increment of 93, 81, and 70% was revealed for the length, surface area and root volume, respectively, in the TBIO Sinuelo cultivar. For all the herbicides tested, when they were applied at the first phenological stage, they induced a decrease in the root parameters. The length (67 mm), surface area (10 mm2) and root volume (0.13 mm3) obviously declined in relation to the application in the first phenological stage (83 and 92).

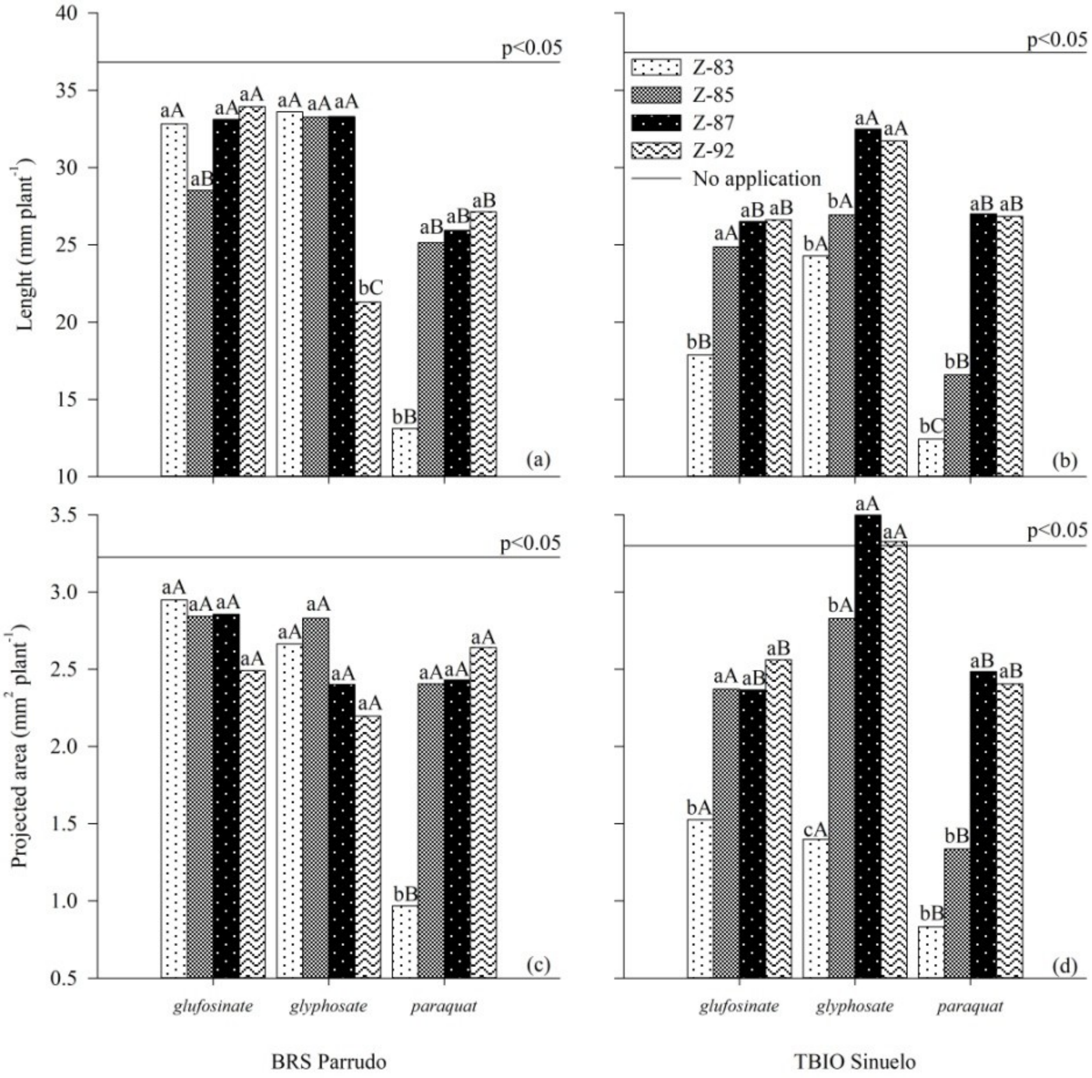

Figure 1. Shoot morphology: shoot length (a, b) and projected area (c, d) of wheat seedlings from plants desiccated in preharvest with nonselective herbicides (HER: glufosinate, glyphosate, and paraquat) in different phenological stages (PS: Zadoks 83, 85, 87, and 92). Distinct lowercase letters (PS deployed in each HER) and uppercase letters (HER deployed in each PS) differ from each other [Scott-Knott ($p < 0.05$)]. Contrast results [Scheffé ($p < 0.05$)] are between treatments with and without herbicide applications (p-value and ^{ns}).

The BRS Parrudo cultivar, however, showed no change in the root parameters in response to the herbicide applications at the preharvest time when compared to the control treatment without herbicide application. Glufosinate-ammonium was the most selective herbicide, which acted independent of the phenological stage at which it was applied. Glyphosate caused a reduction of 13% (length), 15% (surface area) and 16% (root volume) when compared with the applications performed in the first two phenological stages (83 and 85) in relation to the average of the experiments. The herbicide paraquat induced a decrease that could range from 83 - 75 mm, 75 - 13 mm^2 and 0.13 - 0.01 mm^3 for the length, surface area and root volume, respectively.

Hydrogen peroxide content in the roots

The hydrogen peroxide (H_2O_2) level escalated in the treatments provided with herbicides (Figure 3). An increase of 0.06 μmol g^{-1} in the concentration of fresh matter was noted in the cultivar TBIO Sinuelo. The BRS Parrudo cultivar showed an increase of 1.5 times, but no significant difference; however, the p-value for this source of variation was 0.08. Among the herbicides, paraquat produced 86% (BRS Parrudo) and 31% (TBIO Sinuelo) greater concentrations when compared to the other two active ingredients investigated.

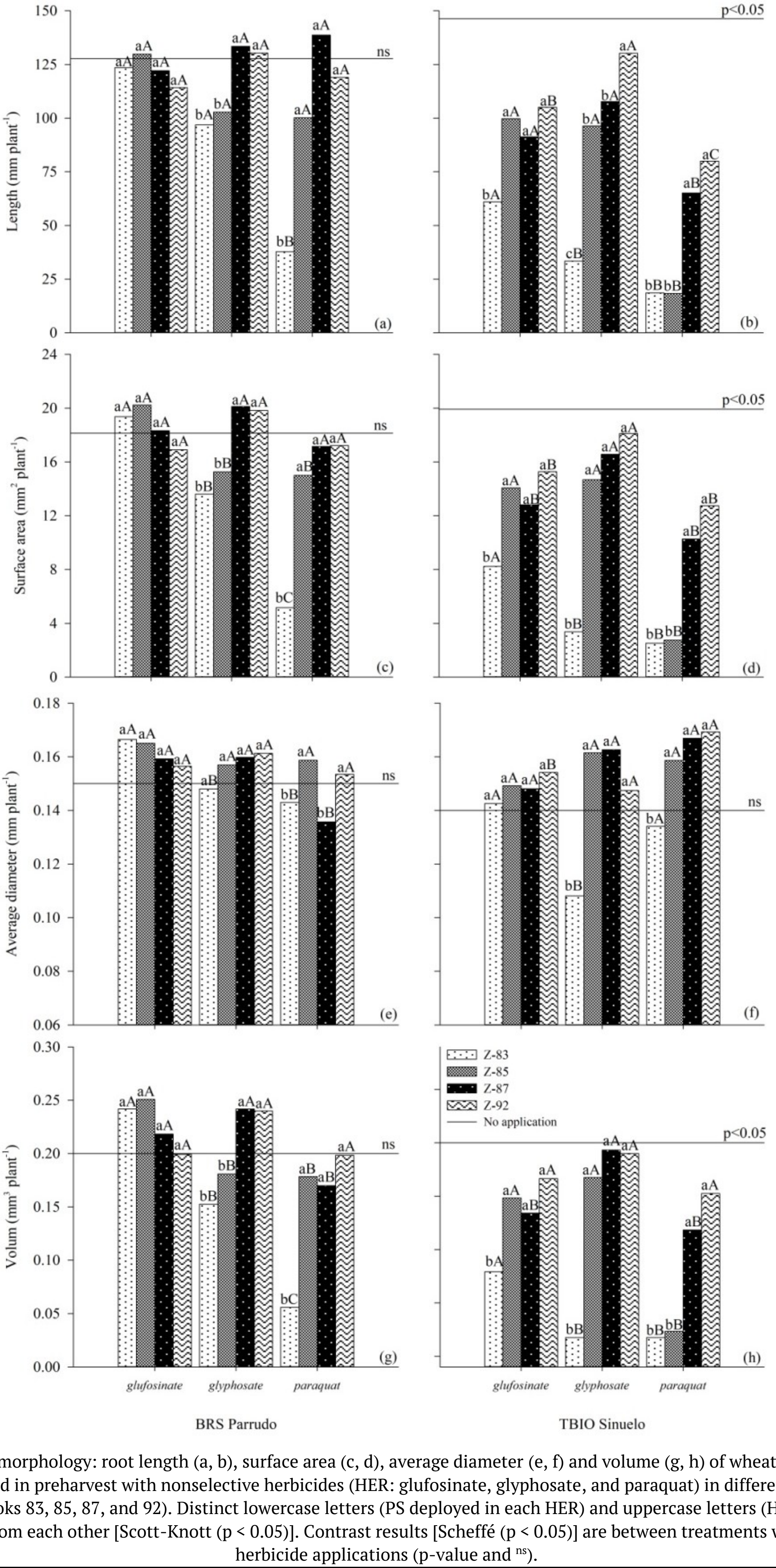

Figure 2. Root morphology: root length (a, b), surface area (c, d), average diameter (e, f) and volume (g, h) of wheat seedlings from plants desiccated in preharvest with nonselective herbicides (HER: glufosinate, glyphosate, and paraquat) in different phenological stages (PS: Zadoks 83, 85, 87, and 92). Distinct lowercase letters (PS deployed in each HER) and uppercase letters (HER deployed in each PS) differ from each other [Scott-Knott ($p < 0.05$)]. Contrast results [Scheffé ($p < 0.05$)] are between treatments with and without herbicide applications (p-value and ^{ns}).

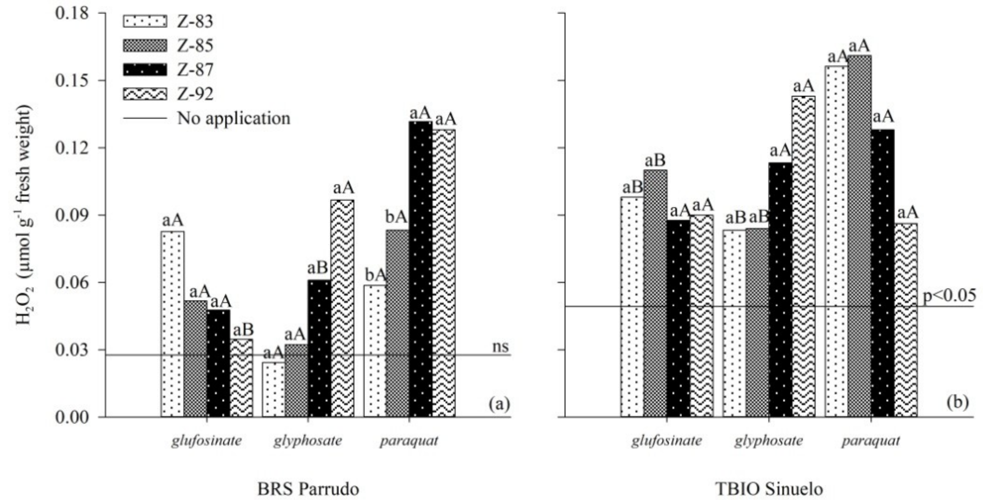

Figure 3. Hydrogen peroxide content of wheat seedlings from plants desiccated in preharvest with nonselective herbicides (HER: glufosinate, glyphosate, and paraquat) in different phenological stages (PS: Zadoks 83, 85, 87, and 92). Distinct lowercase letters (PS deployed in each HER) and uppercase letters (HER deployed in each PS) differ from each other [Scott-Knott ($p < 0.05$)]. Contrast results [Scheffé ($p < 0.05$)] are between treatments with and without herbicide applications (p-value and ^{ns}).

Superoxide dismutase enzyme activity

Superoxide dismutase (SOD) activity was completely unaffected by the herbicide treatments (Figure 4). In the aerial portion of the seedlings of the BRS Parrudo and TBIO Sinuelo cultivars, the enzyme activity increased, particularly with the herbicides glyphosate (2 and 43%) and paraquat (24 and 35%), in comparison to the third herbicide tested. In the seedling roots, when the two herbicides mentioned above (glyphosate and paraquat) were applied, they induced an increase of 71 and 76%, respectively, in the enzyme activity for the cultivar TBIO Sinuelo. The seedling roots of the BRS Parrudo cultivar showed no effect independent of the herbicide application and phenological stage at which the herbicide was applied.

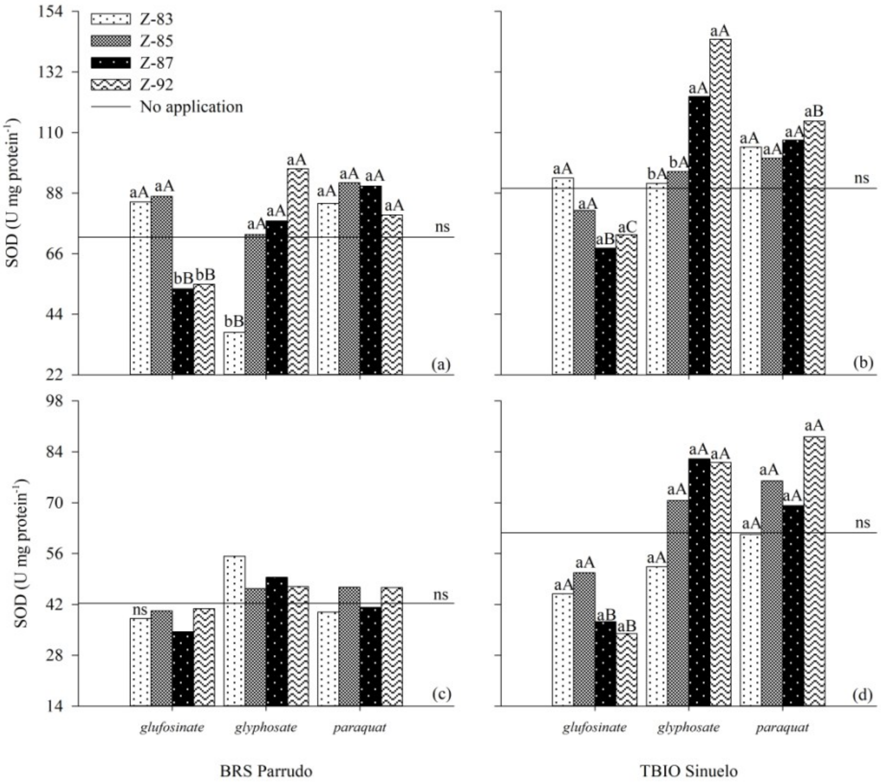

Figure 4. Superoxide dismutase enzyme activity in the shoot (a, b) and root (c, d) of wheat seedlings from plants desiccated in preharvest with nonselective herbicides (HER: glufosinate, glyphosate, and paraquat) in different phenological stages (PS: Zadoks 83, 85, 87, and 92). Distinct lowercase letters (PS deployed in each HER) and uppercase letters (HER deployed in each PS) differ from each other [Scott-Knott ($p < 0.05$)]. Contrast results [Scheffé ($p < 0.05$)] are between treatments with and without herbicide applications (p-value and ns).

Activity of the guaiacol peroxidase enzyme

The herbicide treatments affected guaiacol peroxidase (POD) enzymatic activity (Figure 5). In the area of the seedling where no herbicide had been applied, the enzymatic activity was 34 and 43% less for the cultivars BRS Parrudo and TBIO Sinuelo, respectively. The application of the herbicide glyphosate was observed to boost the enzymatic activity to 45 and 43% in both cultivars. The herbicide glufosinate-ammonium increased the enzymatic activity by 57% in the first cultivar, whereas the herbicide paraquat 57% caused the same effect in the second cultivar. In the seedling roots of the BRS Parrudo cultivar, the treatment without any herbicide application demonstrated lowered enzymatic activity by 40%. The means increased after the glyphosate and paraquat treatments to 174 and 247 mg protein^{-1} enzyme units, respectively. The cultivar TBIO Sinuelo was completely unaffected by the herbicide application.

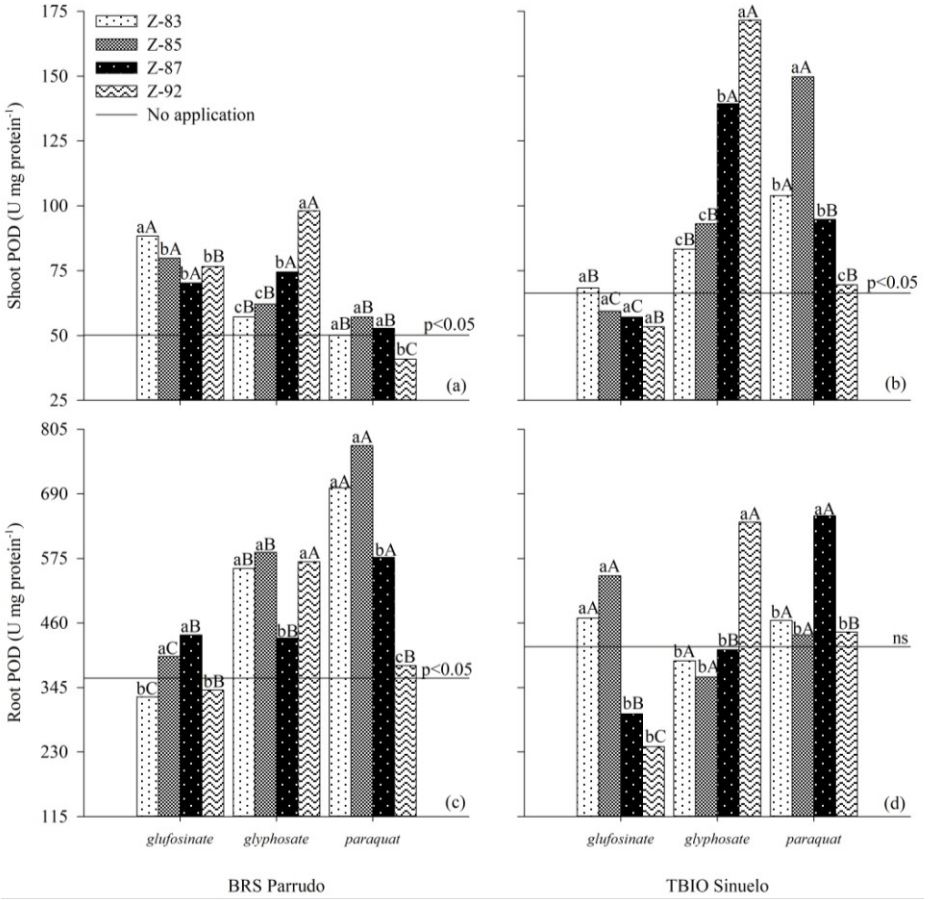

Figure 5. Guaiacol peroxidase enzyme activity in the shoot (a, b) and root (c, d) of wheat seedlings from plants desiccated in preharvest with nonselective herbicides (HER: glufosinate, glyphosate, and paraquat) in different phenological stages (PS: Zadoks 83, 85, 87, and 92). Distinct lowercase letters (PS deployed in each HER) and uppercase letters (HER deployed in each PS) differ from each other [Scott-Knott ($p < 0.05$)]. Contrast results [Scheffé ($p < 0.05$)] are between treatments with and without herbicide applications (p-value and ^{ns}).

Discussion

It is possible that preharvest desiccation could disrupt germination and consequently produce normal wheat seedlings. Under controlled conditions, an average 8 to 26% decrease was recorded depending upon the cultivar used. However, based on the phenological stage at which the application was performed and the active ingredient employed, the seeds could become completely nonviable, revealing only 24.5% germination (Fipke et al., 2018). To more clearly comprehend the causes for this reduction, the integrity of the seeds needs to be assessed. Some morphological and physiological characteristics, such as the quantity and remobilization mode of the accumulated reserves in the seeds, are estimated in the seedlings (Soltani, Gholipoor, & Zeinali, 2006).

The findings of the current study revealed that the decrease in the length and projected area of the aerial portions may be linked to the quantity of accumulated reserves present in the seeds. Earlier results demonstrated that in those treatments with herbicide application, the seed mass suffered adverse effects (data not shown). Wheat plants produced from larger seeds yielded greater leaf emission rates and morphological features related to the crop (Bredemeier, Mundstock, & Büttenbender, 2001). At four days post-germination, the area was basically composed of the coleoptile, which, under the soil conditions of germination, protects the first wheat leaf that emerges. The lateness in the emission of the first leaf may be connected to this present research, as it is another indicator that shows that the herbicide treatments caused formation problems in the seedlings (on average, 12.9 mm less in the length of the aerial portion). This study revealed that the wheat seedling length with eight days of sprout growth showed a 47.9 mm reduction when the paraquat herbicide was used (Bellé et al., 2014). Additionally, in soybean, preharvest desiccation induced by the herbicide glufosinate-ammonium negatively affected both germination and seedling growth because the soluble protein and soluble sugar were slowly mobilized (Delgado, Coelho, & Buba, 2015).

The wheat plant architecture has undergone modifications through various breeding programs so that the resources obtained may be maximized, prioritizing the ratios of the longer and thinner roots and lower shoot to root length (Chapagain, Super, & Riseman, 2014). An increase in the length and surface area of roots can be used to indicate genotypes with greater soil exploration capacity, thus being more efficient in the absorption of water and nutrients (Batista, Furtini Neto, Deccetti, & Viana, 2016). In the present experiment, the morphological plant root characteristics showed a decline in herbicide-applied treatments, particularly in the cultivar TBIO Sinuelo. This clearly demonstrates that the herbicides applied during the preharvest period were present in the seeds because when the new seedlings were generated, the meristematic region was impacted by the residues. When 2 kg ha^{-1} of glyphosate was used, Jaskulski and Jaskulska (2014) reported a 25.7 mm decrease in the root length in comparison to the treatment without herbicide application. For plants, chichimec acid acts as a precursor of several essential aromatic compounds; hence, when glyphosate is applied to the plants, high levels of this byproduct are observed because the route is blocked. (Bresnahan et al., 2003). Thus, to locate the sites of the accumulation of this compound in the plant because of its systemic character, these same authors recorded that on the seventh day post application (368 ppm), the root system was the organ most affected.

As the evidence revealed changes in the morphological parameters of the seedlings, the biochemical and physiological parameters were assessed. The ROS levels and antioxidant enzyme activities were found to be directly related to the signaling and defense against stressors, including responses to water deficit, salinity, extreme temperatures, heavy metals, and pathogen attacks, among others (Barbosa et al., 2014). In this context, due to the forced maturation induced by the chemical desiccation caused by the herbicides, the present research found that these seeds produced seedlings high in H_2O_2 concentration in the aerial portions. In the leaves, the chief organelles that produced this compound, in order of significance, are the peroxisomes, chloroplasts and mitochondria (Bhattacharjee, 2010). Among the processes involved in H_2O_2 generation, the electron transport chain found in the photosystems is the chief generator. The herbicide paraquat inhibits photosystem I. The molecule behaves as an electron acceptor in a site near ferredoxin, causing a loss of the reduced substances and thereby producing a biochemical imbalance in the plant (Oliveira Jr., 2011). This herbicide was observed to induce the highest H_2O_2 concentrations.

The dismutation of the superoxide radical ($O_2^{\bullet -}$) by the enzyme SOD, which controls the ROS levels in the mitochondria, chloroplasts and cytosol (Mittler, 2002), induces H_2O_2 production. Regulating the SOD enzyme activity indicates a battle against the oxidative stress triggered by the biotic and abiotic stressors and significantly influences the survival of the plants under stressful environmental conditions (Gill & Tuteja, 2010). The herbicide residues present could behave as a stress-causing factor for the seedlings. The high activity of this enzyme was evident in the aerial portion of the seedlings, even in the treatment where the herbicides were not applied. The effect was anticipated to be directly proportional to the H_2O_2 levels; however, the inference drawn was that this byproduct was related to other sources. For instance, photorespiration is one of the metabolic pathways that induced increased H_2O_2 generation in photosynthetic cells (Barbosa et al., 2014). Furthermore, the regulation of H_2O_2 levels can be linked to physiological processes in the plant, such as senescence, photorespiration, photosynthesis, stomatal movement, growth and development (Gill & Tuteja, 2010).

The peroxidases, principally found in the cellular wall and vacuole, play a critical role in detoxifying the excess H_2O_2 in the plant. This is often used as an indicator of biotic and abiotic stress, as it enables the early identification of the morphogenic processes occurring during cell differentiation, plant growth and multiplication, in addition to others (Barbosa et al., 2014). The present work revealed high activity levels of the POD enzyme in all the treatments using herbicides and demonstrated that the roots show more intense activity compared with the aerial portion. A definite relationship was observed between the H_2O_2 levels in the aerial portion of the seedlings and enzyme activity because, by deduction, it appears that the enzyme is incapable of dismantling all the H_2O_2 generated. From the behavior of the SOD and POD enzymes, it can be confirmed that a relationship does exist, using the data drawn from the aerial portion of the seedlings.

This study facilitated the verification that morphological and physiological effects of the practice of preharvest desiccation are restricted to grain production, based on the aspect of herbicide residues and human health. Stress-related physiological processes were affected by the presence of ROS and possibly the seed reserve supply.

Conclusion

The shoot and root morphology are negatively influenced, showing a rise in enzymatic activity and hydrogen peroxide concentration in the seedlings from the desiccated plants.

Acknowledgements

The authors show their gratitude towards the Coordination of Improvement of Higher Education Personnel (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES) and the National Council of Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq) for providing the research grant.

References

Barbosa, M. R., Silva, M. M. de A., Willadino, L., Ulisses, C., & Camara, T. R. (2014). Geração e desintoxicação enzimática de espécies reativas de oxigênio em plantas. *Ciência Rural*, *44*(3), 453-460. DOI: 10.1590/S0103-84782014000300011

Batista, R. O., Furtini Neto, A. E., Deccetti, S. F. C., & Viana, C. S. (2016). Root morphology and nutrient uptake kinetics by australian cedar clones. *Revista Caatinga*, *29*(1), 153-162. DOI: 10.1590/1983-21252016v29n118rc

Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry*, *44*(1), 276-287. DOI: 10.1016/0003-2697(71)90370-8

Bellé, C., Kulczynski, S. M., Basso, C. J., Edu Kaspary, T., Lamego, F. P., & Pinto, M. A. B. (2014). Yield and quality of wheat seeds as a function of desiccation stages and herbicides. *Journal of Seed Science*, *36*(1), 63-70. DOI: 10.1590/S2317-15372014000100008

Bhattacharjee, S. (2010). Sites of generation and physicochemical basis of formation of reactive oxygen species in plant cell. In S. D. Gupta (Ed.), *Reactive oxygen species and antioxidants in higher plantas* (p. 1-30). Enfield, NH: Science Publishers.

Brasil. (2009). *Regras para análise de sementes*. Brasília, DF: MAPA/ACS. Retrieved on Sep. 29, 2018 from http://www.agricultura.gov.br/assuntos/insumos-agropecuarios/arquivos-publicacoes-insumos/2946_regras_analise__sementes.pdf

Bredemeier, C., Mundstock, C. M., & Büttenbender, D. (2001). Efeito do tamanho das sementes de trigo no desenvolvimento inicial das plantas e no rendimento de grãos. *Pesquisa Agropecuária Brasileira*, *36*(8), 1061-1068. Retrieved on Sep. 29, 2018 from http://seer.sct.embrapa.br/index.php/pab/article/view /6230/3295

Bresnahan, G. A., Manthey, F. A., Howatt, K. A., & Chakraborty, M. (2003). Glyphosate applied preharvest induces shikimic acid accumulation in hard red spring wheat (*Triticum aestivum*). *Journal of Agricultural and Food Chemistry*, *51*(14), 4004-4007. DOI: 10.1021/jf0301753

Chapagain, T., Super, L., & Riseman, A. (2014). Root architecture variation in wheat and barley cultivars. *American Journal of Experimental Agriculture*, *4*(7), 849-856. DOI: 10.9734/ajea/2014/9462

Cruz, C. D. (2013). GENES - a software package for analysis in experimental statistics and quantitative genetics. *Acta Scientiarum. Agronomy*, *35*(3), 271-276. DOI: 10.4025/actasciagron.v35i3.21251

Del-Buono, D., Ioli, G., Nasini, L., & Proietti, P. (2011). A comparative study on the interference of two herbicides in wheat and italian ryegrass and on their antioxidant activities and detoxification rates. *Journal of Agricultural and Food Chemistry*, *59*(22), 12109-12115. DOI: 10.1021/jf2026555

Delgado, C. M. L., Coelho, C. M. M. d., & Buba, G. P. (2015). Mobilization of reserves and vigor of soybean seeds under desiccation with glufosinate ammonium. *Journal of Seed Science*, *37*(2), 154-161. DOI: 10.1590/2317-1545v37n2148445

Empresa Brasileira de Pesquisa Agropecuária [EMBRAPA]. (2017). *Informações técnicas para trigo e triticale*. Retrieved on Sep. 29, 2018 from http://ainfo.cnptia.embrapa.br/digital/bitstream/item/155787/1/Informacoes-Tecnicas-para-Trigo-e-Triticale-Safra-2017-OL.pdf

Fipke, G. M., Martin, T. N., Nunes, U. R., Deak, E. A., Stecca, J. D. L., Winck, J. E. M., ... Rossato, A. C. (2018). Application of non-selective herbicides in the pre-harvest of wheat damages seed quality. *American Journal of Plant Sciences*, *9*(1), 107-123. DOI: 10.4236/ajps.2018.91010

Giannopolitis, C. N., & Ries, S. K. (1977). Superoxide dismutases: Occurrence in higher plants. *Plant Physiology*, *59*(2), 309-314. DOI: 10.1104/pp.59.2.309

Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, *48*(12), 909-930. DOI: 10.1016/j.plaphy.2010.08.016

Jaskulski, D., & Jaskulska, I. (2014). The effect of pre-harvest glyphosate application on grain quality and volunteer winter wheat. *Romanian Agricultural Research*, *31*(1), 283-289.

Krenchinski, F. H., Cesco, V. J. S., Rodrigues, D. M., Pereira, V. G. C., Albrecht, A. J. P., & Albrecht, L. P. (2017). Yield and physiological quality of wheat seeds after desiccation with different herbicides. *Journal of Seed Science*, *39*(3), 25-261. DOI: 10.1590/2317-1545v39n3172506

Loreto, F., & Velikova, V. (2001). Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. *Plant Physiology*, *127*(4), 1781-1787. DOI: 10.1104/pp.010497

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science*, *7*(9), 405-410. DOI: 10.1016/S1360-1385(02)02312-9

Oliveira Jr., R. S. (2011). Mecanismos de ação de herbicidas. In R. S. Oliveira Jr., J. Constantin, & M. H. Inoue (Eds.). *Biologia e manejo de plantas daninhas* (p.141-192). Curitiba, PR: Omnipax.

Soltani, A., Gholipoor, M., & Zeinali, E. (2006). Seed reserve utilization and seedling growth of wheat as affected by drought and salinity. *Environmental and Experimental Botany*, *55*(1–2), 195-200. DOI: 10.1016/j.envexpbot.2004.10.012

Zadoks, J. C., Chang, T. T., & Konzak, C. F. (1974). A decimal code for the growth stages of cereals. *Weed Research*, *14*(6), 415-421. DOI: 10.1111/j.1365-3180.1974.tb01084.x

Zeraik, A. E., Souza, F. S. De, Fatibello-Filho, O., & Leite, O. D. (2008). Desenvolvimento de um spot test para o monitoramento da atividade da peroxidase em um procedimento de purificação. *Quimica Nova*, *31*(4), 731-734. DOI: 10.1590/S0100-40422008000400003