English Version

Differential of expression in Isoenzymas seeds and seedlings F1 of barley[1]

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Abstract:

The present work had as objective to evaluate the isoenzimatic patterns of Acid Phosphatase (ACP), Alcohol Dehydrogenase (ADH) and Glutamate Oxalacetate Transaminase (GOT) of two barley cultivars (MN 721 and Scarlett). The seeds were picked in three times with different percentage corresponding to three different steps of their maturation. The seed humidity was reduced to 13% and stored in cold camera. The seeds and the plantlet were analyzed of all cultivars and times. The three isoenzimatic systems analyzed presented variations in the expression, as it was expected when compared between seeds and seedlings. The water content of seeds influences the enzyme pattern of the GOT enzyme. ACP bands were detected in all the treatments evaluated, however, in seeds with lower intensity.

Key words: *Hordeum vulgare* L.; electrophoresis; protein extraction; ACP; ADH and GOT.

Introduction

Barley is cultivated in Brazil since the decade of 1930, and its production is concentrated in the South region, with register of crops also in the states of Goiás, Minas Gerais and São Paulo (ARIAS, 1996). It is the forth most important cereal in cultivated surface in the world, after wheat, rice and maize. It is a typical species of cold climate, earlier and more tolerant to low temperature than other cereals, which enables the exploitation of other species in the same property (YALÇIN, et al., 2007). The cultivated area with this cereal in Brazil is approximately 109 thousand hectares (IBGE, 2008). Regarding this and the economic response in relation to other winter crops, many producers showed interest in the inclusion of barley in their production system.

The parameters used as indicative of the appropriate moment for the barley seed sampling is their humidity degree and the aspect of the plants. However, these parameters may vary due to climate, temporal and genetic factors, and are not, thus, indicatives totally secure from the ideal point to the harvest. Barley is characterized for being highly sensitive to rainfall precipitation in the moment of the harvest, mainly by the injury promoted to seeds by the early germination (REUSS et al., 2003).

According to Dias (2001), the harvest performed in the phase of the physiological maturity (humidity of approximately 30%) would be ideal, since it is when the seed achieves the maximum weight of the dry matter and is found in the maximum of its potentiality, with a minimum deterioration (DELOUCHE, 2005). Although, in this phase not only the humidity level in seeds is high, but also the plant is still with a large number of green leaves, which would make it almost impossible the procedure of the harvest (BARROS e PESKE, 2006). The seeds remain in field until achieve a level of humidity appropriated to the harvest, subject to climate conditions not always favorable for the preservation of their quality (BARROS e PESKE, 2006). In this process, biochemical alterations which conduce to a commitment of their metabolic activities may occur, i.e., a process of seed deterioration, increasing the process of respiration and energy expenditure.

According to research data, the monitoring of these changes may be done with aid of molecular

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markers, since, besides favoring useful data over structure and genetic diversity of the plant population, they enable the detection of the enzyme activity in different plant stages (ALFENAS et al., 1998).

The analysis of the isozyme activity is constituted in one technique of biochemical markers widely used since the decade of 1960. Isoenzymes may be considered variations of a certain enzyme inside an organism, which present the same subtract specificity. In accordance with Peirce and Brewbaker (1973), the band intensity and the isozyme profile are specific for a determined part of the plant, tissue and development stage.

The possibility of use isozyme markers as tool in the determination of biochemical alterations due to the deteriorating process of the seeds were already emphasized by many authors (BRANDÃO Jr. et al., 1999; SANTOS et al., 2004; 2005).

The enzymes related to the physiologic quality of the most researched seeds are acid phosphatase, dehydrogenase and transaminases (CARVALHO et al., 2000). The enzyme acid phosphatase has the function the hydrolysis of phosphomonoesters is a great number of vegetal chemical reactions, among them, the formation of sucrose during photosynthesis (TANKSLEY, 1983). The enzyme dehydrogenase alchohol is one of the main fermentative enzymes, which converts the acetaldehyde into ethanol, considered less toxic to the plant since it do not cause injuries to the libid bilayer of the cell membranes and for being permeable (CHANG et al., 2000). The enzyme glutamate oxaloacetate transaminase has an important participation in reactions of transamination, during the elimination of Nitrogen of the aminoacids and in the formation of Keto groups for the Krebs cycle and gluconeogenesis (TANKSLEY, 1983).

Facing this situation, it is crescent the necessity of using methods which enable to evaluate, quickly and efficiently, the physiological quality of seeds and, this way, enable the decision-making referent to the harvest, processing, storage and marketing.

The goal of the present work was to evaluate the expression of the acid phosphatase isozyme (ACP), alcohol dehydrogenase (ADH) and glutamate oxaloacetate transaminase (GOT) in seeds and seedlings of two cultivars of barley (MN 721 e Scarlett), obtained in different crop periods.

Material and methods

The research was performed in the Laboratory of Seed Analysis and Bio-seeds from the Federal University of Pelotas (UFPel). The studied cultivars were "MN 721" and "Scarlett". MN 721, obtained through AmBev (American Beverage Company), presents wide adaptation, including in soils with low fertility. Scarlett is cultivar of Argentinean origin, and has very high yield, surpassing the Brazilian most productive varieties, adapting to climates either cold or hot.

The total experimental area used was approximately 0.5 ha, divided in two subareas. In each subarea there was a cultivar and both were harvested in the three distinct periods (118, 129 and 140 after the physiologic maturity). Samples took from the subarea were composed by ten subsamples (withdrawn from ten different points inside the subarea), drawn randomly, mixed to have a good homogeneity, it was obtained the work sample for the analysis weighting approximately four kilograms of seeds by collecting date and cultivar.

The seed collecting was carried out when they achieved humidity degree lower than 30%, when plants at 118, 129 and 140 days after seeding (harvest periods were determined through the percentage of seed humidity). The seed humidity, to cultivar MN 721, was 56% in the first, 18% in the second and 13% in the third sample, and to cultivar Scarlett it was 26% in the first, 19% in the second and 13% in the third sample. The seeds were dried in oven with temperature of 35-40 °C with forced air circulation, until they achieved 13% of humidity. They were then stored in cold chamber, at temperature of 5 to 10 °C for a period of 7 days, to break dormancy, according to the Rules for Seed Analysis (BRASIL, 1992).

The isoenzymes which were evaluated: acid phosphatase (ACP), alcohol dehydrogenase (ADH) and glutamate oxaloacetate transaminase (GOT), in seeds and also seedlings with 7 days of germination, from both cultivars and three periods of seed picking.

The seeds from each sample were placed to germinate and their seedlings (whole and crushed), with seven days for all the analyzed samples, used to

extraction and electrophoreses. It was also analyzed dry seeds (25 seeds per harvest period for each cultivar) (crushed), not germinated, withdrawn from cold chamber and transported to extraction.

The germination test was effected, to each cultivar an sample, with four replications of 50 seed sowed in paper towel moistened with distilled water in the proportion of 2.5 times the weight of the dry paper, being conduced in constant temperature of 20 °C, according to the Rules to the Seed Analysis (BRASIL, 1992). Ten seeds and 10 seedlings from each cultivar and sample were collected randomly and macerated separately in porcelain mortar.

For each one of the samples, 200 mg of the extract obtained was placed in eppendorf tube, plus extraction solution (gel buffer + 0.15% of 2-mercaptoethanol) in the proportion 1:2 (p/v). Electrophoresis was performed on polyacrylamide gels 7%, placing 20μL of each extract/sample in holes made with the aid of an acrylic comb. Three applications (replications) of each sample were performed. It was used the buffer system described by Scandalios (1969). The gels were placed to migrate in vertical electrophoresis tanks, maintained in cold chamber with temperature between 4 and 6 °C.

The electrophoretic migrations were performed with a difference of potential of 10 Vcm-1, until the front line, formed by bromophenol blue, achieved 9 cm from the point of application. The gels were revealed, for the acid phosphatase enzyme systems, Glutamate Oxalacetate Transaminase, or alcohol dehydrogenase, according to Scandalios (1969) and Alfenas (1998). Gels were then fixed in solution 5:5:1, of distilled water: methanol: acetic acid.

The interpretation of the results was based on the visual analysis of the electrophoresis gels, considering the presence/absence, as well as the intensity of each electrophoretic band in each isoenzyme system evaluated.

Results and discussion

When the enzyme pattern was analyzed for the enzyme GOT, it was verified an increase of the band intensity when the analyzed material was the barley seeds. This fact was also detected in the different germination stages (Figure 1).

Analyzing the gels of the glutamate oxalacetate transaminase system (Figure 1), it is also not possible to conclude about the increase or reduction of the band intensity in the seeds over the time. Although, it can be clearly seen the existence of three bands in gradient of increasing intensity from the point of application (beginning of the gel). Each one of these bands shows intensity increase, in different samples of seedlings of older ages. The band intensity increased considerably as the process of harvest was late, i.e., with unit close to 13%. According to Conn and Stumpf (1980) this enzyme takes part in the process of degradation and synthesis of amino acids, presenting an important role in the seed germination. Because the enzyme is directly involved in the N metabolism, it is possible that variations occur as the synthesis and degradation of amino acids occur during the germination process.

The enzyme GOT has a fundamental role in the protein metabolism, not only during the germination, but also during the entire plant life cycle. In the seed, independent on the period of the harvest the enzyme is present and active. In the seedling the expression of the enzyme did not present activity in both cultivars when harvested 118 DAS. When the harvest was held 129 DAS the enzyme was present only in the cultivar Scarlett, however, ate 140 DAS it was observed in both cultivars.

These data indicate that this enzyme has activity directly related to the physiologic quality of the seeds (COSTA et al., 2008). Probably the absence at 118 DAS indicates that the physiologic quality was not high, by the fact that seeds still presented high levels of water. By contrast, 129 DAS the difference between cultivars probably occurred due to the genetic difference of them. Finally, 140 DAS the level of seed humidity had already reached levels which did not interfere in the physiologic quality of seeds.

The expression of the enzyme acid phosphates (ACP) in the different treatments may be observed in Figure 2. It was detected ACP bands in all the treatments evaluated, however, in seeds with a lower number of bands (2 bands). In seedlings it was found 3 bands in cultivar MN 721 and in the cultivar Scarlett. Malone et al. (2007) researched in rice periods from 0 to 10 days of germination, the

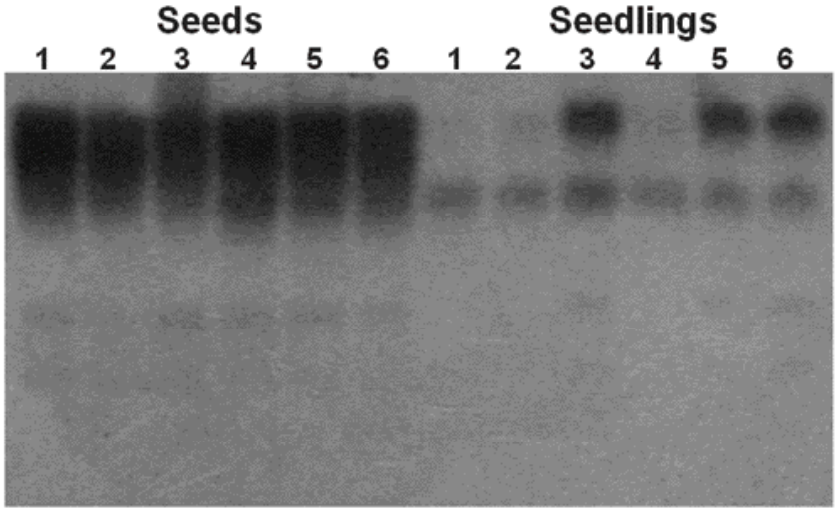

Figure 1. Electrophoretic pattern obtained with the isoenzyme glutamate oxalacetate transaminase system in two barley cultivars harvested in three periods, in seed and seedlings with emergence of seven days. 1- MN 721 118 days after sowing, 2- MN 721 129 days after sowing, 3- MN 721 140 days after sowing, 4- Scarlett 118 after sowing, 5- Scarlett 129 days after sowing and 6- Scarlett 140 after sowing. Laboratório de Bio Sementes/UFPel – Pelotas, 2007.

isoenzyme expression of ACP, and also observed the increase in the number of bands, with the advance of the germination period. According to data obtained by this author, in the day zero of germination the seedlings of rice expressed 2 bands of ACP and at 10 days of germination it was verified an increase to 4 bands. This enzyme has been widely characterized in plants, and its activity increases in plants with present Phosphorus deficiency.

According to Lefebvre et al. (1990), the increase in the activity of ACP under low Phosphorus concentration has been related to a big number of species and plant organs. Thus, the intensity of expression of the acid phosphatase enzyme increases as the content of Phosphorus in soil decreases and when the nutritional requirements of the plantlets are higher. This enzyme also participates of reactions of hydrolysis of esters, and may act over membrane phospholipids and cause peroxidation of these lipids. In the mitochondrial membranes, since they are rich in unsaturated lipids, may present intense peroxidation of these lipids, interfering in respiration. Authors as Brandão-Junior et al. (1999) only verified activities from the acid phosphatase in maize and corn seeds which were in advanced degree of deterioration.

In seedlings, the activity of the acid phosphatase was higher, consequently less damage for imbibition and increased lipid peroxidation may have occurred. Even though it passed 22 days (118 DAS – fist harvest and 140 DAS – last harvest) the seed did not properly get old, but lost water, and was subject to climate conditions, and cultivars responded differently to this condition.

ADH is an enzyme which acts in the respiratory process, removing substances toxic to seeds, as acetaldehyde and ethanol, which are produced when cells begin to respire anaerobically. The expression of the enzyme alcohol dehydrogenase (ADH) in seeds was well pronounced (Figure 3), which suggest intense activity of anaerobic respiration. However, authors as Faria et al. (2003) did not observe activity of ADH in seedlings.

ADH is an enzyme which acts in the respiratory process, removing substances toxic to the seeds, as acetaldehyde and ethanol, which are produced when cells start to breathe anaerobically (FARIA et al., 2003). According to Aldasoro and Nicolás (1980), during the initial stages of the germination, the degradation of amide is performed in a process almost completely anaerobic, until

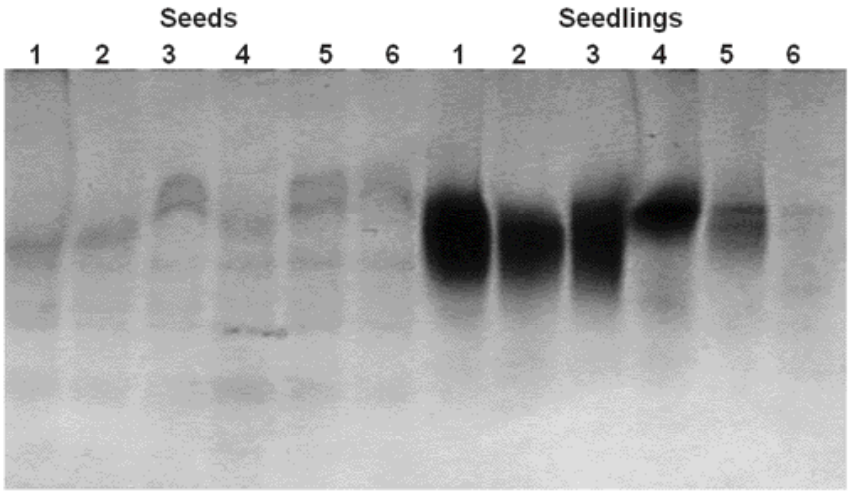

Figure 2. Electrophoretic pattern obtained with the isoenzyme acid phosphatase system in two barley cultivars harvested in three periods, in seed and seedlings with emergence of seven days. 1- MN 721 118 days after sowing, 2- MN 721 129 days after sowing, 3- MN 721 140 days after sowing, 4- Scarlett 118 after sowing, 5- Scarlett 129 days after sowing and 6- Scarlett 140 after sowing. Laboratório de Bio Sementes/ UFPel – Pelotas, 2007.

the seed coat is ruptured by the output of the embryonic axis. Confirming these evidences in the isoenzyme to ADH it was observed high activity with exclusive expression in seeds evidencing that as the germination process advances and the aerobic process of energy generation begins to be predominant, the enzyme ADH is no longer necessary. The expression decreased practically to zero with the process of seedling emergence.

In the three isoenzyme systems analyzed

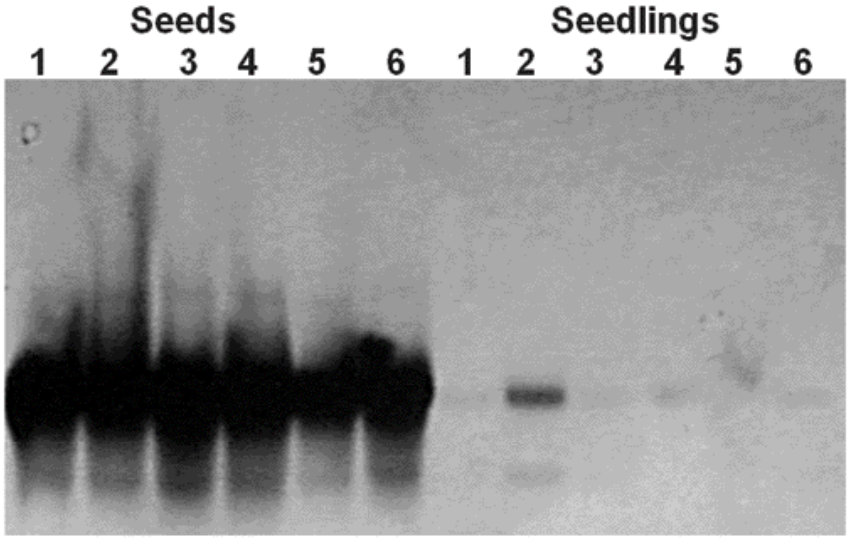

Figure 3. Electrophoretic pattern obtained with the isoenzyme Alcohol Dehydrogenase system in two barley cultivars harvested in three periods, in seed and seedlings with emergence of seven days. 1- MN 721 118 days after sowing, 2- MN 721 129 days after sowing, 3- MN 721 140 days after sowing, 4- Scarlett 118 after sowing, 5- Scarlett 129 days after sowing and 6- Scarlett 140 after sowing. Laboratório de Bio Sementes/UFPel – Pelotas, 2007.

there was pattern of differential expression in seeds and during the process of germination, performed in different periods of harvest. In a general way, it may be observed that the isoenzyme pattern expressed in seeds, did not differ between cultivars in all the protein systems analyzes, when compared to those expressed by developing seedlings. This may be attributed to the fact that the program of seed development and during the germination correspond to different processes of cell differentiation and profile of genic expression.

In the dry seed the metabolic activity is extremely low, occurring just biosynthetic and catabolic reactions necessary for the cell respiration. In developing seedlings during the process of germination, various biosynthetic and catabolic reactions are triggered, as the intense production of ATP, the protein degradation and reserve polysaccharides, the synthesis of new mRNAs, the restructuring and repair of membranes and damaged organelles, which considerably modifies the isoenzyme expression.

The seeds presented more homogeneous results, i.e., did not differ concerning the harvest period and among cultivars, contrasting with seedlings, which presented heterogeneous results regarding the parameters above mentioned.

The results obtained revealed that, depending on the enzyme system used, there is a differential expression of isoenzymes in seeds and seedlings F1 of barley.

Thus, the joint analysis of several isoenzyme systems, performing the extraction of proteins in two development stages (seeds and seedlings), are recommended, since it will enable the verification of modifications which occur from seed to seedlings and also the influence of humidity during the harvest.

Conclusion

There are differences in the expression pattern of the enzymes ACP, ADH and GOT and between seeds and seedlings.

The water content in seeds influences the GOT enzyme pattern.

It was detected ACP bands in all the treatments evaluated, however, in seeds with a lower intensity.

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