ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 02, pp.26440-26444, February, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

PHYSIOLOGICAL QUALITY AND EXPRESSION OF ISOCITRATE-LYASE ENZYME IN SOYBEAN SEEDS SUBMITTED TO SALINE STRRESS

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ARTICLE INFO

Article History:
Received 24th November, 2015
Received in revised form
06th December, 2015
Accepted 23rd January, 2016
Published online 27th February, 2016

Key words:
Glycinemax,
Enzyme expression,
Abiotic stress.

ABSTRACT

The period of germination is a determinant factor that influences the establishment of seedlings in field, mainly in those regions with adverse climate conditions. Due to this, the breeding programs has creating or adapting new cultivars for these conditions, being the saline soils one of the mainly concerns. Facing this, the objective in this work was to verify the physiological quality and the expression of the enzyme isocitrate-lyase in soybean seeds submitted to saline stress, were used seeds of five cultivars of soybean (Savana, Conquista, Valiosa, BRS Celeste and Baliza), and five saline concentrations (0, 25, 50, 75 and 100 mol.m-3 of NaCl) and with these seeds were realized the germination test, realizing the first count of germination with five days and the final count with eight days. Was also evaluated the enzymatic expression of isocitrate-lyase. It was conclude that, all cultivars presented lower percentage of germination when placed in conditions of higher concentration of salinity. The cultivar Savana is that which presents higher tolerance to the salinity and the cultivar Conquista is the most susceptible. The expression of isocitrate-lyase varies with the increase of saline concentration. The soybean seeds of cultivars Savana and Conquista presented higher expression of isocitrate-lyase enzyme according the increase of salinity.

Citation: **Rafael Toledo de Moraes Antoniolli, Pedro Augusto Vilela Amui, Heloisa Oliveira dos Santos *et al.*, 2016.** "Physiological quality and expression of isocitrate-lyase enzyme in soybean seeds submitted to saline strress", *International Journal of Current Research,* 8, (02), 26440-26444.

INTRODUCTION

With the increase of worldwide population there was a huge demand for food, and consequently one of the cultures which has been highlighted to supply this necessity of food is the soybean. The biggest part of utilization of soybean it is in the ration manufacturing for animals, oil production and recently has been increasing the consume in human feed (Brasil, 2007). To achieve this high production like the observed to soybean, is necessary the utilization of a good technological package, and this includes to guarantee the quality of seeds used (Roessing, *et al.,* 2005). Seeds must be adapted to different regions and climates, not least because, the climate changes are occurring all the time, what can affect the Brazilian agriculture, and is due to this the companies and institutions of researches must be attentive to the next years, cause nowadays one cultivar that is adapted to a certain region, could change their adaptability over the crop cycles (Fancelli; Dourado Neto, 2000). To be adapted or even to combat the environment impacts is necessary firstly to know them to later define which techniques must be used to minimize the effect of these changes in the world agricultural scenario. Some regions suffer with the increases of temperature, other with the deficit of water and exists those regions which the climate changes affects the salinity of soils, which generates huge problems in agriculture, mainly in the germination of seeds (Verslues *et al.,* 2006).The salinity is the concentration of salts in soil, caused by an intense evapotranspiration, where exists inefficient drainage or bad use of water of irrigation (Ashraf *et al.,* 2008). Many soybean breeding programs beyond a lot of researches which work with this problem, are verifying the resistance and/or the susceptibility of cultivars, looking for introduce news materials resistant to the salinity soils, and for this, is necessary seeds with high quality and vigor (Eloi *et al.,* 2011). It is important to highlight that each cultivar of soybean presents specifics patterns of proteins that are synthetized, depending of the conditions of salinity that the seeds are found

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during the germinative process. With this, the objective in this work, was to verify the physiological quality and the pattern of expression of the enzyme isocitrate-lyase in soybean seeds submitted to different concentrations of salinity during the germination.

MATERIALS AND METHODS

This study was conducted at Central Laboratory of Seeds, Department of Agricultural of Universidade Federal de Lavras (UFLA), in Lavras-MG. Were used seeds of soybean cultivars savana, conquista, valiosa, BRS celeste and baliza, which were produced in experimental area of Universidade Federal de Lavras with soil classified like distrophic red latosol, with manual seeding. The multiplication of seeds was realized in a randomized block design with three replications. The experimental units had 4 lines of 5 meters each one, being considered only the 2 central lines like useful area. The thinning was realized keeping one population of 16 plants per linear meter. The manual harvesting was realized based on the phenological stages (R7 and R8) according with Fehr and Caviness (1977). The drying was realized in shadow, until seeds achieve 13% of water content. Were used seeds retained in sieves with circular opening between 5, 55 mm and 6, 35 mm for standardization of seeds seize for posterior realization of evaluations. The water content was determined by the oven method at 105°C for 24 hours, using two replicates of 50 seeds from each treatment (Brasil, 2009). The results were expressed in percentage. In germination test, for each treatment were used four replications of 50 seeds. Seeds were distributed between three germitest papers moistened with solutions of NaCl with different concentrations according Table 1. Was used the amount of solution equivalent to 2,5 times the weight of dry paper. Following, the rolls were kept in germinator type B.O.D regulated to the constant temperature of 25ºC.

The evaluation was realized at eighth day after the installation of the test by determining the percentage of normal seedlings (Brasil, 2009). The first count was realized together to the germination test, by determining the percentage of normal seedlings at fifth day after the test installation. The results were expressed in medium percentage of normal seedlings of four replications. In the biochemical evaluation, samples of 25 seeds of each treatment were collected and macerated in presence of PVP (polyvinylpyrrolidone) and liquid nitrogen in small container and afterwards stored at -86ºC temperature. The seeds were collected 48 hours after the germination test installation. For the enzyme extraction was added the extraction buffer (TrisHCl 0,2 M pH 8 + 0,1% of β-mercaptoethanol) in the proportion of 250µL for 100mg ofseeds powder. The material was homogenized in vortex and kept in refrigerator during 12 hours followed by the centrifugation at 14000 rpm for 30 minutes at 4ºC and them, applied in polyacrilamide gel. The electrophoretic run was realized in a discontinuous polyacrylamide gel system at 7,5% (separating gel) and 4,5% (concentrating gel) using Tris-glycine pH 8,9 as standard buffer in the gel electrode system. In each gel channel, was applied 60 µL of the sample supernatant and the running was performed at 120 V for 5 hours. At the end of running, the gel were revealed for theisocitrate-lyase, according the protocols established by Alfenas *et al.,* (2006). The evaluation of the gel was realized on transilluminator, being considered the variation of intensity of bands. Was used the completely randomized experimental design in a factorial scheme of (5x5), being five soybean cultivars (Savana, Conquista, Valiosa, BRS Celeste and Baliza) and five different levels of salinity (0, 25, 50, 75 and 100 mol.m^{-3}). The datas, previously submitted to the normality tests and homocedasticity of variances, were submitted to analysis of variances and the averages were compared by the Scott-Knott test at 5% of probability. The statistical analyzes were realized with aid of SISVAR® statistical program (FERREIRA, 2011). The evaluation of the enzimatic patterns was made according to the intensity of the bands.

RESULTS AND DISCUSSION

The medium water content of seeds in the moment of tests was of 14, 1 with maximum variation of 1%. There was equilibrium hygroscopic, what means when seeds were placed in cold chamber was with 13% of moisture and when the germination test was initiate was with 14%, occurring equilibrium of moisture according the relative humidity and the temperature in cold chamber. Analyzing each saline concentration to different cultivars was observed that in the concentration of 0 mol.m^{-3} of NaCl the cultivars Savana and Baliza presented higher percentage of germination in the first count, cause did not differ significantly, being superior the cultivars Conquista, Valiosa and BRS Celeste. In the concentrations of 25, 50 and 75 mol.m^{-3} the cultivar Savana presented better results when compared to the others. Already in the concentration of 100 mol.m^{-3}of NaCl the cultivars Savana and Baliza were superior to the others cultivars. When it was compared each cultivar, can be observed that the cultivar Savana and Conquista presented similar results with higher responses of germination in the first count in the concentration of 0 mol.m^{-3}, similar results in 25 and 50 and inferior results in 100 mol.m^{-3}.

The cultivar Valiosa presented higher percentage of germination in 0, did not differ in 25, 50 and 75, and presented inferior results in the saline concentration of 100 mol.m^{-3}. Analyzing the cultivars BRS Celeste it was observed superiors results in 0 and 25 mol.m^{-3}, did not differing significantly, and inferior results in 75 and 100 mol.m^{-3} of NaCl that also did not differ. The cultivar Baliza presented higher percentage of germination in the first count in 0 mol.m^{-3} and did not differ in 75 and 100, with results inferiors (Table 2). Being this, was possible to observe that the cultivars Savana and Baliza presented results superiors of germination in the first count when compared to the cultivars Conquista, Valiosa and BRS Celeste.

Table 1. Different concentrations, quantity, conductivity and osmotical potential of NaCl used to moisten the germitest paper to submit to salt stress

NaCl Concentration (mol.m^{-3})	Quantity of NaCl (g/L)	Conductivity (mS DM^{-3})	Osmotical Potential (MPa)
0	0	0,08	0
25	1,46	0,42	-0,2
50	2,93	2,41	-0,4
75	4,39	5,04	-0,6
100	5,85	9,53	-0,8

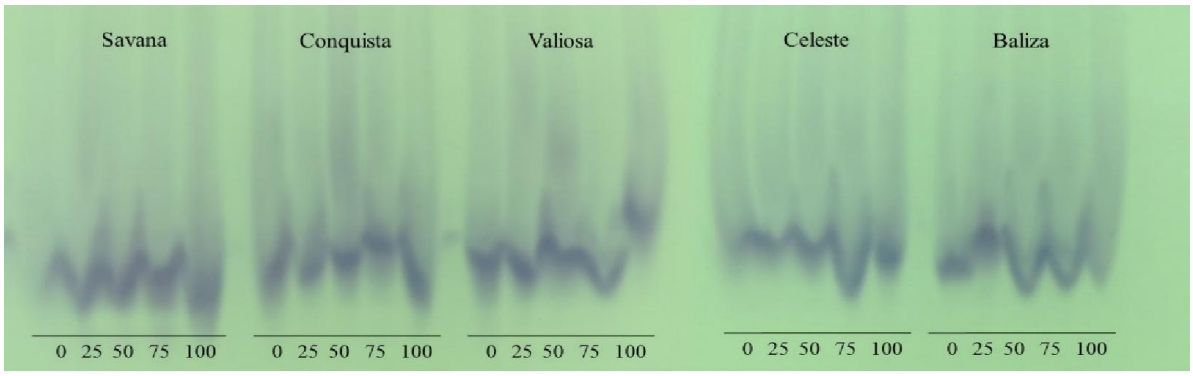

Figure 1. Pattern of expression of isocitrate-lyase enzyme in function of five cultivars (Savana, Conquista, Valiosa, BRS Celeste e Baliza), under five levels of salt stress (0, 25, 50, 75 and100 mol.m-3 of NaCl)

Table 2. Percentage of normal seedlings in the first count of germination at five days of five soybean cultivars, submitted to five levels of salt concentrations.UFLA, Lavras – MG, 2014

Concentration (mol .m^{-3})	Cultivars				
	Savana	Conquista	Valiosa	BRS Celeste	Baliza
0	90aA	54bA	56bA	52bA	88aA
25	80aB	42cB	44cB	44cA	72bB
50	74aB	38cB	42cB	40cB	66bB
75	64aC	24dC	38cB	36cC	54bC
100	52aD	2cD	30bC	34bC	48aC
CV (%)			17,3		

Means followed by the same lower case letter in line, and capital letter in column, do not differ at 5% probability by Scott Knott test.

Table 3. Percentage of germination of five cultivars of soybean, submittted to five levels of salt concentration. UFLA, Lavras – MG, 2014

Concentrations (mol .m^{-3})	Cultivars				
	Savana	Conquista	Valiosa	BRS Celeste	Baliza
0	100aA	100aA	100aA	98aA	98aA
25	96aB	88cB	90bB	88cB	92bB
50	94aB	72cC	64cC	84bB	88bB
75	92aB	64dD	60dC	70cC	78bC
100	88aC	30dE	50cD	58bD	60bD
CV (%)			6,4		

Means followed by the same lower case letter in line, and capital letter in column, do not differ at 5% probability by Scott Knott test.

All the cultivars presented lower germination in the first count with the increase of the concentrations of NaCl, however it is emphasized that the cultivar Conquista decreases drastically the percentage of germination from 50 mol.m^{-3} of NaCl. According Torres (2007) the excess of salt causes significant reduction of vigor, like also of the germination of seeds. Tonin *et al*. (2000), working with maize seeds and Braccini *et al.,* (1996), with soybean seeds observed that with the increasing of the saline concentration on substrate for germination, there is a reduction on vigor, affecting directily the percentage of normal seedlings in the first count of the germination test. Similar results were found in this present work. Carvalho *et al.* (2012), working with conventional soybean seeds and their derivated RR, observed that in situations of higher salinity, the reduction of the germinative powder of seeds was drastic, being greatly affected in criticality conditions (120 mmol. dcm-3). As the salinity of the substrate incresed, there was decreases of vigor of soybean seeds for all cultivars, being the cultivars Savana and Baliza those that presented lower sensibility with the increase of salt concentration. When comparing the different concentrarions of NaCl it can be observed that in 0 mol.m^{-3}there was no significant difference of percentage of germinations between the cultivars Savana, Conquista, Valiosa, BRS Celeste and Baliza. In concentrations of 25, 50, 75 and 100 mol.m^{-3} of NaCl it can be observed that the cultivar Savana was superior to all the other cultivars, obtaining with this, one higher percentage of germination. Analyzing each cultivar, it can be observed that all 5 cultivars presented higher percentage of germination when submitted to 0 mol.m^{-3} of NaCl, and all those also presented lower result of germination when submitted to higher concentration of salinity. The cultivar Savana presented higher percentage of germination in 0 mol.m^{-3}, did not differing significantly in 25, 50, and 75 mol.m^{-3}, and it was inferior in 100 mol.m^{-3}.

The cultivar Conquista presented significant difference in all the concentrations, being superior in 0 mol.m^{-3} and inferior in 100 mol.m^{-3}. Analyzing the cultivar Valiosa, it was observed higher percentage of germination in 0 mol.m^{-3}, did not differing in 50 and 75, in 100 mol.m^{-3} there was a lower percentage of germination when compared to the others. The cultivars BRS Celeste and Baliza presented similar results, being superior in 0 mol.m^{-3}, did not significantly differing in 25 and 50, and in the saline concentration of 100 mol.m^{-3} was observed a lower response of germination, being inferior to the others concentrations of NaCl (Table 3). It is noted that all cultivars presented a higher percentage of germination when submitted to lower levels of salinity, and with the increase of the concentration, these values, were decreasing. It can highlight the cultivar Savana for presenting a lower variation of germination with the increase of saline concentration and the cultivar Conquista for reducing germination when submitted to high level of salinity when compared to the other cultivars, being considered the cultivar Savana the most resistant, and the cultivar Conquista the most susceptible to the conditions of saline stress. The increase of salt concentrations on substrate determines the reduction of water potential, resulting with this in a lower capacity of absorption of water by seeds, what influences in the germinative capacity and in

the development of seedlings (Rebouças *et al.,* 1989). Santos *et al.* (1992), working with different genotypes of soybean under conditions of saline stress observed that the percentage of seeds non germinated was elevated in the lowest values of osmotic potential, suggesting that the toxic effects of salts provoked inhibition of germination. Based on results, is possible to infer that with the increase of saline concentration, it has significant reduction of germination and vigor. Is possible to observe also, that exists variation in relation to the sensibility of saline concentration between the tested materials, being the cultivar Savana, that which presented better results when compared to the others cultivars tested in this work, could being used in breeding programs aiming greater adaptability in regions which presents soils with problems of salinity.

All the cultivars evaluated are different, what means, are distinct genetic materials, presenting with this, chemical composition different, genetic constitution different and this can infer directly in the expression of the enzyme isocitrate-lyase. It is important to highlight that the results of enzymatic analyzes is an important tool complementary to the germination and vigor tests. Was possible to observe activity of isocitrate-lyase in seeds of all cultivars of soybean in all concentrations of NaCl. However, presented a higher or lower expression of this enzyme in function of the concentration of salt. In cultivar Savana, was observed higher expression of this enzyme with the increase of the saline concentration. For cultivar Conquista was observed reduction in the activity of this enzyme until the concentration of 75 $mol.m^{-3}$ of NaCl, however when placed in the concentration of 100 $mol.m^{-3}$ of NaCl, was observed an increase in the activity of this enzyme. The cultivars Valiosa, BRS Celeste and Baliza presented variations of expression, however all cultivars presented a lower expression when placed in the concentration of 100 $mol.m^{-3}$ de NaCl. With this, was observed, that the isocitrate-lyase enzyme presents high activity of expression when placed in adverse conditions, cause the cultivar Savana was the most resistant and the cultivar Conquista was the most susceptible, and it was in this cultivars the higher expression of the isocitrate-lyase enzyme expression (Figure 1). The isocitrate-lyase is a key-enzyme in soybean seeds, cause it is involved in the regulation of glyoxylate cycle and in the metabolism of lipids stored in oleaginous seeds, it is also involved in the development of glyoxysomes activity, cause that it is justified such behavior, what means, the increase in the activity/expression when seeds are exposed to adverse conditions. However, Bewley and Black (1994), claim that the activity of this enzyme increases during the germination of seeds, obtaining values maximum when occurs the maximum of proportion of lipids degraded and in the synthesis of sucrose, fact did not observe in this work.

Conclusion

All cultivars presented lower percentage of germination when placed in conditions of higher concentration of salinity. The cultivar Savana is that which presents higher tolerance to salinity and Conquista is the most susceptible. The expression of enzyme isocitrate-lyase varies with the increase of salt concentration. Soybean seeds of cultivars Savana and Conquista presented higher expression of isocitrate-lyase enzyme according the increase of salinity.

Acknowledgments

To Capes (Higher Education Personnel Improvement Coordination); National Council for Scientific and Technological Development (CNPq) and the Foundation for Research of the State of Minas Gerais (FAPEMIG).

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