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Original Article

EFFECTS OF GERMINATION TIME OF SOYBEAN TO THE ACTIVITY OF SOYBEAN UREASE EZYME

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ABSTRACT: *The seed germination activity of the enzyme will increase. Urease content in soybeans is relatively high. Investigating the factors affecting the germination process in order to find the time to obtain the highest active enzyme urease. Soybeans were germinated at temperatures (20^{o}C, 24^{o}C, 28^{o}C, 32^{o}C) and humidity (75%, 80%, 85%, 90%, 95%) then surveyed over time. The optimum temperature and humidity for the activity of urease enzyme in soybeans was 28^{o}C, 80% in first day of germination.*

Keywords: *germination, soybean, enzyme, urease*

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INTRODUCTION

Urea was firstly detected in human urine by Hillaire M. (1773). The first enzyme that catalyzes the hydrolysis of urea was obtained by Miquel musculus in 1874 and in 1890, from the decomposition of urine, named urease [2]. Urease has essential role in soybean germination and nitrogen metabolism within seeds. Those functions related to protein metabolism during germination time of the arginase [11]. Urease hydrolyzes not only urea but also acetamide, formamide, N-methylurea, semicarbazide, and hydroxyurea [4].

Presents in most of plants, urease particularly abundant in grains, beans such as soybean (*Glycine max*) containing 0.012%/dry matter weight, and jack bean (*Canavalia ensiformis*) containing 0.07 to 0.14 % [2]. The other nuts are also studied to obtain replaceable urease extracted from jack bean such as watermelon seeds (*Citrullus*), pumpkin seeds (*Cucurbita maxima*) (Damodaran and Sivaramakrishnan, 1937) [5].

However, the active ingredients in seeds were affected by the germination, especially the enzyme in some grains such as barley, phytase increased with germination time and the highest obtained activity after 4 days of germination at temperatures of 20^{o}C and 25^{o}C in Sung's research [6].

The best genetic data of plant ureases are available for soybean (Glycine max). Two urease isoenzymes, a tissue-ubiquitous and embryo-specific encoded by two separate genes, as well as regulatory proteins encoded by unlinked genes were identified in soybean. The embryo-specific urease is an abundant seed protein in many plant species, including soybean, jack bean and Arabidopsis while the other type of urease (called

ubiquitous) is found in lower amounts in vegetative tissues of most plants [1].

Metallocenters serve essential biological functions such as transferring electrons, stabilizing biomolecules, binding substrates and catalyzing desirable reactions. Synthesis of these sites must be tightly controlled because simple competition between metals may lead to misincorporation with loss of function and because excess cytoplasmic concentrations of free metal ions can have toxic cellular effects [9].

A number of medical and ecological significances of microbial ureases has been described. The significance of the enzyme includes: to serve as a virulence factor in human and animal infections of the urinary and gastrointestinal tracts, play role in recycling of nitrogenous wastes in the rumens of domestic livestock, and its application in environmental transformations of nitrogenous compounds, involve urea based fertilizers [1].

MATERIALS AND METHODS

Soybeans were purchased at Thu Duc wholesale market, selected best and new soybean harvested 3-4 months and already removed small sizes, insected, and impurity ones.

50 soybean seeds were soaked in 25ml water for 16 hours at temperature of 28°C. Next they were put on the surface of the substrate (coconut fiber and minced perionyx excavatus) and moistened with distilled water according to moisture's survey.

Soya beans were incubated at temperatures of 20, 24, 28, 32 ° C. Grain moisture is controlled at 75, 80, 90, 95%. Moisture control method: seeds are tested for moisture at 8 h, moisture loss is added.

The soluble protein content and enzyme activity will be determined from the first to the fourth day. Method of Braford was selected to determine the soluble protein content [3]. In addition, method by Nessler method is also used for determination of urease activity [10].

The data were processed by Statgraphic software.

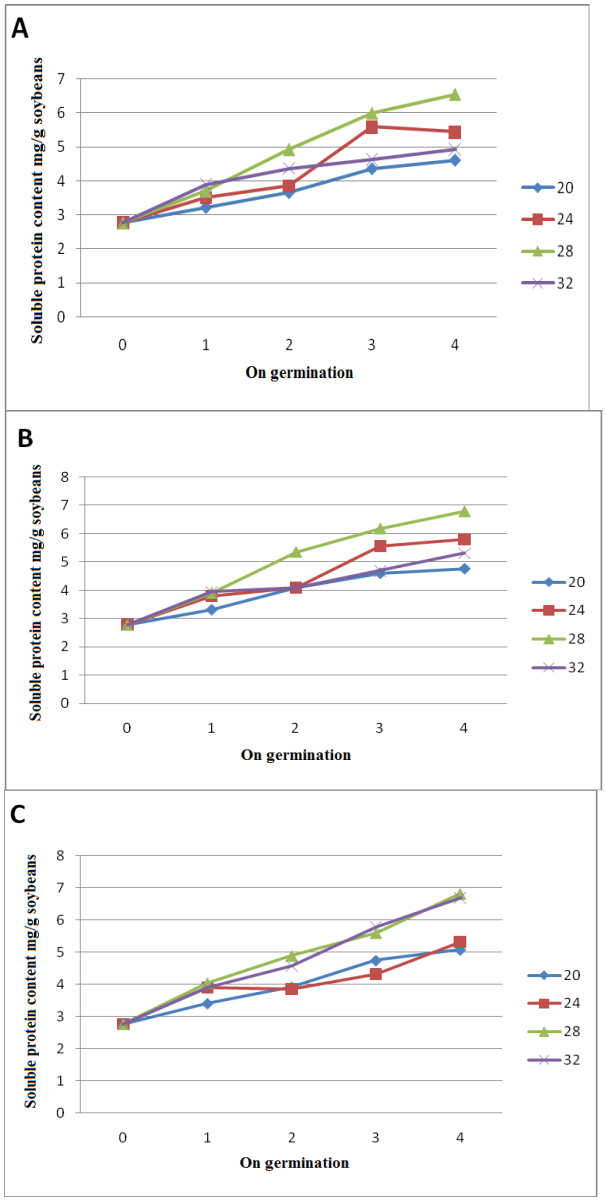
RESULTS AND DISCUSSION

Results of data processing indicated the significant differences as in table 1 and 2 which analyze the effects of moisture content and temperature onto protein contents of germination rate. The modification of protein contents of soybean seeds indicated that with the incubation temperature of 20°C, 24°C, humidity of 75%, 80% and 85%, the protein contents will be increased more slowly than with the temperature of 32°C and 28°C, humidity 90% and 95%.

Soybean seeds germinated at 95% humidity with various rates of temperature, the protein content in the seeds are higher than at other moistures. The highest protein content is obtained at 28°C.

In comparison of protein content in germinated soybeans and in normal ones, they also increase until 4th day.

In figure 1, when the temperature increases from 20°C to 28°C, the soluble protein content increases together with the germination time (H.G. Sung, *et al.*, 2005) [7]. When soybeans were germinated at temperature of 32°C, the germination slowed down due to high temperature is not suitable for the development of germinated soybeans. Temperature suitable for germination lower than 32°C is also confirmed by the study of Delouche (1953). The optimum temperature for germination of



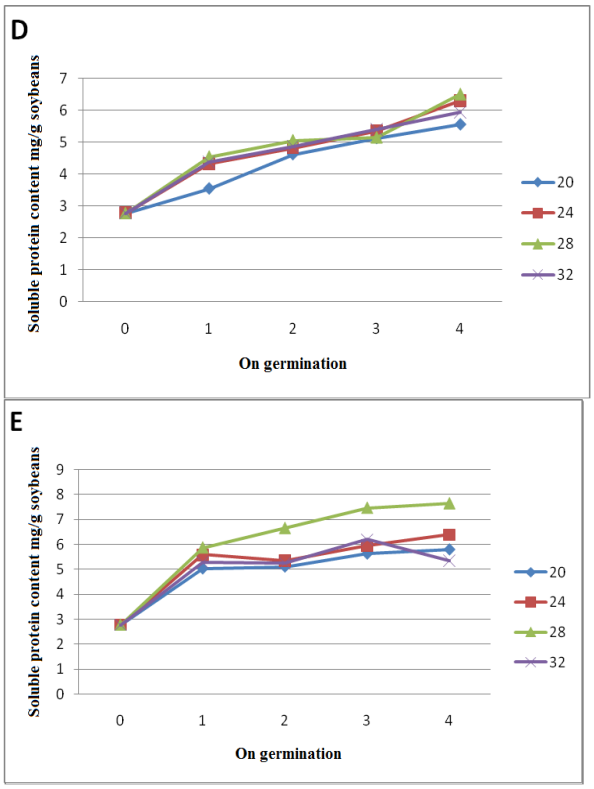


Figure 1. Evolution of the soluble protein content of germinated soybean depends moisture and germination temperature : A 75%; B 80%; C 85%; D 90%, respectively; E 95% humidity.

Table 1. Effect of moisture to protein during germination

On germination	75%	80%	85%	90%	95%
1	3.54^{a}	3.69^{ab}	3.79^{abc}	4.04^{bcd}	5.37^{ghi}
2	4.2^{cd}	4.4^{de}	4.32^{d}	4.84^{ef}	5.59^{hij}
3	5.14^{fgh}	5.26^{fghi}	5.12^{fg}	5.26^{fghi}	6.3^{k}
4	5.38^{ghi}	5.67^{ij}	5.98^{jk}	5.93^{jk}	6.31^{k}
				F=26.43	P<=0.05

Table 2. Effect of temperature to soluble protein content during germination

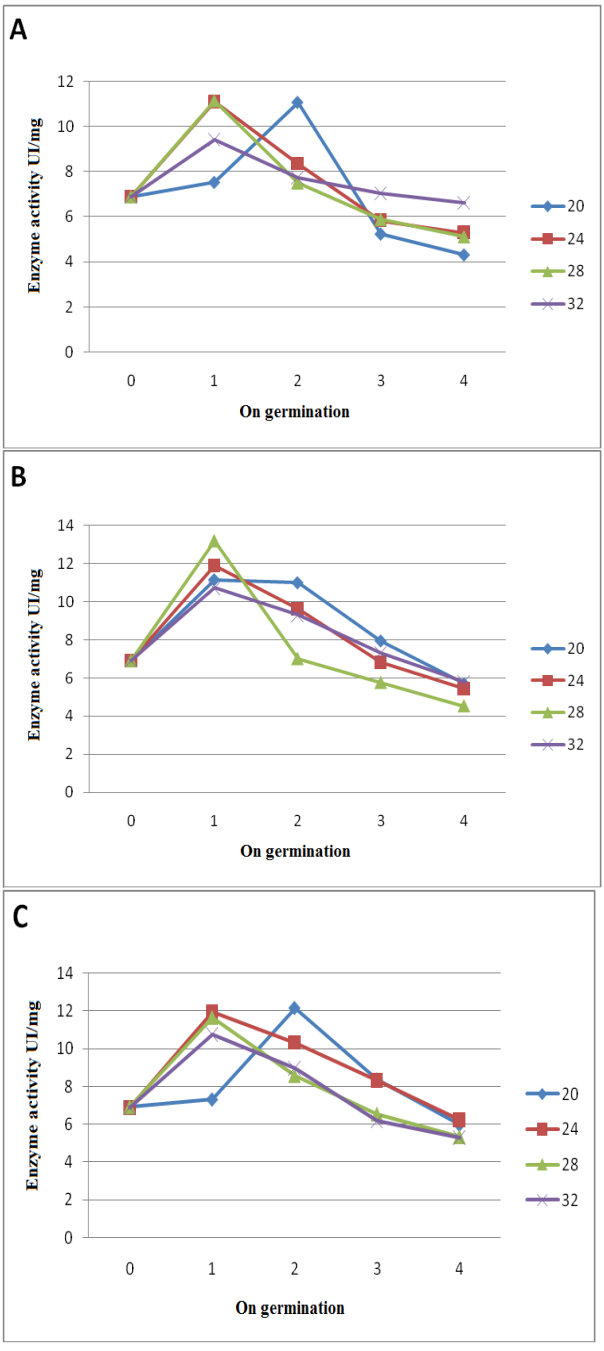
On germination	20°C	24°C	28°C	32°C
1	3.7^{a}	3.92^{ab}	4.43^{c}	4.29^{bc}
2	4.28^{bc}	4.39^{c}	5.38^{fg}	4.63^{cd}
3	4.89^{de}	5.36^{fg}	6.08^{h}	5.34^{f}
4	5.16^{ef}	5.79^{gh}	6.86^{i}	5.57^{fg}
			F=28.49	P<=0.05

some various soybeans is 30°C [6]. In our experiments, with different humidities and temperature of 28°C resulted in highest protein content demonstrated that 28°C is the optimum temperature for the development of the germinated soybean.

According to the experimental results, the activity of the urease enzyme peaked 1 day after germination at any experimental temperature and humidity rates, exception of temperature of 20°C on 2nd day.

The activity of urease enzyme began to decline at prolonging time while protein content continues to increase. This can be explained by demand of protease aggregation; urease has been activated in order to provide NH_3 as raw materials in the early stages, then proteolytic protease forces to increase the protein content.

The germination stimulates the synthesis of biologically active substances and enhances the response of the enzyme for the metabolites stored in grains 8.



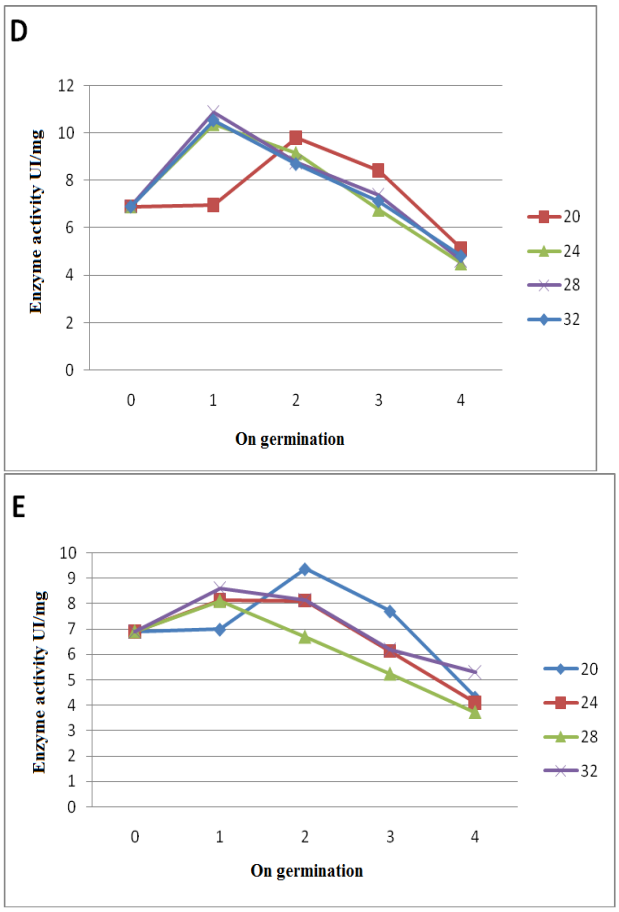


Figure 2. Evolution of the enzyme activity of germinated soybean depending moisture and germination temperature: A 75%; B 80%; C 85%; D 90%, respectively; E 95% of humidity.

Results of data processing indicates the significant differences, as in table 3 and 4, and analyzes the effects of moisture content and temperature rate on the activity of enzyme urease of germinated soybean.

To the figure 2, the effect of moisture on the activity of urease enzyme at 80% moisture is higher than 75%, 90% and 95% moisture, respectively. At 75% moisture content, the activity of the urease enzyme obtained lowest.

At 75% moisture content, the activity of the enzyme urease lowest due to the amount of water is not enough to provide for the metabolic of seed.

At a temperature of 24°C, 28°C and 32°C enzyme activity varies almost the same, difference 20°C. At a temperature of 28°C is the highest enzyme activity compared with the enzyme activity at different temperatures.

Table 3. Effect of temperature to activity enzyme content during germination

On germination	20°C	24°C	28°C	32°C
1	7.29^{ef}	10.71^{hi}	11.0^{j}	10.01^{h}
2	10.68^{hi}	9.12^{g}	7.72^{f}	8.61^{g}
3	7.53^{ef}	6.78^{de}	6.16^{cd}	6.77^{cd}
4	5.08^{ab}	5.11^{ab}	4.67^{a}	5.56^{bc}
			F=55.88	P<=0.05

Table 4. Effect of moisture to activity enzyme during germination

On germination	75%	80%	85%	90%	95%
1	9.81^{ij}	11.74^{k}	10.4^{j}	9.5^{hij}	7.31^{ef}
2	8.67^{gh}	9.29^{ef}	10^{ij}	9.1^{gh}	8.08^{fg}
3	6.0^{cd}	6.97^{de}	7.35^{ef}	7.42^{ef}	6.31^{de}
4	5.34^{bc}	5.36^{bcd}	5.71^{bcd}	4.76^{ab}	4.35^{a}
				F=36.96	P<=0.05

CONCLUSIONS

Research on urease activity from germinated soybean was obtained effectively. The activity of urease enzyme of germinated soybean is higher than of raw ones. It can be used for extracting process of urease enzyme with available materials.

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