Role of Enzymes in Seed Germination

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Abstract : The seed germination is the process in which embryo found in the seed develops into plumule and radicle. Seeds absorb water and which swells the inactive tissues and start the cell division. The radicle arises from micropylar, which moves downward and into the soil. These radicles later on change into roots those provide supply of water and nutrients to the plants throughout their life. Enzymes such as amylase, protease, and lipase are liable for solubilizing spare food material in the form of starch, protein and lipid correspondingly in seed and deliver energy and other fundamentals food material to germinating embryo. The seed proteins are catalyzed by proteases enzymes and break it into amino acids and peptides that are transferred to growing embryo. The Amino acids obtained from the metabolism of the proteins are further used in the biosynthesis of enzymes, hormones, proteins, pyrimidines and purines bases. The starch is catalyzed by enzyme amylase that provides the food materials for growth and development of embryo. Similarly lipases are enzymes those are responsible for metabolism of the triacylglycerols into glycerol and fatty acids. This is also the source of energy for growing embryo. The further detail about the seed germination and role of various enzymes is described further in detail.

***IndexTerms* – enzymes, seed germination, hydrolysis, proteins**

1. Seed Germination

Seeds are heterogeneous storage reserves with wide array of storage compounds that include various soluble carbohydrates, starch polymer, storage proteins and lipids. These stored reserves comprise 70% of the world's caloric intake in the form of food and animal feed produced through sustainable agriculture, which contributes to food and nutritional security. Seed systems biology remains an enigmatic subject in understanding seed storage processes, maturation and pre-germinative metabolism. The embryonic plant which is enclosed in covering is commonly known as seed. It is produced during the reproduction in plants. In addition, mature ovules also called as seed. In suitable environmental conditions, seed germinate to form young plants. The germination of the seed is a complex process and is defined by several authors in different ways (Higashiyama *et al.*, 2003). According to physiologists germination can be defined as the emergence of the radicle via seed coat but seed analysts defined the process of seed germination as the emergences and the development of essential structure from embryo which develop into plants under suitable condition, while some other botanists defined seed germination as the emergence and growth of embryo to young plants by rapture of seed coat. But later Mayer and Shain, (1974) described the definition of germination of seed a series of steps that usually occurs earlier than the development of the radicle from the seed coat. These definitions indicated that seeds are in rest or dormant stage before development of the embryo. The rest stage commonly known as inactive stage has low metabolic activities. These conditions remain until environmental circumstances activate the resumption of growth. Few seeds can germinate after fertilization while some others seed become inactive or dormant and wait for favorable environmental condition. Depending on plants species the dormant period may remain for few days to several years.

A developing seed consists of triploid endosperm and diploid embryo, which are enclosed by the maternal seed coat. In dicots, the embryo encompasses most of the space in the seed along with major lipid and protein reserves (Borisjuk *et al.* 2013). In cereals, the endosperm is the major storage sink where starch and storage proteins are accumulated (Olsen 2001). Vast seed genomics resources have been created from various model organisms (North *et al.* 2010; Olsen 2001; Sreenivasulu and Wobus 2013).

2. Mechanism of the Seed Germination

There are two kinds of seed germinations found, and neither appears to be related to seed structure. These two types are illustrated by the germination of bean and pea seeds. Although these seeds are similar in structure and are in the same taxonomic family, their germination patterns are quite different. Epigeal germination is characteristic of bean and pine seeds and is considered evolutionarily more primitive than hypogeal germination (Larry and Miller, 1995). During germination, the cotyledons are raised above the ground where they continue to provide nutritive support to the growing points. During root establishment, the hypocotyl begins to elongate in an arch that breaks through the soil, pulling the cotyledon and the enclosed plumule though the ground and projecting them into the air. Afterwards, the cotyledons open, plumule growth continues and the cotyledons wither and fall to the ground. While hypogeal germination is characteristic of pea seeds, all grasses such as corn, and many other species. During germination, the cotyledons or comparable storage organs remain beneath the soil while the plumule pushes upward and emerges above the ground. In hypogeal germination, the epicotyl is the rapidly elongating structure. Regardless of their above-ground or below-ground locations, the cotyledons or comparable storage organs continue to provide nutritive support to the growing points throughout germination (Larry and Mille, 1995).

The process of the seed germination can be divided into several steps that include activation of enzymes by imbibition of water, development of the radicle to root for absorption of water from soil and development of the plumule to shoot for photosynthesis. When germination starts seeds absorb water and produce various enzymes that help in breakdown of the reserve food into simpler molecules. These products are transported to emerging seedling for their development. (Millerd and Thomson, 1975) Various minerals, proteins and enzymes perform a significant role in many ways at different steps in seed germination. It has been noted that when seed began to germinate it increases the minerals, proteins, vitamins and enzymes from 25 to 4,000 percent (Azulay, 1997).

Seeds have reserve food in the form of stored proteins, carbohydrates, phosphate and lipid that act as the carbon skeleton and energy source (Mayer *et al.*, 1974). The seed imbibition activates various biochemical processes like activation of synthesis of hydrolytic enzyme. These hydrolytic enzymes play a vital role in catalysis of reserved food such as proteins, carbohydrates, lipids, hemicellulose, polyphosphates and other food materials into simple available food for embryo uptake. Few enzymes are also produced after the uptake of the oxygen such as activation of the mitochondrial enzymes that is involved in electron transport chain and Krebs cycles (Bewley JD and Black M, 1985). The detailed mechanism and role of these hydrolytic enzymes are explained below.

3. Hydrolysis of the protein during seed germination

The metabolism of the seed protein is the necessary step in seed germination which is a several step process and takes places with the help of Proteolytic enzymes (Shutov and Vaintraub 1987). Gepstin and Han, (1980) reported that the activity of the Proteolytic enzymes increased in beans during first seven days of seed germination. It is also observed that the protease inhibitors which are protein in nature are being disappeared. The hydrolysis or catalysis of protein that is stored in seed release free amino acid. These free amino acids help in biosynthesis of the protein in endosperm and embryo that ultimately precedes germination process. Yano *et al.*, (2001) developed a technique known as disulfide proteome technique that is used for visualization of redox changes in proteins. Rice bran is analyzed by using disulfide proteome technique and resulting in identification of dienelactone hydrolase, embryo-specific protein 2 (ESP2), putative globulin globulin-1S-like protein such as putative target of thioredoxin (it supports the hypothesis that thioredoxin triggers cysteine protease with a simultaneous unfolding of its substrate during seed germination. Dunaevsky and Belozersky, (1989) reported that the seed of the buckwheat mainly have 13S globulin which consists of acidic and basic subunits and has molecular mass about 57.5 to 23.5 kDa. The Proteolytic enzymes hydrolyze the 13S globulin step by step and the products of this reaction are used by the growing seedling. The first step of degradation of 13S globulin is accomplished with the help of metalloproteinase with the cleavage of about 1.5 % peptide bonds. This step takes first three days of seed germination. While in the second step of 13S globulin degradation carboxypeptidase and cysteine proteinase (appear in germinating seeds) hydrolyze the products of metalloproteinase into small peptides and amino acids. The carboxypeptidase facilitates the hydrolysis of storage protein and works in assistance with cysteine proteinase. In last step aspartic proteinase hydrolyzes the proteins present in the seed (Belozersky and Dunaevsky, 1990).

4. Hydrolysis of starch during seeds germination

Most plants' grains contain about 70–80% starch. In crops, various hydrolysis enzymes are synthesized in the scutellum or aleurone in response to germination indication. Numerous improved seed systems were hand-me-down to sense the induction process and recognize possible aspects regulating enzyme induction in the absence of embryo (Paleg, 1960). Among numerous researches which concerned with studying the importance of α-amylase function during germination of seed in different environmental stress such as drought stress heat stress etc., few important researches are summarized as follows. The promotion of water stressed germinating seeds is a result of high α-amylase activity directly but it might be related to adaptive strategy to water deficit since its activity is required for solutes accumulation and decrease osmotic potential. In addition, α-amylase synthesis inhibition might be not a mechanism by which drought prevents the germination of *Agropyron desertorum* seeds (Kepczynski, 1986).

5. Hydrolysis of storage seed lipids during germination

Normally oilseeds consist of two parts, the kernel (main part) and the seed covering (enclosed the kernel and also known as tegument or husk). The kernel contained two parts that are the endosperm and the embryo. The activities of the Lipase enzymes are investigated during seed germination where oil contents are of maximum value (Paques and Macedo 2006). Triacylglycerols accumulated in oleosomes are in the range between 20 to 50% of dry weight. As germination continues, triacylglycerols are degraded or hydrolyzed to give energy which is obligatory for the biosynthesis of sugars, amino acids (such as aspartate, asparagine, glutamate and glutamine) and carbon chains are obligatory for embryonic growth and development (Quettier and Eastmond, 2009). Various activities of the lipase enzymes and lipid level were observed in numerous germinating seeds (Hutton and Stumpf, 1969). The most important hydrolytic enzymes associated with the lipid catalysis during germination of the seeds are the lipases which catalyze the hydrolysis of ester carboxylate bonds and liberating organic alcohols and fatty acids (Barros *et al.*, 2010; Pereira *et al.*, 2003) and also involved in esterification (reverse reaction) or even several trans-esterification reactions (Leal *et al.*, 2002).

Villeneuve, (2003) and many other researchers divided the specificities of lipases into three main groups; first group is substrate specificity in which glycerol esters indicate the natural substrates while 2nd group is called regioselective and includes the further subgroups such as *non-specific lipases* which hydrolyze the triacylglycerols and make glycerol and fatty acids in arbitrary way with synthesis of monoacylglycerols and diacylglycerols as transitional products; *specific 1.3 lipases* which catalyze the hydrolysis at C1 and C3 glycerol bonds in triacylglycerols with delivering of fatty acids, 1.2-or 2.3-diacylglycerols, unbalanced intermediates 2-monoacylglycerols and *specific or selective type fatty acid* that hydrolyze the ester bond of a specific fatty acid or a specific group of fatty acids at any position of triacylglycerol. The 3rd group enantioselective which recognize enantiomers (Enantiomers are chiral molecules that are mirror images of one another) in a racemate or racemic mixture. The enantioselective specificities of lipases depend on the type of substrate (Castro and Anderson, 1995).

6. Role of Beta -1, 3-glucanases during seed germination

The β-1, 3-Glucanase is an enzyme that catalyzes β-1, 3-Glucan. The thick layer of β-1, 3-Glucan is found as cover on seed which later on degrades during germination by the activities of the β-1,3-Glucanase. The β-1,3-Glucanase play a very important role in dormancy, regulation and germination of seeds. In many plants species this envelope of β-1, 3-Glucan delays the radicle emergence (Simmons, 1994). The β-1, 3-Glucanase induced transcriptionally in the micropylar endosperm of tomato, tobacco, and other seeds of family *Solanaceae* before radicle emergence. Induction of β-1, 3-Glucanase and seed germination is closely linked in response to plants hormones and environmental aspects e.g. both are inhibited by abscisic acid and enhanced by gibberellins. According to Simmons, (1994) β-1, 3-Glucanase digest the β -1,3- glucan callose (plants polysaccharide between the cell wall and plasma membrane of various tissues). Callose is present in bulky quantities in several dicot species mostly found in the layers of seed cover (Nguyen *et al.*, 2002). The important possible role of the callose in plants include chemical and physical separation of developing gametes bud dormancy, cell division, defense from osmotic and environmental stress maintain plasmodesmatal traffic, pollen tube development and interaction between plants and microbes (Doblin *et al*., 2001; Sivaguru *et al.*, 2000; Scherp *et al.*, 2001; Rinne *et al.*, 2001). Leubner *et al.*, 1995 reported a working hypothesis about β-1, 3-Glucanase that it take part in degradation of cell wall as a result endosperm rupture and arise radicle projection.

7. Hydrolysis of phytic acid during seed germination

The seed stores phosphorus in the form of phytic acid that is also known as inositol hexophosphate in cereals and legumes (Jacela, 2010). Phytic acid also known as antinutrient because it has the ability to bind with cations such as Ca, Mg, Fe, Mn, K, Zn through ionic link to make a mixed salt commonly known as phytin or phytate and it also form complexes with protein. Phytate or phytin reduces the digestive ability of seed (Lott, 1995).

According to Shi *et al.*, (2005) a high amount of stored phytate is not necessary for seed viability and germination or seedlings growth. Phytin in germinating seeds is broken down by an acid phosphatase enzyme known as phytase with liberating of cations, phosphate, and inositol that are consumed by the seedlings or young plants.

Silva and Trugo, (1996) discussed these results as evidence of metabolism in germinating seed. It was observed that about 87% of inositol hexophosphate (IP6) breaks down during the first six days of seeds germination. In this deference, Ogawa *et al.*, (1979) hypothesized that the initial axiferous IP6 digestion is vital for metabolic action of the inactive tissue through providing minerals and Pi for metabolic and physiological desires, such as, enzymes of starch metabolism. In addition, IP6 associated compounds like pyrophosphate-having inositol phosphates (PP-IP) perform a significant role in providing Pi for ATP synthesis during the initial steps of seeds germination before whole dependence on mitochondrial aerobic respiration that is the main source of ATP synthesis for seed germination.

8. Conclusion

From above inferences, it is concluded that the seed germination is the essential phenomena for the survival of the plants species. Different environmental conditions like temperature, moisture, and soil etc. effect the seed germination process The enzymes also play a very significant role in seed germination, growth and development. Without enzymes the process of germination cannot continue. Seed’s cotyledons comprise stored food materials which provide energy for growth and development. The stored food is commonly found in the form of starch (insoluble sugar molecule) which needs to be converted into its soluble form before it is used for growth and development of embryo. Amylase enzymes are used for catalysis of starch which is produced in the seeds after the absorption of water from the soil. The starch necessities to be changed into soluble sugar with help of enzymes for the seeds to make use of. Amylase is produced in the seeds after the absorption of water from the soil. It breaks starch to make maltose that facilitates the process of germination. Similarly, others enzymes also facilitate the germination process such as lipase in the breakdown of lipids, protease help in breakdown of protein found in seeds and β-1, 3-Glucanase in catalysis of β-1, 3-Glucan.

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