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# THE DYNAMICS OF BIOCHEMICAL PARAMETERS IN *ZEA MAYS* DURING THE GERMINATION PERIOD

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***Key words:*** *Zea mays, germination, enzymatic activity*

## INTRODUCTION

The multiplication of plants has always caused a special interest, aiming the understanding of biochemical and physiological aspects concerning the mobilization of nutritive substances from seeds, tubers, etc. and their use by the new plants. This process is extremely complex, to its development competing internal as well as external factors. The internal factors contain the morphological integrity of seeds and tubers, also as the optimum content in nutritive elements, meanwhile the external factors regard the conditions the soil offers: humidity, temperature, mineral salts, acid-base balance etc.

Generally, during germination, the catabolic processes develop at high speed. Some of them lower completely the stock substances, in order to assure the proper energy for the future biosynthetic processes, and others only divide partially these biomolecules, up to their structural basic unities (monosaccharides, fat acids, glycerol, amino acids, acetyl-CoA etc.), which will serve as precursors in the biosynthetic processes, that will form the glucids, lipids, proteins and all the other biomolecules essential in the development of the embryo and the plantlet, up to the moment when photosynthesis begins (BURZO *et al*., 1999).

At cereals, the starch synthesis, realized in endosperm, develops following an unique mechanism (GUAN and PREISS, 1993; BLAUTH *et al*., 2002; GENSCHEL *et al*., 2002; DINGES *et al*., 2003; JAMES *et al*., 2003), the starch seen as the main spare polysaccharide, which means during germination it should be a perfect correlation between the total amylolytic activity and the degradation speed of the starch.

The objectives of this study have aimed the determination of the concentration in starch and proteins, correlated with the dynamics of the amylase, neutral protease, alkaline and acid lipase activity from the corn caryopses that have been germinated in laboratory conditions.

The obtained experimental results prove a differenced dynamics both of enzymatic activity and proteins as well as starch concentration, depending on the time of germination.

## MATERIAL AND METHOD

The germination of the seeds represents a large step in the life of plants, prepared by all qualitative accumulations, determined by them, but released by external factors: humidity, temperature, air.

Inside the *Zea mays* beans, there are located nourishing substances (like starch, proteins, lipids), deposited in albumen or in cotyledons, which, in a latency state, are necessary at a low scale, but they are priceless as a stock during first growth.

The experimental researches have been performed on germinated caryopses of *Zea mays*, submitted to germination in laboratory conditions, after a preceding treating with peroxide 3%, for removing to eventual pathogenic germs or different substances, which could influence the germination, leaving them afterwards at soaking for 24 hours.

For the determination of the amylolytic activity, it has been used the Noelting-Brenfeld method, based on the reduction of the free maltose, resulted from the enzymatic hydrolysis of starch, with 3,5-dinitrosalicilic acid; the activity of the vegetable lipase has been determined depending on the increase of the acid value in the incubation environment, as a consequence of the release of fat acids from the triacylglycerols used as a substratum; the activity of the proteases has been determined using the Kunitz method, consisting in the quantitative dosing of hydrolysis products, released by protein as a result of neutral protease action; the proteins have been dosed with the Lowry method, based on the reaction they give wit copper ions in an alkaline environment, resulting a compound that reduces the Folin-Ciocâlteu reagent, followed by a change of color in product, to blue. The starch concentration has been determined through a polarymetric method (ARTENIE and TĂNASE, 1981; ARTENIE *et al*., 2007; COJOCARU, 2005).

## RESULTS AND DISCUSSIONS

For the evaluation of biochemical phenomena that occurs during germination, we resorted, as a first objective, to the determination of total amylase activity as against the dynamics of the starch content, knowing the fact that the starch represents

the main spare polyglucide that, during germination, is mobilized to obtain the energy essential in the development of different subsequent metabolic processes, which means during germination it should be a perfect correlation between the total amylolytic activity and the degradation speed of the starch (CIORNEA, 2008).

As one can observe from the graphic representation, there are no perfect similitude between the dynamics of the starch concentration and the total amylasic activity. On the one hand, the total amylolytic activity has a progressive growth during first period, while the starch concentration reaches a progressive decline, on the other hand the starch concentration continues to decrease until the end of the analyzed period, even though the enzymatic activity diminishes visibly (Fig. 1).

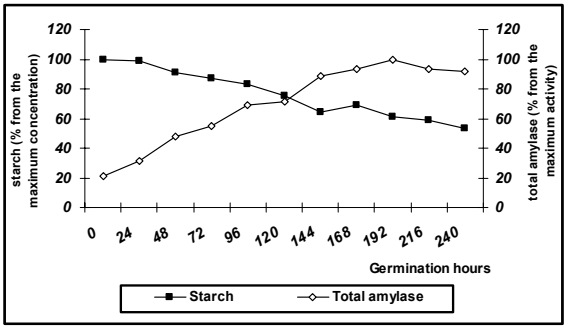


Fig.1. Representation of the correlation between the starch concentration and the total amylolytic activity during germination at corn

Starting from the idea that the two amylases present different acting mechanisms and, in the same time, they have different functions in the process of spare starch mobilization, we proposed further on to separately determine the α- and β-amylase activity, to clarify the way the starch is hydrolysed, under the catalytic action of these two enzymes. A proper analysis of the process of starch mobilization through hydrolysis during germination can be achieved only by following closely their catalytic activity. This is the reason for our choice to continue this study with an analysis of the two amylases activities, in comparison with their total amylolytic activity.

As one can observe from the presented data (Fig. 2), there are differences between the α- and β-amylase activity and, respective, between their amount and the total amylasic activity. The dynamics of the two amylases activities is identical only at the beginning of the germination period, namely when the absolute values are very low but their subsequent evolutions are very different and their total amylasic activity does not represent the amount of the two amylases activity. This evolution confirms the data collected from the scientific literature, according to which the vegetable β-amylase is found mainly insoluble, this providing the fact that the determined catalytic activity in the watery extracts does not totally reflect the real catalytic potential of the cellular enzymatic equipment (CIORNEA, 2008).

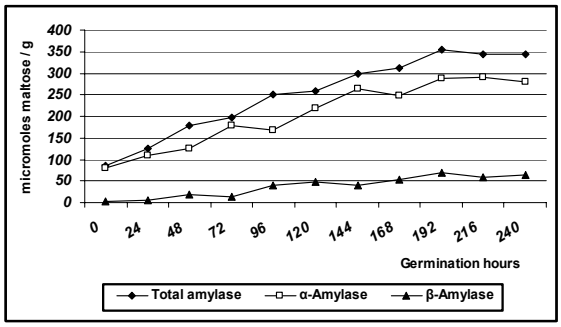


Fig. 2. The dynamics of the total, α- and β-amylase activity during germination at corn

During the germination of the seeds, the division of spare proteins of endosperm or cotyledons takes place at high speed and leads to the formation of amino acids and others simple nitrates substances that are used in the biosynthesis of developing embryo's proteins. The degrading of the proteins is also observed in the plants process of ageing, when the proteins of vegetative organs divide, the nitrates compounds obtained migrating to the reproductive organs, where the protein synthesis develops intensely.

Comparatively representing the correlation between the concentration of proteins and the activity of neutral protease, one can state that, on the same direction with the increase of medium value of proteic concentration, it is recorded in the same time a decrease of neutral protease activity, that reaches the medium value of 95.9 U/100 g, after 168 hours of germination, very close to the initial one (Fig. 3).

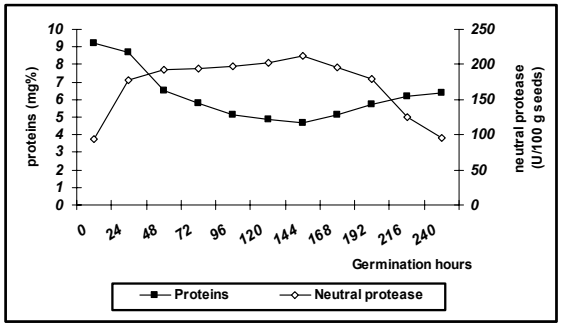


Fig. 3. Representation of the correlation between the concentration of proteins and the neutral protease activity during germination at corn

Regarding also the concentration of the lipids in the caryopsis of germinated corn in laboratory conditions, one can observe major fluctuations depending on the degree of germination in the same time with broad oscillations of the lipases activity.

This way, in the soaked sample, at the beginning of the germination, the activity of the alkaline lipase

registers the medium value of 149.5 ml/100 g seeds, and the acid one a much lower medium value of only 24.3 ml/100 g seeds.

At 24 hours since the beginning of the germination, a rapid growth of alkaline lipase activity distinguishes to the medium value of 233.6 ml/100 g seeds, the acid lipase increasing to the medium value of 74.4 ml/100 g seeds.

To the end of the analyzed germination period, the enzymatic activity presents lowering values, mentioning the fact that the acid lipase activity represents only a third of the alkaline one (Fig. 4).

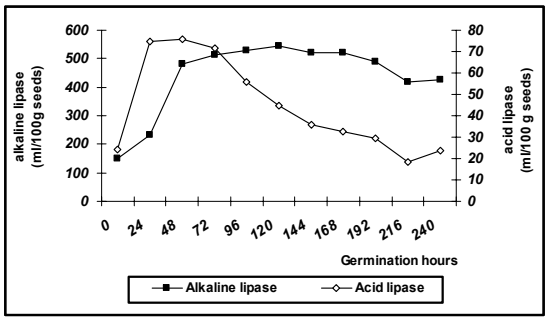


Fig. 4. The dynamics of the alkaline and acid lipase activity during germination at corn

## ABSTRACT

From biochemical point of view, the germination process consists in the mobilization of storage substances that is realized by the hydrolyze of proteins, polysaccharide and lipids under the action of specific enzymes until the their basic structural units (amino acids, monosaccharide, fatty acids etc.) and their utilization as precursors in the metabolic processes that assures the development of plantlet until the unleash of the photosynthetic process.

For the accomplishment of the experimental part we evaluated the dynamic of total amylase activity, α- and β-amylase, neutral protease, alkaline and acid lipase at maize (*Zea mays*) during germination and the correlation of fond values with the dynamic concentration of their specific substances.

The obtained dates emphases a high enzymatic activity that should demonstrate the fact that, during the germination period, the main storage substances are mobilized with an amazing speed with the purpose of energy production, energy that is necessary for embryo growing till the moment of unleash of the biosynthetic process.

## CONCLUSIONS

The experimental researches that we accomplished have led us to the following general conclusions:

1. The amylolytic activity presents an increasing dynamics, having minimum values at the beginning of the germination, attaining the maximum value after 192 hours, directly correlated with the dynamics of concentration in starch, as main spare polysaccharide, that records a progressive decrease during the entire period of time studied.
2. The activity of neutral protease and alkaline, as well as acid lipases (that represents an indicator of the proteins and lipids mobilization during germination) presents a diverse dynamics, totally different from amylase. In the first days it is noticed a strong increase of these enzymes activity, in a relation of reversed proportionality with the concentration of specific substrata, lowering afterwards in the second half of the analyzed period.
3. The activity of the enzymes taken into study demonstrates the fact that the main spare substances are mobilized at high speed in the first days of germination, after that they record a decrease of their activity, also probably caused by the biosynthetic processes that extend.

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