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Dehydrogenase Enzyme Activities in Germinating Cowpea (*Vigna Unguiculata* (L) *Walp*)

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Abstract

Investigations through enzymatic assays on germinating cowpea (*Vigna unguiculata* (L) *Walp*) seeds reveal that dehydrogenases contributed to the catalyses of stored products in the anaerobic phase of seed germination. This led to the release of energy used in the process of early seed growth in germinating cowpea seeds. The results showed that dehydrogenase enzymes activities followed the pattern LD>AD>SD in a 60>31>9 percent ratio respectively. The enzyme activity curves suggest an initial burst of dehydrogenase activity preceding the initiation of seed germination and seedling vegetative growth in cowpeas.

Keywords: Cowpea, Dehydrogenases, Enzyme activity, Germination

1. Introduction

Germination can be defined as the resumption of active growth by the hitherto quiescent embryo within a seed, resulting in the rupture of the seed coat and emergence of the radicle and Plumule constituting a young plant. A seed is basically a kernel that encloses in itself a small embryonic plant covered by a hard seed coat and some stored food that upon receiving the appropriate climatic conditions, will promote growth of the embryo (Thomas *et al*., 2006). The stored foods in the plant seed include carbohydrates, proteins and lipids. The liberation of these foods are inhibited initially due to the impermeability to oxygen by the seed coat before they get soften and allow air influx. During germination, the stored food in the seed cotyledon is liberated initially by anaerobic respiration. Anaerobic respiration is made possible by the activity of enzymes such as dehydrogenases which catalyze catabolic chemical processes in anaerobic conditions (Turner and Turner, 1975).

Dehydrogenases belong to the class of enzymes known as oxidoreductases. The classification is based on donor-acceptor reactions in which electron molecules are transferred from one molecule (the oxidant) to another (the reluctant). Meyer and Anderson (1952) describe dehydrogenases as enzymes which accomplish intracellular oxidation and reduction by transfer of hydrogen from one kind of molecules to another. Most dehydrogenases use nicotinamide adenine dinucleotide (NAD^+) or nicotinamide adenine dinucleotide phosphate ($NADP^+$) while some make use of Riboflavin (Robert *et al*., 2009). Dehydrogenase enzymes include Alcohol dehydrogenase (EC 1.1.1.1), Lactate dehydrogenase (EC 1.1.1.27), Succinate dehydrogenase (EC 1.3.99.1) etc. Alcohol dehydrogenase (ADH) is one of two proteins in the ethanol fermentation pathway that is responsible for the reduction of acetylaldehyde, which is toxic to plant tissues, to ethanol, resulting in continuous regeneration of NAD^+ in the cytoplasm (Chung and Ferl, 1999). Succinate dehyrogenase, a complex enzyme tightly bound to the inner mitochondrial membrane oxidizes succinate to fumarate (Devlin, 2011). Lactate dehydrogenase catalyses the reversible oxidation of lactate to pyruvate using NAD^+ as a co-enzyme.

Cowpea, (*Vigna unguiculata* (L) *Walp*) of the family Leguminosae is an important food legume crops in the semi-arid tropics covering Asia, Africa, Southern Europe and Central and Southern America (Okigbo, 1986). Cowpea is considered nutritious with a protein content of about 23%, fibre content of 1.8%, carbohydrate content of 67% and water content of 8-9% (Chidda *et al*., 2003). Expected yield of cowpea in Nigeria is between 700-1000kg/ha on a well-managed soil and under good rainfall distribution. Cowpea resistance to drought, its ability to improve soil fertility and prevent erosion makes it an important economic crop in many developing regions. (Udealor, 2002). Cowpea crop is of vital importance to both global and national nutritional security, making the crop to be deserving of intense scientific scrutiny and monitoring. This study was undertaken to investigate the activities of different dehydrogenase enzymes in germinating cowpea. The understanding of enzyme activities in germinating seeds is a vital tool for the control and management of the productivity of many crops.

2. Materials And Methods

2.1 Collection of Sample

Cowpea (Vigna unguiculata (L) Walp) seeds were purchased from Institute of Agricultural research (IAR), Zaria, Kaduna state. The samples were identified by a botanist in the department of Biological Sciences, Nigerian Defence Academy.

2.2 Extraction and Purification of Enzymes

The germinated seeds of cowpea were washed, crushed, measured into a mortar; 40ml of chilled phosphate buffer 0.1m at pH7.5 was added to solubilise the paste. The plant was crushed to liberate the enzymes locked inside the cells. After sieving the residue, the extract was collected, placed in a centrifuging tube and then centrifuged at 26000xg for 15 minutes employing Chaplain and Dennis (1960) method. The supernatant obtained was decanted and used in the enzymatic assay process.

2.3 Enzyme Assay

1ml of enzyme extract was added to Thunberg tubes which were inverted to turn the extract into the knob. To the main tube, 1ml of 1/1000methylene blue, 2ml M/50 of substrate (alcohol, lactic and Succinic acids) and 2ml M/15 phosphate buffer was added. The mouth of the tube was oiled and capped with the cap containing the enzyme extract in the side arm-cap. The Thunberg tube with its content were connected to an elongated rubber tube and fitted to the air pump to evacuate air from the Thunberg tube for 3 minutes. The cap was rotated slowly to 180^{o}after evacuation and the tube was disconnected from the air pump. The tube was transferred into a water bath at 37^{o}C for 10minutes. Upon raising the temperature of the content, the tube and side arm cap mixed. The mixed content was compared with 1/10M concentration of methylene blue solution as the blank in a photometric visual monitor. The end point was when both colours of the side arm cap and main tube content match. After the experiment a few crystals of Na_2So_4 was added to completely reduce methylene blue. The content was placed in a 1cm cuvette and read in a spectrophotometer using wavelength of 580um to measure the absorbance. The graph of enzyme concentration (absorbance) was plotted against days of germination.

3. Results

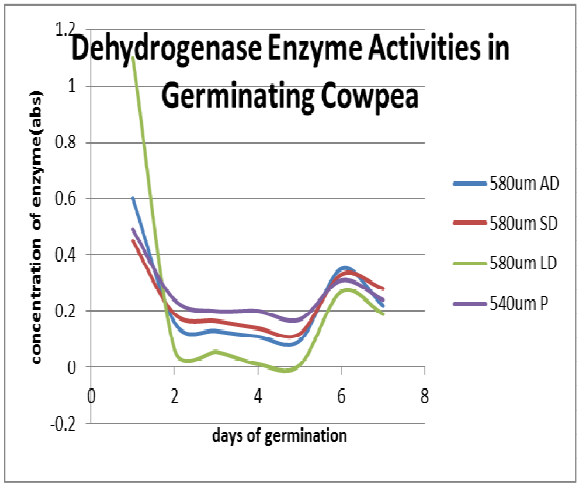

Fig 1: Effects of Dehydrogenase Enzymes Activities in Germinating Cowpea

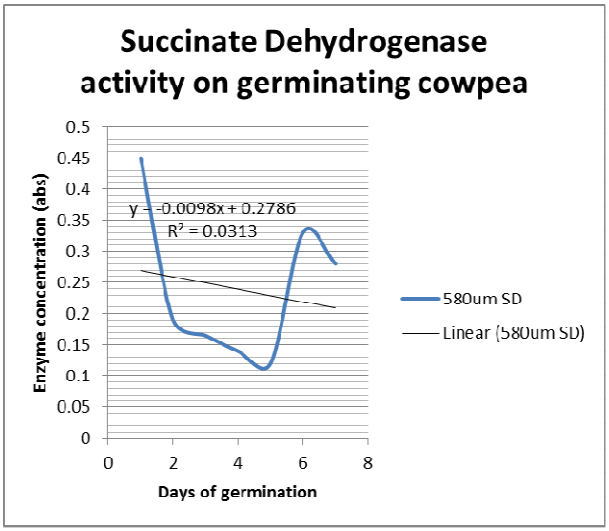

Fig 2: Effect of Succinate Dehydrogenase activity in germinating Cowpea

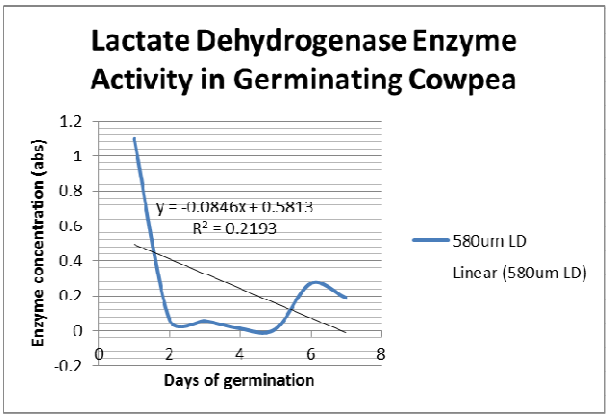

Fig 3: Effect of Lactate dehydrogenase enzyme activity in germinating Cowpea

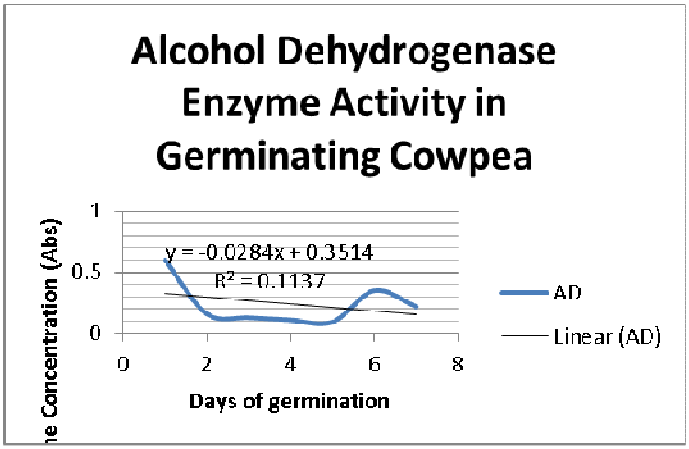

Fig 4: Effect of alcohol dehydrogenase enzyme activity in germinating Cowpea

Keys:

Alcohol dehydrogenase (AD)
Lactate dehydrogenase (LD)
Succinate dehydrogenase (SD)
Phosphate (Blank)

As can be observed from figure 2-4, the profile of dehydrogenase enzymatic activities followed a decreasing pattern from day 0 – 15. Although the profile of enzymatic activity showed initial sharp decrease (up to day 3), a stable reaction rate (day 4 – 10) and a minor increase (day 11-13), the regression analysis of these enzyme reactivity curves followed the same pattern in the figures shown.

Table 1: Regression analysis of dehyrogenase enzyme activities curve.

Enzyme	Substrate	Product	Enzyme Commission number	Percentage (%)	R^2
Lactate dehydrogenase(LD)	Lactate	Pyruvate	EC.1.1.1.28	60.20	0.2190
Alcohol Dehydrogenase (AD)	Ethanol	Acetaldehyde	EC.1.1.1	31.51	0.1137
Succinate Dehydrogenase. (SD)	Succinate	Fumarate	E.C.1.3.99.1	08.59	0.0313

The relative rate of reaction for the component dehydrogenases as showed in table 1 indicate that dehydrogenase enzyme activities followed the pattern LD> AD>SD. LD (60%) was about twice as reactive as AD (31%) while SD contributed only about 9% to the overall reactivity of dehydrogenase enzymes in germinating cowpea.

4 Discussion

Dehydrogenase enzyme activities have been shown to involve the activities of Alcohol dehydrogenase, Lactate dehydrogenase and Succinate dehydrogenase. Ebukanson and Bassey (1992) states that in germinating seeds, stored food, e.g. starch, is hydrolysed to glucose for use in the growth. Reactivity of these dehydrogenases covered barely the first three days of cowpea seed germinations. The enzymes separately mediated the conversion of stored carbohydrates and lipids through the anaerobic respiratory oxidation process. Dehydrogenases function as components in the electron transport chain facilitating the transport of electrons from substrates to oxygen (Robert et al., 2009). While Lactate and Alcohol dehydrogenases are gateway for production of Acetyl-coA, and Succinate dehydrogenase acts upon succinate to produce fumarate and eventually glucose to produce ATP (Adenosine Triphosphate).

Jones *et al*., (1999) observed that anaerobic respiration occurs during resting stages of seeds and the initial stages of seed germination. It was observed that for cowpea, the L-lactate pathway was the preferred pathway for anaerobic dehydrogenation, However the Alcohol pathway was also significant. This evidences that the enzymatic reaction was still substantially within the anaerobic phase. This was further sustained by the low reactivity of succinate dehydrogenase which is required mostly in the glyoxylate cycle of the aerobic respiratory phase.

The research work shows that germination respiration in the seeds of cowpea is initially anaerobic and that Lactate dehydrogenase enzyme is responsible for a substantial part of the dehydrogenase activities. This information would be useful to breeders, agronomists and physiologists, and also represent a useful contribution to the understanding of the physiology of cowpea.

5 Conclusion

As common in other species, anaerobic respiration precedes the commencement of germination process in Cowpea seeds. This phase of germination in cowpea seeds, last only for a short period not exceeding the third day after hydration. Enzymatic assay is one of the veritable tools for the elucidation of physiological process. In

this case the contribution of some of the dehydrogenase enzymes towards the initial breakdown of stored nutrition in cowpea seeds has now been shown.

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