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RESEARCH ARTICLE

BIOCHEMICAL PERFORMANCE, VIGOR AND CHARACTERISTICS OF INITIAL GROWTH OF WHEAT PLANTS UNDER DIFFERENT SOWING DEPTHS

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ABSTRACT

Triticum aestivum L., world's second most produced grain, may have its productivity harmed due to various factors, such as sowing depth. This study aimed to evaluate the biochemical performance, vigor and characteristics of initial growth of wheat plants under different sowing depths. The experiment was performed in a greenhouse (31°48'15" S and 52°24'19" W). The experimental design were randomized blocks and the treatments were the sowing depths of 20, 30, 40 and 50 mm. The experimental unit was each tray with 50 seeds. Results were subjected to analysis of variance ($p<0.05$) and, when significant by F-test, adjusted to orthogonal polynomials. Variables evaluated were: seedling emergence, shoot and root length, shoot and root dry matter, leaf area, shoot to root ratio, isoenzymatic expression of peroxidase and malate dehydrogenase and *a*, *b* and total chlorophyll content. Seedling emergence, shoot dry matter and leaf area reduced with the increasing sowing depth, as the best performance in seedling emergence and the higher leaf area were obtained at 20 mm depth and the shoot dry matter decreased from 30 mm downwards. Chlorophyll *a*, *b* and total contents were higher at sowings depths from 30 to 40 mm. An additional isoenzyme of malate dehydrogenase was expressed at the shoots at the sowing depth of 50 mm while an extra peroxidase isoenzyme was expressed at the roots at the sowing depths of 30, 40 and 50 mm. Deep sowings reduce seed vigor, negatively affecting the initial growth of wheat plants.

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INTRODUCTION

Triticum aestivum L. has an important position in the Brazilian and international markets, as one of the major winter crops, especially in Southern Brazilian (KLEIN *et al.*, 2008). The wheat acreage reached 2.7 million ha in 2014, which is 4.2% of the total Brazilian agricultural area (CONAB, 2016). Several environmental factors and management influence wheat initial establishment and yield. During sowing, one of the aspects that must be considered is the depth of seed deposition, since it may affect seedling emergence (SILVA *et al.*, 2008). Thus, the seed must be placed at depths that allow an intimate contact with the wet soil and a high percentage of emergence (Modolo *et al.*, 2010).

An uneven emergence negatively affects plant stand, moreover, inadequate sowing depths during the initial development causes a negative effect on initial growth characteristics, affecting the synthesis of assimilates and the carbon allocation across different plant structures (PEDÓ *et al.*, 2014). The greater the sowing depth is, the longer is the period required for seedling emergence and thus to reach the soil surface. In this sense, a greater period to emerge results in an increased consumption of endosperm reserves, generating seedlings with lower vigor which will delay the formation of the photosynthetic apparatus (TAIZ and ZEIGER, 2013). It is possible that longer periods necessary for seedling emergence at greater sowing depths negatively modifies some physiological variables of initial growth, for example the shoot to root ratio. The evaluation of initial growth in cultivated species subjected to various environmental stresses is a useful tool to evaluate genotype performance under different

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management conditions. Sowing depth effects on dry matter accumulation, leaf area, isoenzymatic profile and the phenolic content, provide important information due to their relationship to the solar radiation intercepted and taken up by the leaves, which are responsible for the growth and energy production in plants (JENKS and HASEGAWA 2016). The chlorophyll is a key molecule for the execution of photosynthesis and it is composed of a tetrapyrrole ring bind to a magnesium atom in its center and a long (C_{20}) hydrophobic side chain, known as a phytol tail, that is attached to the tetrapyrrole ring and renders the molecule extremely nonpolar (BUCHANAN *et al*., 2015). The first precursor of chlorophyll is δ-Aminolevulinic acid which is converted to protoporphyrin IX through a series of reactions; then, a magnesium atom is inserted and subsequent reactions produce protochlorophyllide which can be reduced to chlorophyllide strictly in the presence of light in angiosperms; finally, chlorophyll *a* is synthetized by esterification of the phytol side chain (BUCHANAN *et al*., 2015).

Hence, at shallower sowing depths, the coleoptile of wheat seedlings could reach the soil surface more rapidly, occurring the formation of the first leaves and enabling the greater synthesis and concentration of chlorophylls *a* and *b*, reducing the time required to the plant to become an autotrophic organism. The chlorophyll content is related to photosynthetic efficiency, due to its role in luminous energy uptake and transport, and to the adaptability and growth under different environments which allows evaluating photosynthetic potential (TAIZ and ZEIGER, 2013). It is worth mentioning that under abiotic stresses such as drought, salt and water, the chlorophyll content reduces (SANCHEZ *et al*., 1983; LIMA *et al*., 2004; MAFAKHERI *et al*., 2010; KHAYATNEZHAD and GHOLAMIN, 2012), therefore, such evaluation allows the determination of the level of stress experienced.

The exposure of plants to stressful environmental conditions causes the increase of reactive oxygen species (ROS) such as superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) (BUCHANAN *et al*., 2015). The distinct subcellular localization and biochemical properties of antioxidant enzymes, their differential inducibility at the enzyme and gene expression level and the plethora of non-enzymatic scavengers render the antioxidant systems a very versatile and flexible unit that can control ROS accumulation temporarily and spatially (VRANOVÁ *et al*., 2002). The isozymes are enzymes that differ in amino acid sequence yet catalyze the same reaction which are encoded by different genetic loci and can often be distinguished from each other by biochemical properties such as electrophoretic mobility (NELSON et. al. 2014). In this sense, the use of electrophoresis is a useful tool to evaluate enzymatic alterations under different conditions. Therefore, the objective of this study was to measure the effects of sowing depth on the biochemical performance, vigor and the characteristics of initial growth in wheat plants.

MATERIALS AND METHODS

The experiment was performed in a chapel greenhouse, located at 31º48'15" S and 52º24'19" W. The evaluations were performed at the Didactic Laboratory of Seed Analyses from the Phytotechny Department. The region presents temperate climate without dry season and hot summer, which is classified as Cfa accordingly to Köppen-Geiger climate classification (PEEL *et al*., 2007).

The wheat cultivar used was TBIO Iguaçu which was sown into polyethylene trays filled with a solodic, haplic, eutrophic planosol (albaqualf) from the Pelotas mapping unit. The soil was previously amended accordingly to soil analysis in which nitrogen fertilization was accomplished using 70 g of urea m^{-3} of soil, phosphorous fertilization through the application of 140 g of triple superphosphate m^{-3} of soil and potassium was provided via 140 g of potassium chloride m^{-3} of soil. Drip irrigation was used to maintain field capacity. The treatments were composed by the following sowing depths 20, 30, 40 and 50 mm, obtained with the use of a ruler, scaled to millimeter. Vigor expression and physiological characteristics of initial growth were evaluated by the following variables:

- Seedling emergency (E): 200 seeds per treatment, distributed in subsamples of 50 seeds. The evaluation was performed 21 days after sowing and the results were expressed as a percentage of emerged seedlings.
- Shoot (L_S) and root length (L_R): obtained from four subsamples of 10 seedlings, by the end of the seedling emergency test. The length of the shoot was measured starting at the root insertion of the basal region to the shoot apex and the root length was measured from the root insertion to the root apex. The results were expressed in cm.
- Leaf area (A_L): four subsamples of ten seedlings were measured in a leaf area measurer equipment, model LI-3100, expressing the results in m² $seedling^{-1}$.
- Shoot (W_S) and root (W_R) dry matter content: obtained from four subsamples with 10 seedlings each, at the end of the seedling emergence test. The seedlings were accommodated inside kraft paper envelops and subjected to kiln drying at 70 ºC for 72 hours. The results were expressed in g.
- Shoot to root ratio (S:R): calculated by the equation $S{:}R=W_S/W_R$.
- Chlorophyll content (*a*, *b* and total): obtained from seedling leaves at the end of the seedling emergence test using four samples of 0.2 g of fresh tissue each treatment. The samples were macerated with mortar and pestle within 10 mL of acetone solution (80%) in a darkroom under green light (ARNON, 1949). The macerated mixture was subjected to filtering and the volume completed to 25 mL. The chlorophyll content was calculated according to LICHTENTHALER (1987) and expressed in mg g^{-1} of fresh weight.
- Isoenzymatic expression: obtained from ten seedlings collected at the end of seedling emergence test. The isoenzyme expression was determined by vertical electrophoresis on polyacrylamide gel. Initially, the frozen samples with liquid nitrogen were macerated using mortar and pestle. Then, 200 mg of each sample was transferred to centrifuge tubes with later addition of the extraction solution (Lithium borate 0.2 M pH 8.3 + Tris citrate 0.2 M pH 8.3 + 0.15% of 2-mercaptoetanol) at 1:2 ratio (*m*/*v*). Finally, malate dehydrogenase and

peroxidase isoenzyme expression were measured by electrophoresis in polyacrylamide gel (7%), using 20 µL of sample extracts.

The experiment was performed under randomized block design, using four treatments and five repetitions. Each experimental unit consisted of four subsamples (replicates) of 50 seeds per polyethylene trays. With the exception of seedling emergence, all other variables were evaluated from seedlings collected in the central rows of the polyethylene trays in order to reduce border effect. The results of isoenzymatic expression were analyzed visually by the presence and intensity of bands. Data from other variables were subjected to the analysis of variance and, when F-statistic was significant $(p<0.05)$, adjusted by orthogonal polynomials.

RESULTS AND DISCUSSION

The shoot length increased until sowing depth of 50 mm (Figure 1A). Whereas the root length decreased at 33 cm depth, with later increase to the depth of 50 mm (Figure 1B). Thus, it is perceptible that a marked decrease in the root length did not affect negatively the elongation of the shoot. The increase in length is the result of cellular elongation (BURGOS *et al*., 2004) thus seeking for the emergence of vegetative structures and leaf area establishment to capture sunlight.

The shoot dry matter increased to the depth of 30 mm and then decreased until 50 mm depth (Figure 1C). The increase in dry matter is explained by the efficient investment of seed reserves, contributing to the rapid emergence and uniform seedling growth, leaf appearance and expansion, aiming at the formation of a light energy capture area during the autotrophic phase. According to Grotta *et al.* (2008), the increase in sowing depth results in lower shoot dry matter in peanut. However, it is worth mentioning that carbon allocation differs between species of different families or even inside the family. Root dry matter was reduced with the increase of sowing depth; at 50 mm, plants presented the lower root dry matter content (Figure 1D).

The stress imposed by thicker layers of soil over the seed, may have affected the translocation and allocation of assimilates at different seedling structures (PEDÓ *et al*., 2014) or yet, due to a delay in leaf area formation and expansion. Similar results were found by PACHECO *et al.* (2009) while studying seedling emergence and growth of cover crops agronomical features at different sowing depths. Seedling emergence was reduced with the increasing sowing depth. A quadratic model explained this pattern with a high coefficient of determination (R^2= 0.99). The decrease was more pronounced in the interval from 20 to 40 mm depth, representing a 20% reduction (Figure 2A).

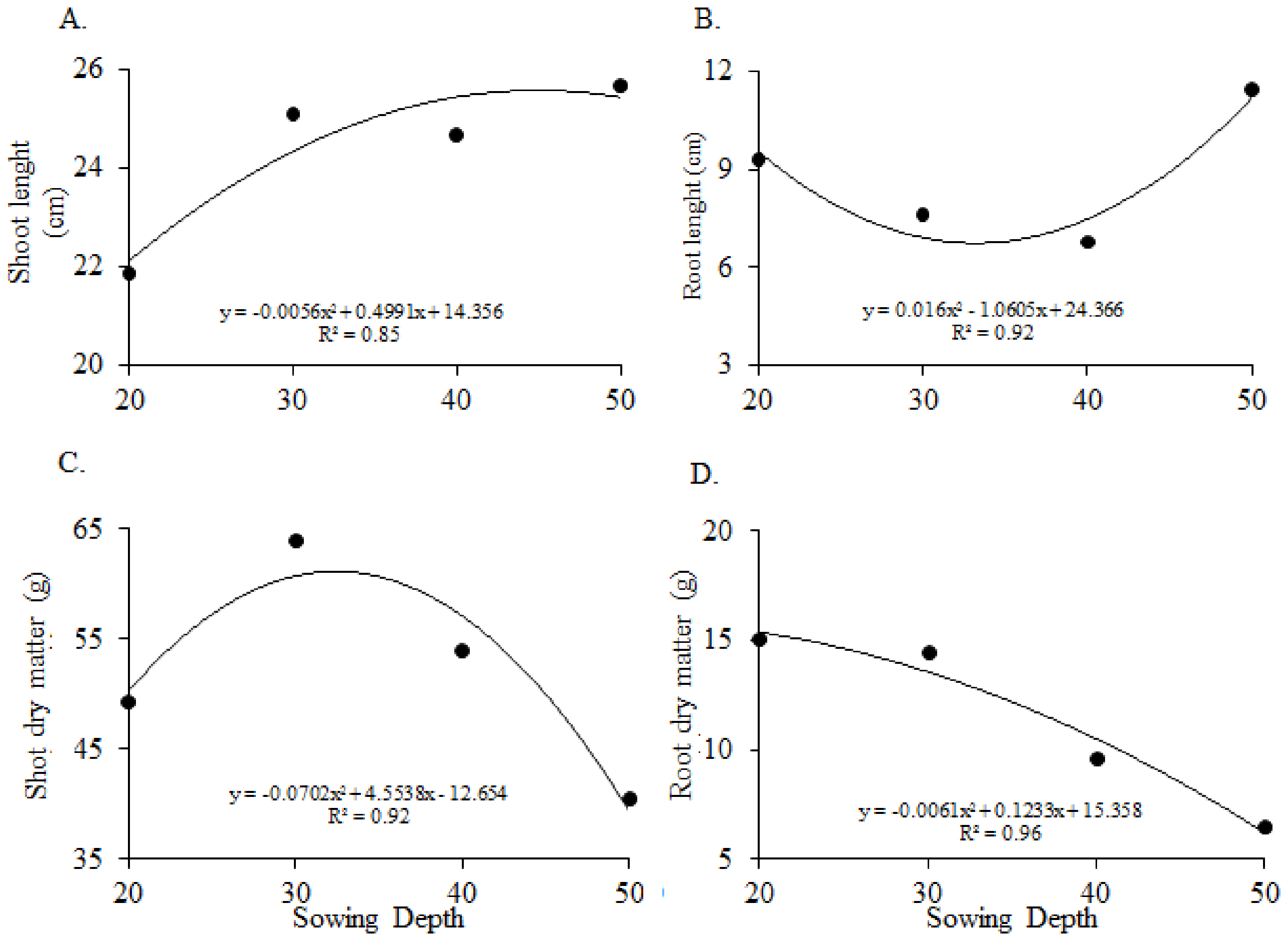

Figure 1 - Shoot length (A), root length (B), shoot dry matter content (C) and root dry matter content (D) of wheat seedlings under four sowing depths. *Statistically different by Tukey test at 5% significance level ($p \leq 0.05$)

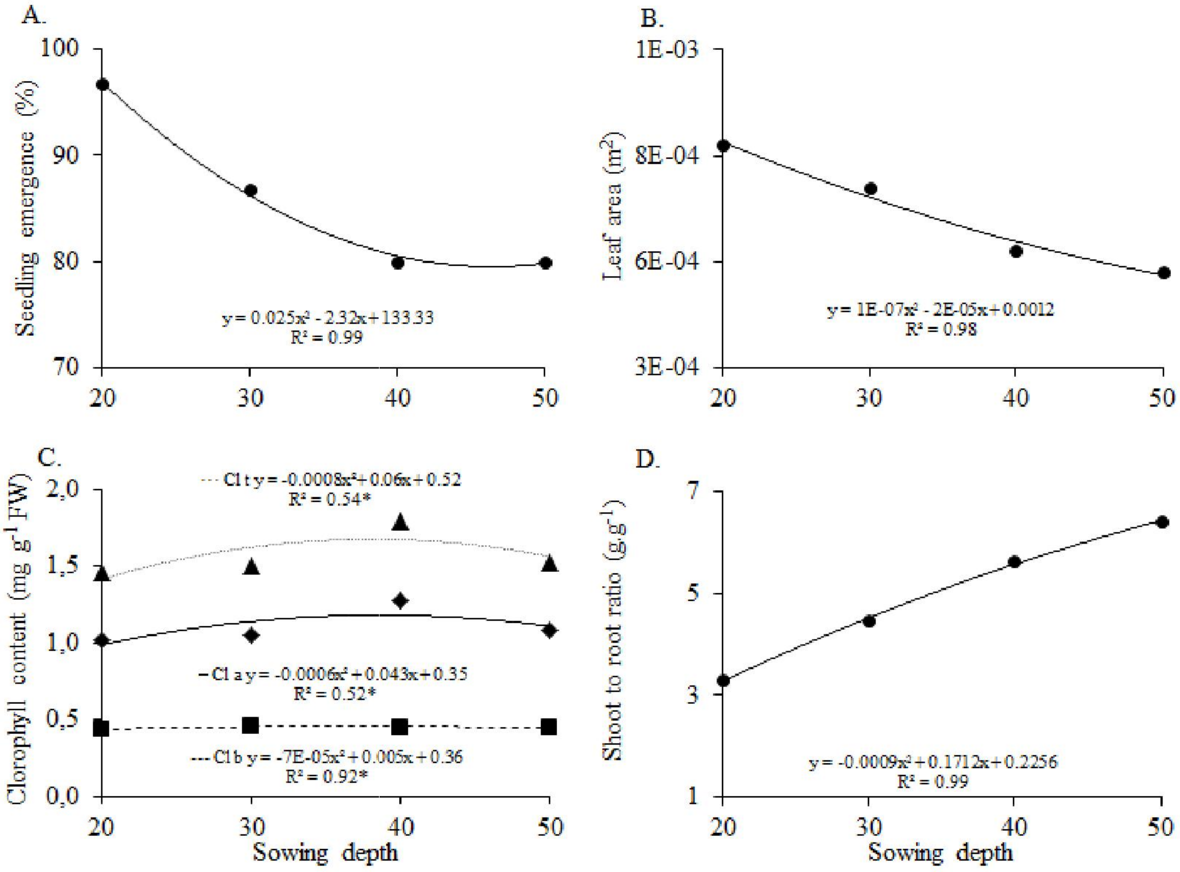

Figure 2 - Seedling emergence (A), leaf area (B), chlorophyll content (C) and shoot to root ratio (D) of wheat seedlings under four sowing depths. ◆ : *a* chlorophyll; ■ *b* chlorophyll; ▲ *total* chlorophyll. *Statistically different by Tukey test at 5% significance level ($p<0.05$)

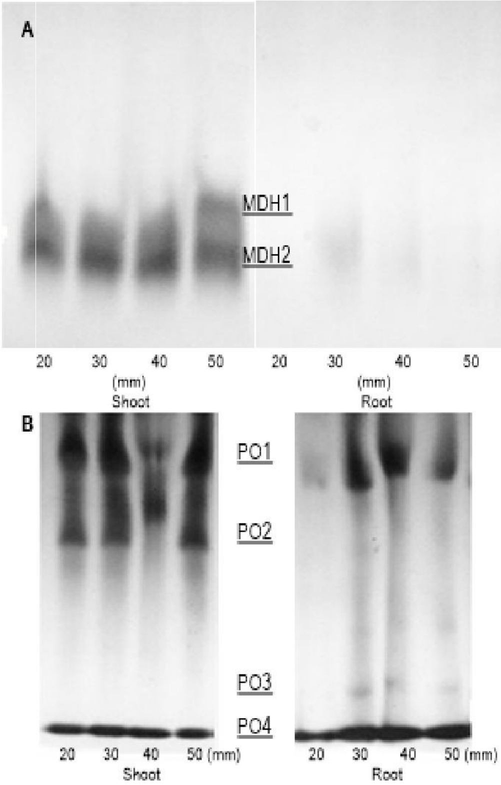

Figure 3 - Isoenzymatic expression of wheat seedlings under different sowing depths. (A) Malate dehydrogenase, (B) peroxidase

The lower seedling emergence with the increasing depth may be due to the physical barrier imposed by a thicker layer of soil over the seeds. SILVA *et al.* (2004) found similar results while evaluating sowing depth on soybean seedling emergence and vegetative development, in which the seedling emergence was greater at shallower sowing depths. Evaluating seedling emergence of cover crop species at different depths, PACHECO *et al.* (2009) verified a significant decrease of seedling emergence at higher sowing depths for the species *Pennisetum glaucum*, *Eleusine coracana* and Cober Crop®, which is a hybrid of *Sorghum bicolor* x *Sorghum sudanense*. The wheat leaf area decreased alongside the increase of sowing depth (Figure 2B). At 50 mm leaf area decreased up to 37% compared with that of seedlings at 20 mm sowing depth. This result represents a decrease of light energy intake, thus reducing carbon assimilation and energy and mass accumulation. While studying different sowing depths on *Pennisetum glaucum* L., PACHECO *et al.* (2009) found similar results. Crop yield will be higher as leaf area increases over time, since photosynthesis is directly related to plant leaf area (MANFRON *et al.*, 2003). The adequate supply of metabolites to the different plant parts is attributed to photosynthetic efficiency.

The leaf chlorophyll *a* content increased to the sowing depth of 40 mm and then decreased, whereas, the chlorophyll *b* content was not amended (Figure 2C). The higher total chlorophyll content was reached within plants at the depth of 40 mm. Both types of chlorophylls are degraded and synthetized continually during vegetative growth and both require light, which can also, under some conditions, cause its photo-oxidation (BUCHANAN *et al.*, 2015). In this context, the rapid emergence at shallower depths may have allowed a greater influence of light period on the photooxidative processes of the chlorophyll molecule. Furthermore, the lower chlorophyll content at deeper sowings can be explained by the longer period required for the formation of the photosynthetic apparatus, as previously hypothesized. The shoot to root ratio increased with sowing depth (Figure 2D). This increase was due, mostly, to the decrease in root dry matter, since shoot dry matter presented a slighter decrease. These results may be due to the smaller leaf area and derived low production of carbohydrates in the leaves, affecting its translocation to the roots and thus decreasing growth and dry matter accumulation, in plants at greater depths. According to PEDÓ *et al.* (2014), the increase on shoot to root ratio on beans (*Phaseolus vulgaris*) indicates a negative effect of sowing depth on assimilate partitioning. Seedling shoots promoted the expression of an allele of the isoenzyme malate dehydrogenase (*MDH*1) at the sowing depth range of 20 to 40 mm (Figure 3a), while at the sowing depth 50 mm two alleles were expressed (*MDH*1 and *MDH*2). On the other hand, the roots did not show any expression. This isoenzyme plays an important role on the citric acid cycle, catalyzing the conversion of malate to oxaloacetate, thus producing NADH, which is a key compound for ATP production (TAIZ and ZEIGER, 2013), along with other intermediate compounds essential to cellular functioning. Thus, the additional isoenzyme expressed at the sowing depth of 50 mm may be related to a higher energetic demand and to the synthesis of the necessary compounds directed to break soil superficial layers. According to MALONE *et al.* (2007), the higher expression of malate dehydrogenase is associated to the synthesis of new tissues at the initial developmental stages. The peroxidase isoenzyme, at the four sowing depths, expressed three alleles (*PO1*, *PO2*, and *PO4*) at the shoot. However, the roots expressed four alleles (*PO1, PO2*, *cPO3* and *PO4*), which were detected at 30, 40 and 50 mm depth (Figure 3b). The peroxidases show multiple isoforms, which were involved within a variety of cell reactions, for example, the phenolic compounds oxidation, polysaccharide link, indole-3-acetic acid oxidation, monomers link and cell elongation (SBALCHEIRO *et al.*, 2009) and the decrease of hydrogen peroxide level, a free radical which leads to lipid peroxidation. The greater number of peroxidase isozymes may be due to the necessity of a greater activity in response to a higher production of free radicals in the roots, caused by deep sowing. The accumulation of these potentially toxic compounds may have caused the loss of selectivity on cell membranes, affecting the wheat seedling vigor expression (Figure 2A) and resulting on low dry matter accumulation (Figure 1C; 1D). It is worth mentioning that the isoenzymatic expression does not correspond to enzyme activity, however, it constitutes an adequate indicative of the plant response to the stress imposed, as verified in this study for the enzymes malate dehydrogenase and peroxidase. The use of greater sowing depths affects negatively the wheat seedlings vigor expression, reducing distinctly the emergence, the leaf area, shoot and root dry matter at 30 mm. However, the increase in sowing depth was accompanied by the rise of shoot length and shoot to root ratio.

Conclusion

The increase of sowing depth negatively affects the seed vigor expression and the initial growth of wheat plants while the leaf area is reduced at greater sowing depths, with a marked decrease from 40 mm depth. Furthermore, chlorophyll *a*, *b* and total present higher contents at the sowing depths from 30 to 40 mm. The number of the isozymes malate dehydrogenase and peroxidase expressed is affected by sowing depth and increases at greater depths.

REFERENCES

Arnon D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiology 24: 1-15.

Buchanan, B.B. *et al.* 2015. Biochemistry and molecular biology of plants. American Society of Plant Physiologists, 2. 1280 p.

Burgos, N.R. *et al.* 2004. Growth inhibition and root ultrastructure of cucumber seedlings exposed to allelochemicals from rye. Journal of Chemical Ecology, 30(3): 671-689. <http://dx.doi.org/10.1023/B:JOEC.0000018637.94002.ba>

CONAB (Companhia Nacional de Abastecimento). 2016. Acompanhamento da safra brasileira de grãos: v.3, safra 2015/16, N. 4, quarto levantamento. Visited on January 16nd, 2016. Available at: < http://www.conab.gov.br/OlalaCMS/uploads/arquivos/16_01_12_14_17_16_boletim_graos_janeiro_2016.pdf>.

da Silva, R.P., Corá, J.E., Filho, A.C., Furlani, C.E.A. and Lopes, A. 2008. Efeito da profundidade de semeadura e de rodas compactadoras submetidas a cargas verticais na temperatura e no teor de água do solo durante a germinação de sementes de milho. Ciência e Agrotecnologia 32(3): 929-937.

Grotta, D. C. C., Furlani, C. E. A., Silva, R. P., Reis, G. N. D., Cortez, J. W. and Alves, P. J. 2008. Influência da profundidade de semeadura e da compactação do solo sobre a semente na produtividade do amendoim. Ciências e Agrotecnologia 32(2): 547-552.

Jenks A.M. Hasegawa P.M. 2016. Plant Abiotic Stress. United States Arid Land Agricultural Research Center USDA-ARS Maricopa, Arizona.

Khayatnezhad m., Gholamin r. The effect of drought stress on leaf chlorophyll content and stress resistance in maize cultivars (*Zea mays*). African Journal of Microbiology Research 6(12): 2844-2848.

Klein V.A., Massing, J. P., Junior, I. J. B., Marcolin, C. D., Vieira, M. L. 2008. Velocidade de semeadura de trigo sob sistema plantio direto. Revista de Ciências Agroveterinárias 7(2): 150-156.

Lichtenthaler H.K. 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods in Enzymology 148: 350-382.

Mafakheri, A., Siosemardeh, A., Bahramnejad, B., Struik, P. C., Sohrabi, Y. 2010. Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Australian Journal of Crop Science 4(8):580-585.

Malone, G., Zimmer, P.D., Meneghello, G.E., Castro, M.A.D.S.D. and Peske, S.T. 2007. Expressão diferencial de isoenzimas durante o processo de germinação de sementes de arroz em grandes profundidades de semeadura. Revista Brasileira de Sementes 29(1): 61-67.

Manfron, P. A., Dourado Neto, D., Pereira, A.R., Bonnecarrére, R.A.G., Medeiros, S.L.P., Pilau, F. G. 2003. Modelo do índice de área foliar da cultura do milho. Revista Brasileira de Agrometeorologia 11(2): 333-342.

Modolo, A.J., Trogello, E., Nunes, A.L., Fernandes, H.C., Silveira, J.C.M.D., Dambrós, M.P. 2010. Efeito de cargas aplicadas e profundidades de semeadura no desenvolvimento da cultura do feijão em sistema plantio direto. Ciência e Agrotecnologia 34(3): 739-745.

Nelson, D.L., Cox, M.M. 2011. Princípios de bioquímica de Lehninger. Porto Alegre: Artmed, 2011. 6. ed. Porto Alegre: Artmed, 2014.

Pacheco, L.P; Pires, F.R., Monteiro, F.P; de Oliveira Procópio, S., de Assis, R.L; Silva, G.P., Petter, F.A. 2009. Emergência e crescimento de plantas de cobertura em função da profundidade de semeadura. Semina: Ciências Agrárias 30(2): 305-314.

Pedó, T., Segalin, S.R., da Silva, TA., Martinazzo, E.G., Neto, A.G., Aumonde, T.Z. and Villela, F.A. 2014. Vigor de sementes e desempenho inicial de plântulas de feijoeiro em diferentes profundidades de semeadura. Revista Brasileira de Ciências Agrárias, 9(1): 59-64.

Peel, M.C., Finlayson, B.L. and McMahon, T.A. 2007. Updated world map of the Koppen-Geiger climate classification. Hydrol. Earth Syst. Sci. 11: 1633-1644.

Sbalcheiro, C.C., Denardin, N.D. and Brammer, S.P. 2009. Alterações de isoenzimas peroxidases em plantas de feijoeiro tratadas com biocontrolador do crestamento bacteriano comum. *Tropical Plant Pathology* 34(1): 29-37.

Silva, R.D., Teixeira, F. and Campos, M. 2004. Efeito da profundidade de semeadura e da carga sobre a roda compactadora no desenvolvimento da soja (*Glycine max*). Engenharia na Agricultura 12(3): 169-176.

Taiz L; Zeiger E. 2013. Fisiologia Vegetal. 5 eds. Porto Alegre. Artmed. 954 p.

Vranova, E., Inzé, D., Van Breusegem, F. 2002. Signal transduction during oxidative stress. Journal of Experimental Botany 53 (372): 1227-1236.

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