Analele Ştiinţifice ale Universităţii „Alexandru Ioan Cuza”, Secţiunea Genetică şi Biologie Moleculară
TOM XVIII, Fascicula 4, 2017

INQUIRY ON AMYLASE ISOZYMES IN BARLEY (*HORDEUM VULGARE*) SEEDS DURING GERMINATION

EUGEN UNGUREANU[1*]

Received: 25 November 2017 / Revised: 30 November 2017 /
Accepted: 23 December 2017 / Published: 30 December 2017

Keywords: germination, isozyme, amylase, barley

Abstract. Barley is an important crop for both human and animal food use because of its high starch seed content. Germination, as a process of developing a new plant, or as part of making alcoholic beverages, make use of starch stored in barley seeds as food for the embryo. Amylases, who broke down the starch, are among the most important enzymes, mostly from human point of view. Analyzing and understanding the whole starch mobilizing process by amylases could be of high value for both the agricultural processes and for biotechnological ones. The amylases from germinating barley seeds have been investigated by electrophoresis for the presence of different isoenzymic forms. Both α and β amylases have been investigated and the results shows that there is more than one isoform in both types of amylases.

INTRODUCTION

Since immemorial times amylases and barley seeds are a part of human life, even if only looking to day to day work or to celebration. As a process taking place in plants, germination is one of the most important processes. Barley is the fourth main among grains, considering the quantity produced each year. Studies shows that it was among the first plants used by humans. Even if today its importance as human food had decreased it remains very important as animal food and base for malting and brewing. (Smith, 1998; Ulrich, 2011)

As substances all enzymes, the well-known biological catalysts, belong to the proteins. (Pratt et al., 2018) It was proved that an enzyme could have more than one form and that each of its many forms is an isozyme. (Markert, 1968; Scandalios, 1969; MacDonald et al., 1972; Soltis et al., 1989) The existence and the identification of different forms of the enzymes is a problem related with the genetic variability of each individual of a species and also could be a problem of localization within a cell compartment or even tissue (MacDonald et al., 1972; Daussant et al., 1994)

Amylases have been well studied from many point of view. It is well known that there are more than one type and in each specific type there are isoforms. (van Onckelen et al., 1969; MacDonald et al., 1972; Brown et al., 1982; Daussant et al., 1994)

The main purpose of the paper was to find if there are any different isozymes of both, α and β amylase, in the extracts made with specific extraction solutions from the probes collected during germination of the seeds from barley (*Hordeum vulgare*).

MATERIALS AND METHODS

For our study we used barley (*Hordeum vulgare*) seeds that have been produced in farms from our region (Nord-Eastern part of Romania). Three kinds of seeds (noted as A, B and C) have been chosen to be subjected to germination. Germination was performed in Petri dishes on sterile material and prior to germination seeds have been checked for integrity then washed thoroughly with water. (Akinyosoye et al., 2014) To ensure that almost no fungi will grow alongside germinating seeds, those were cleaned with 30% H_2O_2 for 10 minutes then washed again, few times, with sterile water. (Deno, 1993) Samples have been collected on a day to day basis. Germination was considered over when newly formed leaves become green. (Bewley et al., 1994; Bewley, 1997) All the samples have been stored in a freezer at -25°C until analysis was done.

To extract amylases, probes have been taken from each sample and extracted 1/10 after they were crushed using a mortar and pestle. Extraction of α-amylase has been done with distilled water. β-amylase was extracted with a pH 8 buffer solution, 50 mM Tris-HCl with added 1mM EDTA. (Artenie et al., 2008) Centrifugation of those extracts was performed in two steps, first at 4000 rpm for 25 minutes, then with smaller samples, at 13000 rpm, both in cold, 4°C. The clear extracts were used for electrophoresis.

Electrophoresis was performed according to Laemli system using native conditions. The migrating gel was 8%. (Sambroock et al., 2001) Following the electrophoresis gels have been incubated for 1 hour in a 2% starch solution at room temperature. Then gels were washed with distilled water and incubated with N/3000 iodine solution. Starch gives a blue color when mixed with iodine and gels become blueish with clear spots where amylase is present, and starch has been hydrolyzed.

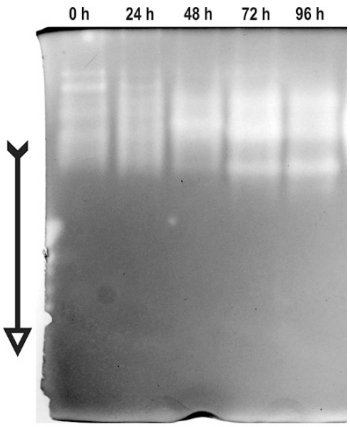
(Artenie et al., 2008) Because the color is not stable in time photos have been taken immediately using a transilluminator and a DSLR. Further analysis of gels was done by comparing the Rf of clear spots representing different isoforms of amylase(s).

RESULTS AND DISCUSSIONS

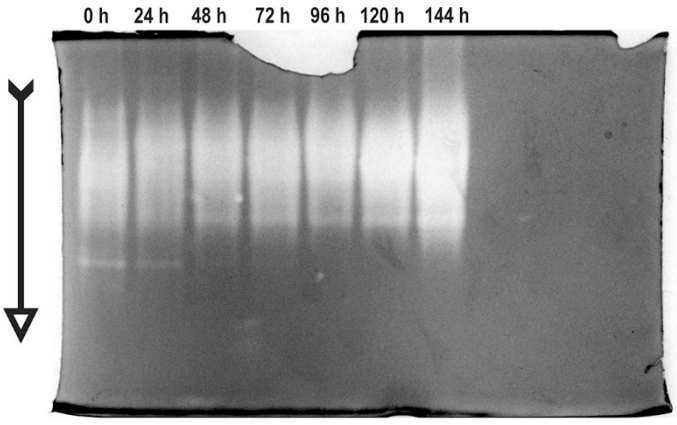
Following the experiments, it is very interesting that each of our type of barley seeds expressed a different time needed for germination. From only 4 days in A variant to 6 days in B variant and 9 days in C variant.

Profiles of α-amylase isozymes from barley (*Hordeum vulgare*) seeds during germination are presented in Figure 1 A, B and C.

Even if it had the shortest germination period A variant exhibit many isozymes compared with variant B and C.



A



B

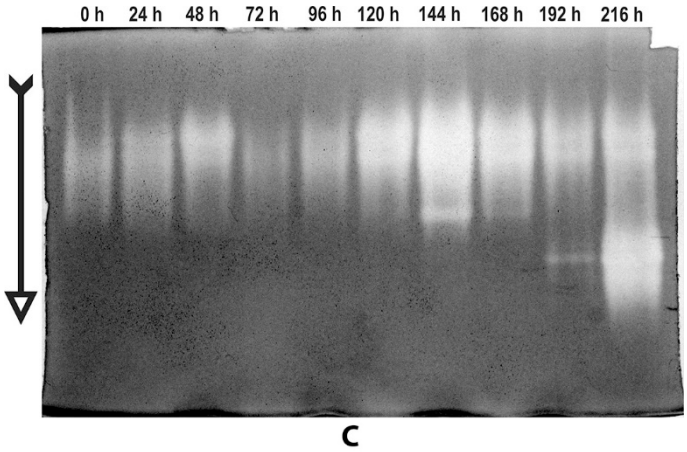


Figure 1 α-Amylase isozymes identified in extracts from barley (*Hordeum vulgare*) seeds during germination

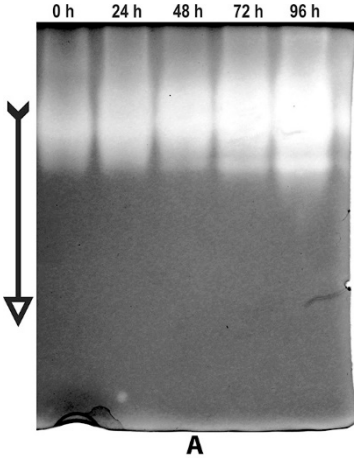
In variant A spots with Rf around 0,26 present an increase in activity during germination (reflected in spot dimensions) and are present from dry seed until the end of germination. Spots with smaller Rf tend to disappear but at the end of process (72 hours and 96 hours) a new spot appear with Rf 0,35.

The simplest profile is found in barley seeds variant B where only a very big spot is clearly visible throughout the entire germination.

Almost same situation is to be found in variant C but here, at the end of germination, maybe because of the newly formed plant, there is some variation.

Looking to all identified spots corresponding to different isozymes of α-amylase form those three variants analyzed we could conclude that there is more than one isozyme in each variant and that during germination profile change.

For β-amylase there have been detected far less spots no matter the variant (A, B or C) of the seeds.



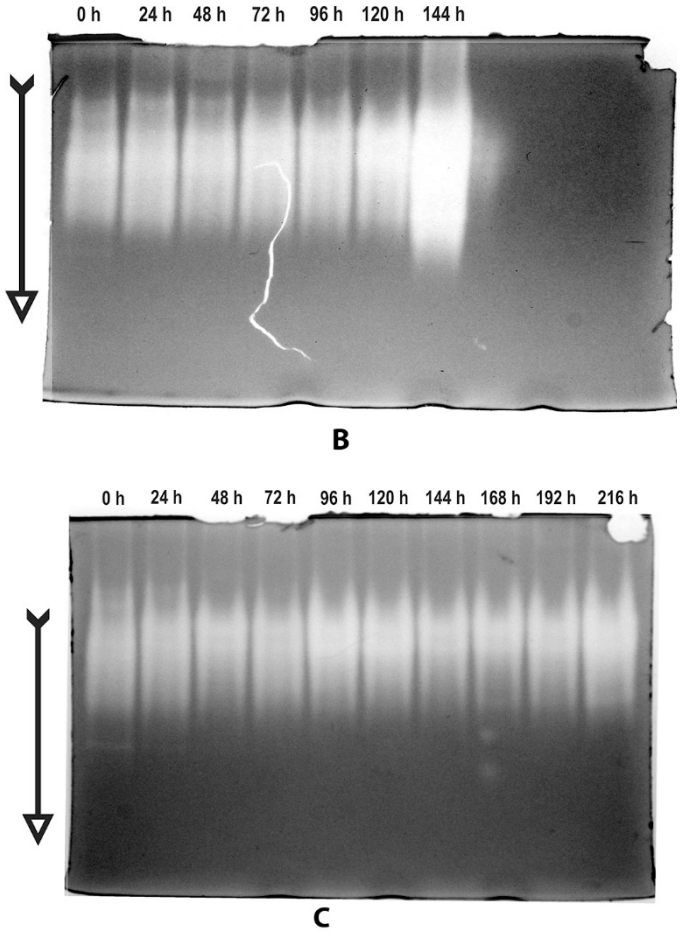


Figure 2 β-Amylase isozymes identified in extracts from barley (*Hordeum vulgare*) seeds during germination

In each case there is a very big spot which somehow masks almost all other (if any) spots. Though, at the beginning of at the end of the process there are some small spots, corresponding to different isoforms of β-amylase.
Future analysis using different acrylamide / bisacrylamide concentrations will be needed to further enhance the separations to achieve a better image over the spectrum of isozymes of amylases during germination of barley seeds.

CONCLUSIONS

There have been detected several isozymes of α-amylase in the extracts made from germinating barley seeds.
The profile of those isozymes is changing through the entire germination process.
β-Amylase shows less diversity concerning the presence of isozymes.

More investigations are needed to find the slightest differences between different isozymes, both in α-amylase but also in β-amylase during germination in barley (*Hordeum vulgare*) seeds.

REFERENCES

Akinyosoye, S.T., Adetumbi, J.A., Amusa, O.D., Olowolafe, M.O., Olasoji, J.O., 2014 - Effect of seed size on in vitro seed germination, seedling growth, embryogenic callus induction and plantlet regeneration from embryo of maize (*Zea mays* L.) seed, Nigerian Journal of Genetics, 28, Issue 2, 1-7
Artenie, Vl., Ungureanu E., Negură, A., 2008 - Metode de investigare a metabolismului glucidic și lepidic – manual de lucrări practice –, Editura PIM, Iași
Bewley, J.D., 1997 - Seed Germination and Dormancy, Plant Cell., 9(7):1055–1066
Bewley, J.D., Black, M., 1994 - Seeds: Physiology of Development and Germination, Plenum Press, New York
Bilderback, D. E., 1971 - Amylases in Developing Barley Seeds, Plant Physiol., 48, 331-334
Brown, A., Jacobsen, J., 1982 - Genetic basis and natural variation of α-amylase isozymes in barley. Genetical Research, 40(3), 315-324
Daussant, J., Sadowski, J., Ziegler, P., 1994 - Cereal β-Amylases: Diversity of the β-Amylase Isozyme Status Within Cereals, In Journal of Plant Physiology, 143, 6, 585-590
Deno, N., 1993 - Seed Germination Theory and Practice, Second Edition, 79
MacDonald, T., Brewbaker, J., 1972 - Isoenzyme Polymorphism in Flowering Plants: VIII. Genetic control and dimeric nature of transaminase hybrid maize isoenzymes, J. Hered., 63, 11-14
Markert, C. L., 1968 - The molecular basis for isozymes, Annals of the New York Academy of Sciences, 151:14–40
Pratt, C., Cornely, K., 2018 - Essentials of Biochemistry, John Wiley and Sons, Inc.
Sambrook, J., Russell, D., 2001 – Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York, USA, 559
Scandalios, J. G., 1969 - Genetic control of multiple molecular forms of enzymes in plants: A review, Biochem. Genet., 3, 37-79
Smith, B.D., 1998 - The Emergence of Agriculture, Scientific American Library, New York
Soltis, D. E., Soltis, S. S., 1989 - Isozymes in plant biology, Dioscorides Press, UK
Ulrich, S. E., 2011 - Barley: production, improvement and uses, Blackwell Publishing Ltd
van Onckelen, H. A., Verbeek, R., 1969 - Formation of α-amylase isozymes during germination of barley, Planta., 88(3):255-60

[1] ”Alexandru Ioan Cuza” University, Iasi, Romania
* aeu@uaic.ro