



EFFECT OF SILICON FERTILIZATION ON THE EXPRESSION OF ISOENZYMES, THE GERMINATION AND SEED YIELD OF WHEAT

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ABSTRACT

This study evaluated the effects of silicon fertilization on expression of isoenzymes, germination and seed yield of wheat. The experimental design was completely randomized with four replications. The treatments consisted of combinations of two sources of silicon and six levels in a factorial AxB, totaling 12 treatments with four replications. The isoenzyme patterns were studied: esterase, glutamate oxaloacetate transaminase, malate dehydrogenase and peroxidase. After harvesting, it was evaluated: number of seeds per plant, thousand seed weight, hectoliter weight and seeds yield. It is concluded that silicon fertilization, with both sources, does not affect the germination of wheat seeds, but further increase in productivity up to a dose of 1720.84 kg ha⁻¹. The source of rice husk ash promotes greater number of ears per plant and higher seed yield per plant than the kaolin. Electrophoretic variation of isozymes is associated with the supply of silicon in the soil in wheat plants.

KEYWORDS: *Triticum aestivum* L., silicon, isoenzyme, electrophoresis, productivity.

EFEITO DA ADUBAÇÃO SILICATADA NA EXPRESSÃO ISOENZIMÁTICA, NA GERMINAÇÃO E NO RENDIMENTO DE SEMENTES DE TRIGO

RESUMO

Objetivou-se com o presente trabalho avaliar os efeitos da adubação silicatada na expressão isoenzimática, na germinação e no rendimento em sementes de trigo. O delineamento experimental foi inteiramente casualizado, com quatro repetições. Os tratamentos consistiram em combinações de duas fontes de silício, em esquema fatorial AxB (Fator A: Caulim e cinza de casca de arroz, Fator B: níveis de 0, 500, 1000, 1500, 2000 e 2500 kg ha⁻¹), totalizando 12 tratamentos, com quatro repetições. Os padrões isoenzimáticos estudados foram esterase (EST, EC 3.1.1.1), glutamato oxaloacetato transaminase (GOT, EC 2.6.1.1), malato desidrogenase (MDH, EC 1.1.1.37) e peroxidase (PO, EC 1.11.1.7). Após a colheita avaliaram-se as seguintes variáveis:

número de sementes por planta, peso de mil sementes, peso hectolítrico e rendimento de sementes. Concluiu-se que a adubação silicatada, com ambas as fontes, não afetam a germinação de sementes de trigo, porém promovem aumento na produtividade, até a dose de 1720,84 kg ha⁻¹. A fonte cinza de casca de arroz promove maior número de espigas por planta e maior rendimento de sementes por planta do que o caulim. Variações eletroforéticas de isoenzimas estão associadas ao fornecimento de silício via solo em plantas de trigo, com exceção da malato desidrogenase (MDH, EC 1.1.1.37).

PALAVRAS-CHAVE: *Triticum aestivum* L., silício, isoenzimas, eletroforese, produtividade.

INTRODUCTION

The increasing of production and productivity of major crops, among them wheat, rice, soybeans, corn and beans are crucial to be met the demands of food caused by population growth. Given this, there are aspects that are essential to achieve this goal, as the use of high quality seeds, plant protection and deployment of technologies for increasing crop productivity, emphasizing the use of nutrients, especially those classified as essential, and the beneficial species of the family Poaceae, such as silicon (FAO, 2002).

The incidence of diseases has been a limiting factor in the full expression of yield potential of wheat in Brazil (BACALTCHUK et al., 2006). Among the research for improvement of agricultural production, have focused on seed technology (VIGANO et al., 2010; TOLEDO et al., 2011). According to COPELAND & MCDONALD (2001), plant nutrition is one of the factors that may influence seed vigor. SÁ (1994) emphasizes that well-nourished plants can produce more seeds with better physiological quality, because they can become more tolerant to adverse weather.

Among the nutrients beneficial to plants is silicon (Si), which provides an increase in growth, reducing the incidence of pathogens, improvement of leaf architecture, reduction in transpiration rate, increasing in photosynthetic rate and improves the quality of physiological seeds, providing an increase in yield (LIMA-FILHO, 2004; LIMA-FILHO, 2005). Several authors have reported the importance of silicon for increased resistance and reduced the incidence of fungal diseases, increase in soil fertility, and reduced pesticide costs and environmental impact (DATNOFF et al., 2007; LIMA-FILHO & TSAI, 2007; VIEIRA et al., 2011).

Plants are quite different in their ability to absorb the Si, which is absorbed in the acid form mono silica (H₄SiO₄) passively or actively, by transporters specific membrane for this purpose. The transport and accumulation of silicon can also be regulated by an active process that is triggered by stimulation of protection against diseases, pests and other stress conditions (PERRY, 2007), which leaves the main body of silicon deposit. In plants, approximately 99% of silicon is deposited in the form of silica and less than 1% is ionic or colloidal fraction, a soluble form. Deposits of silica in plants will result in increased production and product quality (BLAICH & GRUNDHÖFER, 1998).

To assess the quality of plants has been used in electrophoresis study of enzymes, or isozymes with respect not only to changes in quality, but also in genetic and biochemical regulations (ISTA, 1992), and analyzing proteins and nucleic acids, and a

versatile technique, relatively simple, quick and very informative power (ACQUAAD, 1992).

The isoenzymes are products of gene expression and highly influenced by the environment and therefore the management, because the genes controlling their expression are manifested in certain developmental stages and in specific organs and tissues, or under a given stimulus (RAMIREZ et al., 1991). In simple terms, the variations may be considered isoenzymes of a particular enzyme in an organism which exhibit the same substrate specificity. According to MALONE et al. (2006), the intensity of the bands and isoenzyme profiles are specific to a particular part of the plant tissue and developmental stage. It has been as important isozymes for checking the quality of plants and seed: esterases (EST), glutamate oxaloacetate transaminase (GOT), malate dehydrogenase (MDH) and peroxidase (PO) (SPINOLA et al., 2000; MENEZES et al., 2004; SANTOS et al., 2005; VIEIRA et al., 2009).

In this context, the aim with this study to evaluate the effect of silicon fertilization on the expression of isozymes, the germination and seed yield of wheat.

MATERIAL AND METHODS

The experiment was conducted in Seed Laboratory and Bio-Sementes in Plant Science Department of Agronomy College "Eliseu Maciel", Federal University of Pelotas (UFPel/FAEM), in crop 2011 with FUNDACEP Horizonte wheat cultivar.

The wheat plants were grown in pots of 15 liters, 10 seeds were sown per pot, only the remaining four plants per pot (high vigor), filled with sieved soil, collected from the A1 horizon of a Haplic Planossolo Solodic Eutric (STRECK et al., 2008), belonging to map unit Pelotas. The fertilization was performed according to the results of soil analysis and recommendations of the CHEMISTRY AND SOIL FERTILITY - RS / SC (2004). It was used only nitrogen, phosphorus and potassium, applied 14 days before seeding and liming was held thirty days before sowing. After seeding the buckets were irrigated daily maintaining the soil at field capacity.

The treatments consisted of combinations of two basic sources of silicon in a factorial AxB (Factor A: Kaolin and rice husk ash, Factor B: levels of 0, 500, 1000, 1500, 2000 and 2500 kg ha⁻¹), totaling 12 treatments with four replications. The application of silicon was carried out with the seeding.

Manual harvesting was done at the stadium in which 2/3 spikelet color was pale yellow or cream, characterizing the physiological maturity. After evaluating the following variables: number of spikes (NS) and number of seeds per plant (NSP): performed by manual counting of the spikes and seeds in each experimental unit. Yield (Y) was obtained by weighing the harvested seed, and the moisture adjusted to 13%. Hectoliter weight (HW) performed with four replicates and specific scale, with 1 liter of seeds, the results were expressed as kg hL⁻¹. Thousand seed weight (TSW) - were used eight replicates of 100 seeds. For weighing calculate the mean, standard deviation and coefficient of variation. All plots showed a coefficient of variation less than four, so we multiplied the average by 10, and thus gave the thousand seed weight (BRASIL, 2009).

The seed quality was evaluated by tests: Germination (G) - performed with four replicates of 50 seeds for each treatment, germination paper substrate ("germitest"), previously moistened with distilled water, using the ratio 2.5 times the weight of dry

paper, and maintained at 20 °C. The evaluations were performed according to the Rules for Seed Analysis (BRASIL, 2009) to eight days after sowing.

The isoenzyme patterns were studied: esterase (EST, EC 3.1.1.1), glutamate oxaloacetate transaminase (GOT, EC 2.6.1.1), malate dehydrogenase (MDH, EC 1.1.1.37) and peroxidase (PO, EC 1.11.1.7). For this, a plant was collected at 30 days after emergence (shoot) per experimental unit at random and macerated in a porcelain mortar in each treatment. From each sample, 200 mg of mash were placed in Eppendorf tube together with the extraction solution (Tris-0.1 M citrate buffer pH 8.3 + 0.1 M lithium borate pH 8.3 + 0.15% 2-mercaptoethanol) 1:2 (w / v). Electrophoresis was performed in 7% polyacrylamide gels, placing 20µl of each sample in holes made with the aid of a comb acrylic. The enzyme standards were analyzed according to the methodology described by SCANDÁLIOS (1969) and ALFENAS (1998). Gels were placed in stainless electrophoretic vertical kept at room temperature. The electrophoretic migration was performed using a potential difference of 10V.cm⁻¹, until the front end formed by the bromophenol blue reached the bottom edge of the gel. Gels were developed for the above enzyme systems as (SCANDÁLIOS, 1969; ALFENAS, 1998) and fixed in 10% glycerol solution.

Interpretation of results was based on visual analysis of the gels electrophoresis, considering the presence / absence, as well as the intensity of each of the electrophoretic bands. Since the germination data, number of spikes, number of grains per plant, yield, test weight and grain weight were subjected to analysis of variance, which was performed comparing the means for the qualitative factor by Tukey test and the factor quantitative proceeded polynomial regression at 5% probability. When the interaction was significant developments were carried out due, if not meaningful to compare means and polynomial regression was performed with the average levels of the respective factor. For statistical analysis we used the Statistical Analysis System version 1.0 Winstat (MACHADO & CONCEIÇÃO, 2003).

RESULTS AND DISCUSSION

There were no significant differences in the germination of wheat seeds from plants fertilized with kaolin and rice husk ash in the soil (Table 1). Germination at all doses analyzed was above 80%, with the standards required for seed, where the minimum required is 80% (BRASIL, 2005). These results run counter to those obtained by TOLEDO et al. (2011), which used doses of 0, 150, 300 and 450 mg dm⁻³ silicon as potassium silicate in oat seeds, and had greater germination at doses of 300 and 450 mg dm⁻³, similar results were found in the search for VIEIRA et al. (2011), where there is a tendency of increase in germination of rice seeds with application of calcium silicate to the dose of 1600 kg ha⁻¹.

Data of germination, number of spikes per plant, number of seeds per plant, seed yield per plant, hectoliter weight and thousand seed weight are in Table 1, below.

TABLE 1. Germination (G), number of spikes per plant (NS), number of seeds per plant (NSP), seed yield per plant (Y), hectoliter weight (HW) and thousand seed weight (TSW) from wheat fertilization with Kaolin and rice husk ash (RHA) in the soil. Capão do Leão/RS, 2011

Dose (kg ha ⁻¹)	Fonte											
	Kaolin G (%)	RHA	Kaolin NS	RHA	Kaolin NSP	RHA	Kaolin Y (g)	RHA	Kaolin HP (kg. hL ⁻¹)	RHA	Kaolin TSW (g)	RHA
0	98	99	28	29	203	204	42,4	48,3	78,0	78,9	43,7	43,1
500	100	100	24	28	219	215	42,7	46,5	78,4	78,8	43,1	43,2
1000	98	99	25	30	228	226	45,4	48,4	79,0	78,9	43,0	42,7
1500	100	100	26	30	239	236	46,8	50,2	79,1	79,3	42,8	42,6
2000	99	100	26	27	236	245	47,5	48,9	79,2	78,8	42,7	42,8
2500	100	99	25	24	220	238	45,2	47,8	79,4	78,6	42,2	42,7
Avg	99 A	100 A	26 B	28 A	224 A	227 A	45.0 B	48.4 A	78.9 A	78.9 A	42,9 A	42,9 A
V.C.(%)		1,2		10,8		9,8		7,6		0,5		1,8

* Means followed by same capital letter in the line in each response variable, do not differ by Tukey test at 5% probability.

The source of silicon from rice husk ash (RHA) showed a significant increase in the number of spikes and yield to source Kaolin (Table 1). However, the number of seeds per plant, hectoliter weight and thousand seed weight showed no statistical difference between the two sources studied. For all these variables there was no interaction between doses and sources. The results agree with those obtained by LIMA-FILHO & TSAI (2007), where testing different doses of silicon in three wheat cultivars and two of oats, observed a significant increase in the number of spikes in the three wheat cultivars studied, obtaining significant increase in productivity in both species, but more marked in wheat, where the increase in seed production reached 100%. Similar data were found by SINGH et al. (2005) who studied doses and application times of silicon in two consecutive harvests of rice, where the silicon fertilization increased the height, dry matter production, number of panicles per square meter and productivity.

For the variable number of seeds per plant (Figure 1A), there was an increase to the point of maximum efficiency at a dose of 1808.4 kg ha⁻¹ of silicon. The increase seed production may be related to the highest photosynthetic as silicon to the architecture of the plant (DEREN et al., 1994), resulting in a smaller opening angle leaf capitulation allowing more light energy (YOSHIDA et al., 1969), increasing productivity. The data corroborate the results found by LIANG et al. (1994), which found that implementation of silicon in the soil in four consecutive years, increased productivity of rice and wheat from 4.6 to 20.7% and from 4.1 to 9.3% respectively. ZAGO et al. (2010), using soybean foliar application, in the doses 0, 2, 4, 6 and 8 L ha⁻¹ had no significant difference in number of seed per plant.

To assess the yield (Figure 1B), the sources used to promote increase the dose of 1720.84 kg ha⁻¹ silicon, which is its point of maximum. BARBOSA-FILHO et al. (2001), working with three rice cultivars, found that the seed yield increased significantly and linearly with increasing doses of application of silicon, in the order of 0.002% for each mg SiO₂.kg⁻¹ soil. However, these data disagree with those obtained by

CARVALHO-PUPATTO et al., (2004), working with the rice cultivar IAC-202 in field conditions, did not achieve significant results.

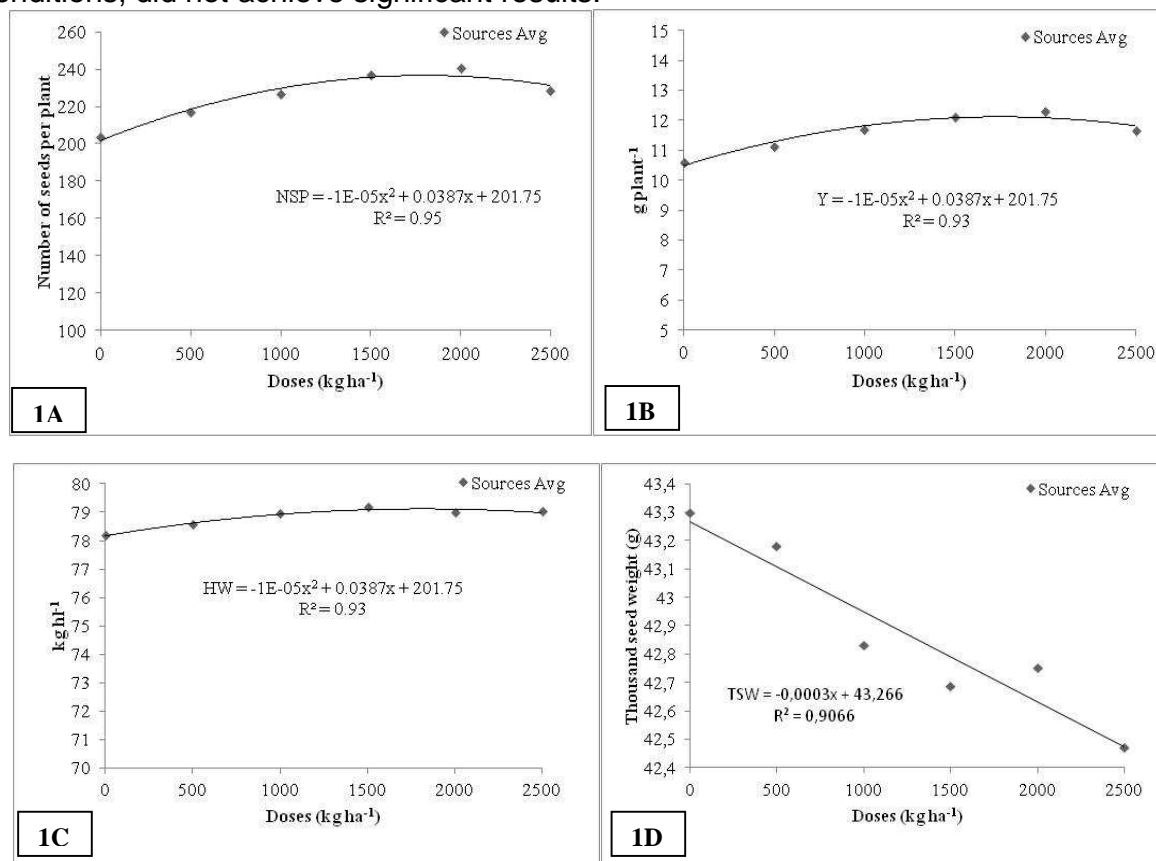


FIGURE 1. Average of the sources of silicon applied to soil in the number of seeds per plant (1A), seed yield per plant (1B), hectoliter weight (1C) and thousand seed weight (1D) of wheat. Capão do Leão-RS, 2011.

As for hectoliter weight (Figure 1C), we observed an increase in this variable to its maximum, which was the dose of 1784.8 kg ha⁻¹ with subsequent reduction. The data are consistent with those found by REIS et al. (2008), who found an increase in hectoliter weight in rice cultivars IAC 201 and IAC 202 with increasing doses of silicon being, however this increase showed a linear increase. However the data presented by ORIOLI-JÚNIOR et al. (2008), working with the rice cultivars IAC 24 and IAC 370, differ from those obtained by this project since no significant differences for these variables as the silicon levels used.

Unlike the increase appears to other variables, the thousand seed weight was reduced in a linear tendence of 3×10^{-4} g per unit dose increase of silicon (Figure 1D). This effect may be due to a possible toxicity of the doses. The results disagree with those found by LIMA FILHO & TSAI (2007), who found positive results for the weight of seeds up to 25%, 44% and 35% for wheat cultivars BR18, BR40 and IPR85, respectively. JUNIOR ORIOLI et al. (2008) using wheat cultivars IAC 24 and IAC 370 showed no influence on the silicon levels evaluated for thousand seed weight.

For electrophoretic patterns are shown in Figures 2 and 3. Notes to the source kaolin was reduced in intensity in the band of esterase (EST, EC 3.1.1.1) at a dose of 2500 kg ha⁻¹ (Figure 2A), but for the source of rice husk ash, there was a reduction significant expression of this enzyme in doses 2000 and 2500 kg ha⁻¹. It is inferred that, because this enzyme is responsible for both the hydrolysis of esters, and lipid metabolism, changes in the patterns of the same evidence the occurrence of deterioration (SANTOS et al., 2004; TUNES et al., 2010), and lipid peroxidation according to BASAVARAJAPPA et al., (1991), an event associated with degenerative process membranes. The data found to yield confirm the effect of seed decay, given that the use of a silicon source rice hull ash in the soil, showed lower expression of the esterase.

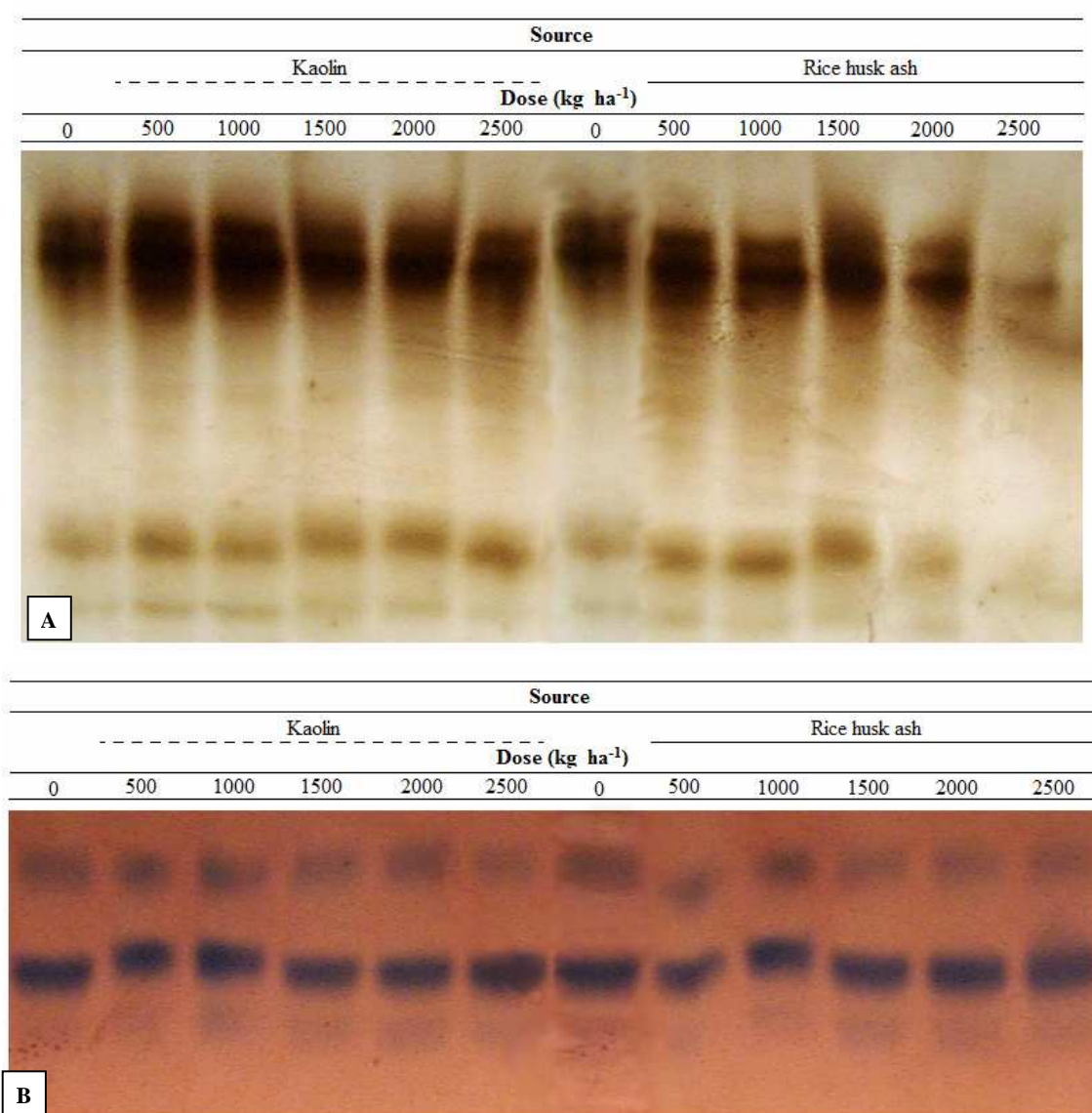


FIGURE 2. Electrophoresis patterns obtained with the EST (2A) and GOT (2B) isoenzyme system in wheat plants subjected to different sources and levels of silicon in the soil.

For the standard isoenzyme glutamate oxaloacetate transaminase (GOT, EC 2.6.1.1), shown in Figure 2B, there is no difference in the intensity of the bands in both sources. This is an enzyme that participates in the process of degradation and synthesis of amino acids (CONN & STUMPF, 1980), presenting an important role in seed germination, agreeing with the results of this study, which showed no difference in germination rates, resulting in a high yield. This enzyme is responsible for the oxidation of amino acids, providing power to the Krebs Cycle or reduced α -ketoglutarate for the synthesis of new amino acids, such as power supply to the developing embryo (VIEIRA et al., 2009). In this function is directly involved in the metabolism of N, it is possible for variations occurring as happens the synthesis and degradation of amino acids during the germination process. Undoubtedly, the enzyme GOT has a fundamental role in protein metabolism, not only during germination, but throughout the life cycle of the plant.

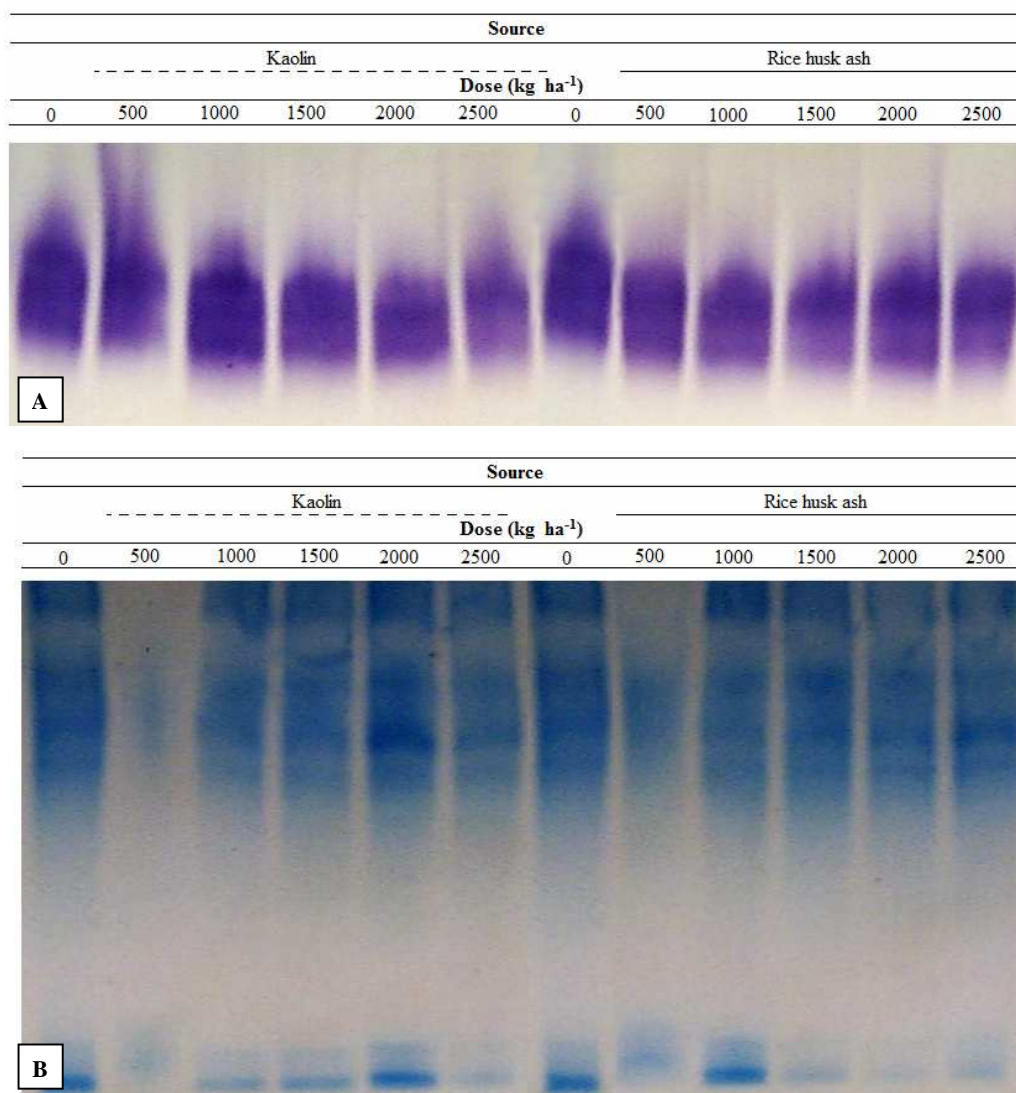


FIGURE 3. Electrophoresis patterns obtained with the system malate dehydrogenase MDH (3A) and PO (3B) in wheat plants subjected to different sources and levels of silicon in the soil.

Analyzing the system for the enzyme malate dehydrogenase (MDH, EC 1.1.1.37) (Figure 3A), no difference is observed in the expression. The malate dehydrogenase enzyme catalyzes the conversion of malate to oxaloacetate, and the important function of NADH for the production of the Krebs cycle and generation of oxaloacetate to biosínteses amino acid. According to SATTTERS et al. (1994), because it is an important enzyme in the cell breathing process, the increase in activity may be due to increased expression of this enzyme in various cellular compartments and / or the induction of enzyme activity expressed by a higher intensity of bands, confirming the data positives obtained in the yield, since an increase in rise of the mean breathing deteriorating process.

For the electrophoresis pattern obtained in the peroxidase (PO, EC 1.11.1.7) (Figure 3B) shows an increase in intensity of bands for the dose of 2000 kg ha⁻¹ Kaolin the source. Peroxidases are proteins of approximately 50 kDa which are present as multiple isoenzymes in plant tissues (JEBARA et al., 2005). Are associated with physiological and biochemical processes such as growth, cell formation, fruit development, ethylene biosynthesis and response to various stresses (MATAMOROS et al., 2003). They participate in processes related to cell wall, such as oxidation of phenols and lignification of plant cells during host defense response to pathogens (DATTA & MUTHUKRISHNAN, 1999). The increased expression of this enzyme as increases the amount of silicon corroborates the data obtained in this work, where the average of the sources had the highest yield at a dose of 1720.84 kg ha⁻¹ Si, implying that a good sanity for plants with better performance.

The use of silicon source for rice husk ash is promising, because it proved superior to kaolin analyzed when the number of spikes per plant and yield, besides being an alternative source and more affordable cost. It was further observed that silicon increased the number of seeds per plant, yield and hectoliter weight with increasing dose.

As for the four different enzyme systems studied, the enzyme esterase (EST, EC 3.1.1.1), glutamate oxaloacetate dehydrogenase (GOT, EC 2.6.1.1) and peroxidase (PO, EC 1.11.1.7), proved to be as promising biochemical markers for assessing the degree of deterioration of wheat plants. It was detected in the electrophoretic profiles of plants subjected to different silicon levels, showing that this may lead to lower levels deteriorative and induction of plant resistance, resulting thus to an increase in productivity.

The results of this study suggest that, depending on the enzyme system used, there is a differentiation of proteins. As a result, the combined analysis of several isozymes systems and recommend modifications to allow verification that occur inside the plant when subjected to some kind of treatment that influences productivity.

CONCLUSIONS

Silicon fertilization, with both sources, does not affect the germination of wheat seeds, but further increase in productivity up to a dose of 1720.84 kg ha⁻¹. The source of rice husk ash promotes greater number of spikes per plant and higher seed yield per plant than the kaolin.

Electrophoretic variation of isozymes are associated with the supply of silicon in the soil in wheat plants, with the exception of malate dehydrogenase (MDH, EC 1.1.1.37).

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