

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Metabolic Processes During Seed Germination

Awatif S. Ali and Alaaeldin A. Elozeiri

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.70653>

Abstract

Seed germination is crucial stage in plant development and can be considered as a determinant for plant productivity. Physiological and biochemical changes followed by morphological changes during germination are strongly related to seedling survival rate and vegetative growth which consequently affect yield and quality. This study is aimed to focus on proceeding of the most vital metabolic processes namely reserve mobilization, phytohormonal regulation, glyoxylate cycle and respiration process under either stressful or non-stressful conditions that may be led to suggest and conduct the more successful experimental improvements. Seed imbibition triggered the activation of various metabolic processes such as synthesis of hydrolytic enzymes which resulted in hydrolysis of reserve food into simple available form for embryo uptake. Abiotic stresses potentially affect seed germination and seedling establishment through various factors, such as a reduction in water availability, changes in the mobilization of stored reserves, hormonal balance alteration and affecting the structural organization of proteins. Recent strategies for improving seed quality involved classical genetic, molecular biology and invigoration treatments known as priming treatments. H_2O_2 accumulation and associated oxidative damages together with a decline in antioxidant mechanisms can be regarded as a source of stress that may suppress germination. Seed priming was aimed primarily to control seed hydration by lowering external water potential, or shortening the hydration period.

Keywords: reserve mobilization, proteolysis, glyoxylate cycle, phytic acid, seed priming, stress tolerance mechanisms

1. Introduction

Seed germination is vital stage in plant development and can be considered as a determinant for plant productivity. It begins by water imbibition, mobilization of food reserve, protein synthesis and consequence radicle protrusion [1]. To sustain a good seedling development,

seed stores a food reserve mainly as proteins, lipids and carbohydrates [2]. Protein and oil bodies are the major reserve in oilseed which represent a source for each of energy, carbon, and nitrogen during seedling establishment [3]. Because the physiology of reserve mobilization during germination and post-germination events is still poorly understood, extensive studies must be performed to know the metabolic mechanisms of reserve food mobilization providing insights into the ability to use such seeds as planting material [4]. Enzymatic hydrolysis of protein, lipid and carbohydrate, and transportation of metabolites is dependent mainly on water availability [5].

Physiological and biochemical changes followed by morphological changes during germination are strongly related to seedling survival rate and vegetative growth which affect yield and quality. Food reserve of starch and protein are mainly stored in the endosperm. In general, germination process can be distinguished into three phases: phase I, rapid water imbibition by seed; phase II, reactivation of metabolism; and phase III, radicle protrusion [6]. The most critical phase is phase II whereas, the essence physiological and biochemical processes such as hydrolysis, macromolecules biosynthesis, respiration, subcellular structures, and cell elongation are reactivated resulting in initiation of germination [7].

Water imbibition by reserve substances in germinating wheat seed stimulates the embryo to produce phytohormones mainly gibberellic acid (GA) which can diffuse to aleurone layer and initiate a signaling cascade resulting in the synthesis of α -amylases and other hydrolytic enzymes. Then, hydrolytic enzymes secrete into the endosperm and hydrolyzed food reserve [8, 9]. Germination is considered a response includes bidirectional interactions between the embryo and endosperm since the endosperm can secrete signals to control embryo growth [10]. Previous studies were investigated the activity of some key enzymes in glycolysis, pentose phosphate pathway (PPP), the tricarboxylic acid cycle (TCA cycle), and amino acid metabolism during germination [11].

Seed germination is particularly vulnerable to environmental stress encountered conditions, specifically salt and water which are widespread problem around the world [12]. High salt and drought tolerance seeds might be showed rapid germination resulting in a good seedling establishment and hence expected to maintain high yield productivity [13]. Water and salt stress conditions affect seed germination with reducing germination rate and delay in the initiation of germination [14]. Under water stress, enzymes activity such as α -amylase in *Cicer arietinum* cotyledons [15] or α - and β -amylase in *Medicago sativa* germinating seeds [16] were reduced. In contrast, water stress conditions led to an increase in the activity of α -amylase in *Hordeum vulgare* seedlings [17], β -amylase in *Cucumis sativus* cotyledons [18], cytosolic glyceraldehyde-3-phosphate dehydrogenase in *Craterostigma plantagineum* plants [19] and protease in *Oryza sativa* seedlings [20]. Salt stress causes ion toxicity, osmotic stress and reactive oxygen species (ROS) stress [21]. ROS reacts with cell macromolecules [22] and lipids [23], and disrupt diverse physiological and biochemical processes, such as hormonal imbalance and reduced use of reserves [24]. Plants develop ROS-scavenging mechanisms include enzymatic and non-enzymatic antioxidant systems [25] that protect plants against oxidative damage. Therefore, improvement the activity of antioxidant enzymes in plants organs is necessary for increasing plant's salt tolerance. Species and varieties/cultivars varied in their ability for

salt tolerance mechanism. Comparing with adult plant, the mechanisms of stress tolerance in germinating phase are poorly interpreted and might be related to a series of factors that are inherent to the species and environment [26, 27].

Phytohormones have essential role in inducing plant acclimatization to change in environmental conditions by mediating growth, development, source/sink transitions, and nutrient allocation [28]. Phytohormones are considered the most important endogenous substances for modulating physiological and molecular responses [28]. They include auxin (IAA), cytokinins (CKs), abscisic acid (ABA), ethylene (ET), gibberellins (GAs), salicylic acid (SA), brassinosteroids (BRs), and jasmonates (JAs). The strigolactone (SL) are relatively new phytohormones.

Genetically and physiological studies have been demonstrated the effective roles of the plant hormones ABA and GAs in regulation of dormancy and germination [29]. To counteract the adverse effects of abiotic stress, seed priming methods have been applied to improve germination, uniformity, improve seedling establishment and stimulate vegetative growth in more field crops [30, 31]. Wheat seeds were priming to increase germination characteristics and stress tolerance. As seeds imbibe water, metabolic processes initiate with an increase in respiration rate [7]. Early developmental stages of seedling require fueling energy before it becomes autotrophic [32].

Seeds store mineral nutrients as sucrose or amino acids which are synthesized into starch or proteins during development to be used in early seedling emergence. Phosphorus is taken up by plants as phosphate and translocate to developed seeds where it is stored in phytic acid form mainly (about 75%).

2. The role of hydrolytic enzymes in seed germination

On seed hydration, separate intercellular bodies of seed stored carbohydrates, proteins, lipid and phosphate act as energy source and carbon skeleton [33]. Seed imbibition triggered many metabolic processes such as activation or freshly synthesis of hydrolytic enzymes which resulted in hydrolysis of stored starch, lipid, protein hemicellulose, polyphosphates and other storage materials into simple available form for embryo uptake. Also, consumption of an elevated level of oxygen may be induced activation/hydration of mitochondrial enzymes, involved in the Krebs cycle and electron transport chain [34, 35].

2.1. Hydrolysis of storage seed proteins

Proteolytic enzymes have the main role in using stored protein in metabolism of germinating seeds which proceed through many stages [36]. According to Gepstin and Ilan [37], proteolytic activity in germinating beans increased during the first 7 days which partially dependent on the embryonic axis. Proteases and peptidases have been detected in many seeds during germination whereas; plant protease and amylase inhibitors which are proteinaceous in nature are being disappeared [38]. Antitryptic and antichymotryptic activities were observed to be markedly reduced in the endosperm of finger millet on germination which might be

attributed to the proteolytic activity in hydrolysis of the inhibitory proteins [39]. Hydrolysis of stored proteins produced free amino acids, which support protein synthesis in endosperm and embryo and so proceeding of germination process [40]. Schlereth et al. [41] recorded an initial little decrease in free amino acids at the beginning of vetch seeds imbibition which is attributed to leakage from the axis, but remain without change during late germination stage.

A disulfide proteome technique was developed by Yano et al. [42] to visualize redox changes in proteins. This technique was used to analyze rice bran resulting in identification of embryo-specific protein 2 (ESP2), diene lactone hydrolase, putative globulin, and globulin-1S-like protein as putative target of thioredoxin, which support the hypothesis that thioredoxin activates cysteine protease with a concurrent unfolding of its substrate during germination [43].

In buckwheat seeds, the main storage protein constituent about 16% of total seed protein is the 13S globulin with molecular mass of about 300 kDa and consists of acid and basic subunits with molecular masses ranging from 57.5 to 23.5 kDa [44]. During seed germination, 13S globulin is hydrolyzed by proteolytic enzymes through stages and the products are used by the growing seedling. The first stage of the 13S globulin degradation resulted from a limited proteolysis activity of metalloproteinase with the cleavage of about 1.5% of peptide bonds. This stage proceeds during the first 3 days of germination. It takes place during the first 3 days of germination [45]. Metalloproteinase activity is controlled by a proteinaceous inhibitor (Mr—10 kDa), present in dry buckwheat seeds in a complex with the enzyme which dissociated by bivalent cations liberated from phytin hydrolysis process. Phytin is present in buckwheat seeds in sufficient amount in the form of globoids disposed in protein bodies [46].

During the second stage of 13S globulin degradation; the products of metalloproteinase protein activity hydrolyzed into small peptides and amino acids at acid pH (5.6) by cysteine proteinase and carboxypeptidase which appear in germinating seeds [47]. It was clear that cysteine proteinase is able to hydrolyze only the modified 13S globulin but not the native. The role of carboxypeptidase is to facilitate the flow of storage protein hydrolysis and works in cooperation with cysteine proteinase. At latest stage when pH becomes more acidic (5.0) in the vacuoles, aspartic proteinase which is present in dry seeds is involved into the course of hydrolysis protein bodies.

2.2. Hydrolysis of storage seed starch

Carbohydrates represent the most storage food constituent in cereal grains, whereas it contains about 70–80% starch, about 15% protein, less than 5% lipids, minerals and vitamins. In cereals, most hydrolysis enzymes are produced in the aleurone or scutellum in response to germination signals. Several modified seed systems were used to detect the induction process and identify potential factors controlling enzyme induction in absence of the embryo [48].

Chrispeels and Varner [49] observed that isolated aleurone failed to synthesize α -amylase in a manner quantitatively similar to distal half seeds led to correction by adding calcium to the medium. The role of calcium might be expected to involve amylase stability, and to have a much more complex involvement in regulating enzyme activities [50]. Because of *de novo* amylase synthesis during seed germination to stimulate the stored starch mobilization for

providing young plant till photosynthesis will be initiate, amylase has been showed high activity [51]. Parys et al. [52] showed that the amylase activity is regulated by the concentration of reducing sugars in vivo in both cotyledons and axis. At the time, the amylase activity in the cotyledons increased gradually and reached a maximum on the 5th day of germination process, while the starch decreased and soluble sugars increased [53].

Many studies which concerned with studying the essentiality of α -amylase activity during seed germination under drought stress and could be summarized as follows; the promotion of drought 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 [54, 55]. In addition, α -amylase synthesis inhibition might be not a mechanism by which drought prevents the germination of *Agropyron desertorum* seeds [54]. GAs can alleviate the drought stress-caused inhibition of seed germination through regulation of α -amylase [19].

2.3. Hydrolysis of storage seed lipids

Generally oilseeds composed of two parts, the kernel which is main part and the seed covering that enclosed the kernel and called the husk or tegument. The kernel comprised two parts which are the embryo and the endosperm. Lipase activity is investigated during seed germination where it is maximum value [56, 57]. Triacylglycerols is stored in oleosomes and comprise in range from 20 to 50% of dry. As germination proceeds, triacylglycerols are hydrolyzed to produce energy which required for the synthesis of sugars, amino acids (mainly asparagine, aspartate, glutamine and glutamate) and carbon chains required for embryonic growth [58].

Lipid level and lipase activity were studied in various germinating seeds. It was showed that β -oxidation takes place 4 days after germination of Castor been seeds [59]. The major hydrolytic enzymes concerned with the lipid metabolism during germination are the lipases which catalyze the hydrolysis of ester carboxylate bonds and releasing fatty acids and organic alcohols [60, 61] and the reverse reaction (esterification) or even various transesterification reactions [62]. The ability of lipases to catalyze these reactions with great efficiency, stability and versatility makes these enzymes highly attractive from a commercial point of view.

Villeneuve [63] and others classified lipases specificities into three main groups; the 1st group is **substrate specificity** in which glycerol esters represent the natural substrates, the 2nd group is called **regioselective** and involves the subgroups *non-specific lipases* that hydrolyze the triacylglycerols into fatty acids and glycerol in a random way with production of mono- and diacylglycerols as intermediate products (**Figure 1**); *specific 1.3 lipases* which catalyze the hydrolysis at C₁ and C₃ glycerol bonds in triacylglycerols with liberating of fatty acids and unstable intermediates 2-monoacylglycerols and 1.2-or 2.3-diacylglycerols 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** could identify enantiomers in a racemic mixture. The enantio specificities of lipases depend on the type of substrate [64].

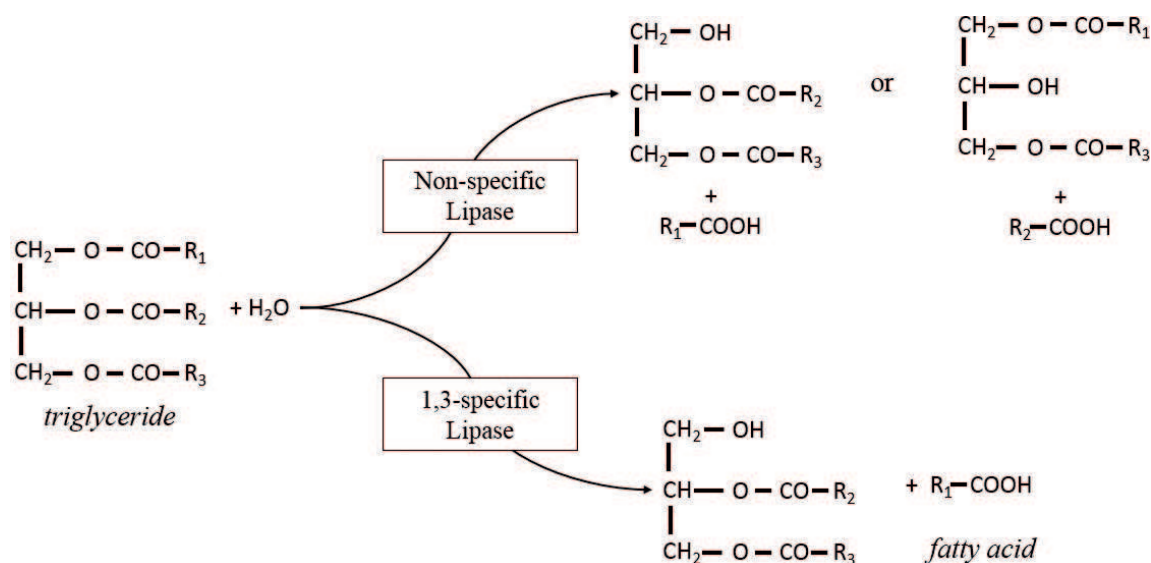


Figure 1. Regioselective: non-specific and 1,3 specific lipases catalyze the hydrolysis of triglycerides in different manners with the production of fatty acids.

The induction of lipase activity during germination might be dependent on factors from embryo [65]. Early study of Shoshi and Reeves [66] showed the presence of two lipases in the endosperm of Castor bean seed, acid lipase in dry seed and alkaline lipase during germination. On the other hand, storage tissues of all the oilseeds except Castor bean contained only lipase activity which increased during germination [67].

Because of sucrose is the substrate for lipid biosynthesis in developing seed and the end product of lipid degradation, it might be primarily considered as regulatory factor in studying the mechanisms of lipid metabolism [58, 68]. In addition, asparagine and nitrate are considered regulatory factors in lipid metabolism of lupine [69]. In lupin germinating seeds, the level of asparagine can reach 30% of dry matter, and it is a main transport form of nitrogen from source to sink tissues [70]. Borek et al. [71] reported that asparagine controls the metabolism of carbohydrate as it caused a significant decrease in soluble sugars and increase in starch in organs of germinating lupin seed. In contrast, nitrate is not a favorable source of nitrogen in protein metabolism in lupin seeds [72] and rather does not influence the carbohydrate metabolism [71]. Nitrate similarly as N sucrose, is regarded as a factor which can regulate plant metabolism by changes in the expression of some genes [73].

Storage lipid mobilization in germinating seeds begins with hydrolysis of triacylglycerols in oleosomes by lipases into free fatty acids and glycerol. Then fatty acids undergo β -oxidation in peroxisomes. Next, glyoxylate cycle will proceed partially in the peroxisome and partially in the cytoplasm. Three of the five enzymes of the glyoxylate cycle (citrate synthase, isocitrate lyase and malate synthase) are located in peroxisomes, while two other enzymes (aconitase and malate dehydrogenase) operate in the cytoplasm [74]. Succinate transported from peroxisome to mitochondria and here is converted to malate via the Krebs cycle. Malate in turn, after transport to the cytoplasm, is converted to oxaloacetate. Finally, gluconeogenesis and the synthesis of sugars are the processes which are a form of carbon transport especially in germinating seeds proceed [58, 75].

2.4. Hydrolysis of phytic acid during seed germination

The greatest storage form of total phosphorus (about 50–80%) is phytic acid ($C_6H_{18}O_{24}P_6$) and also known as inositol hexophosphate (IP6) in legumes and cereals seeds [76]. Phytic is regarded as antinutrient because it has the ability to form complexes with proteins and bind with cations (especially Fe, Ca, K, Mn, Mg, Zn) via ionic association to form a mixed salt called phytin or phytate with the reduction of their digestive availability [77]. On the other hand, phytate may play an important role as an antioxidant by forming iron complex that cause a decrease in free radical generation and the peroxidation of membranes, and may also act as an anticarcinogen, providing protection against colon cancer [78]. Because of it was regarded as antioxidant, anticarcinogen or vitamin like substance, it is essential to measure and manipulate phytate content in food grains such as beans [79, 80].

One of the major breeding objectives is the development of crop cultivars with low seed phytin content. It was found that the increase in *myo*-inositol and reduced amounts of *myo*-inositol phosphate intermediates in the seeds of maize mutants with a phenotype of reduced phytic acid had a little effect on plant growth and development [81]. These findings might suggest that a high level of stored phytate is not necessary for seed viability and germination or seedlings growth.

Phytin is mainly stored in protein bodies in seeds called globoids in the aleurone layer and scutellum cells of most grains. Phytic acid has a strong ability to chelate multivalent metal ions, specially zinc, calcium, iron and as with protein residue. Seed phytate content depend mainly on the environmental mainly plant phosphorus fertilization [82]. It has been shown the important genetic variability in the phytate content of beans and it appears to be a trait controlled by several genes [83]. Also, a correlation between phytate and protein contents was found [84], so the protein content of grains can be considered another factor that regulates phytate content.

Phytin in germinating seeds is hydrolyzed by an acid phosphatase enzyme called phytase [85], with releasing of phosphate, cations, and inositol which are utilized by the seedlings. It was found little changes in extractible P_i in hazel seeds during chilling accompanied with IP6 mobilization that might be suggested the rapid conversion of P_i into organic form [86]. These results were discussed as evidence of active metabolism in germinating seed [87]. In agreement, phytase is strongly and competitively inhibited by P_i , while the decrease in phytase activity coincided with maximal IP6 turnover [88]. It was found that about 87% of IP6 is digested during the first 6 days of germination [89]. In this respect, Ogawa et al. [90] postulated that the early axiferous IP6 digestion is essential for metabolic activity of the resting tissue via supplying P_i and minerals for physiological and metabolic requirements, for example, enzymes of starch metabolism. In addition, IP6 related compounds such as pyrophosphate-containing inositol phosphates (PP-IP) play a potential role in providing P_i for ATP synthesis during the early stages of germination before complete dependence on aerobic mitochondrial respiration the mainly source of ATP production [91].

In stressed seeds, many vital processes such as germination, growth, respiration and other related processes are affected which consequently can trigger other effects on metabolic activities particularly the enzymes of phosphate metabolism that play an important role in

germination and seed development [92]. Phosphate metabolism is one of negatively affected processes under different stressful conditions [93]. Under stressful conditions, the restriction of growth and phosphorus availability resulting in enhancement the activity of phosphatases to produce P_i by hydrolysis the insoluble phosphate form that modulate mechanism of free phosphate uptake. In agreement, Olmos and Hellin [94] reported that acid phosphatases activity increased to sustain P_i level which enables it to be co-transported with H^+ down a proton motive force gradient.

3. Effect of abiotic stress on metabolic activities during seed germination

Abiotic stresses including salt, drought, heavy metals, pollutants, heat, etc., potentially affect seed germination and seedling growth. Depending on the stress intensity and genetic background, germination is delayed or suppressed. Plants have developed unique strategies including a tight regulation of germination ensuring species survival [95]. It was well known that stress exposure would produce early signals such as change in intracellular Ca^{2+} , secondary signaling molecules such as inositol phosphate and ROS as well as activation of kinase cascades.

Seed imbibition triggers many biochemical and cellular processes associated with germination involve the reactivation of metabolism, the resumption of cellular respiration and the biogenesis of mitochondria, the translation and/or degradation of stored mRNAs, DNA repair, the transcription and translation of new mRNAs, and the onset of reserve mobilization [7, 96]. These processes are followed by ROS (mostly H_2O_2) accumulation as a result of a pronounced increase in the intracellular and extracellular production during early stages [97, 98].

ROS function as cellular messengers or toxic molecules on seed hydration [99]. ROS caused seed damage accompanied with a loss of seed vigor and as a repercussion of aging [100]. The highly activity of respiration during germination results in superoxide anion production during electron leakage from the mitochondrial electron transport chain followed by dismutation to H_2O_2 . Other sources of ROS are NADPH oxidases of the plasma membrane, extracellular peroxidases, β -oxidation pathway in glyoxysomes [97]. H_2O_2 is along-lived ROS that can diffuse easily through membranes and that can reach targets far from production sites, and is recognized as an important signaling molecule [101]. H_2O_2 is considered as strong oxidizing agent, it could interact with most biomolecules resulting in oxidative stress that causes cellular damage. It causes lipid peroxidation which in turn affects polyunsaturated fatty acids (PUFAs) found in membranes or reserve lipids. Also, H_2O_2 cause oxidation of nucleic acids (DNA, RNA) and proteins [97]. Induction of DNA oxidation by H_2O_2 resulted in the accumulation of 7, 8-dihydro-8-oxoguanine (8-oxo-dG), which has been shown to cause the accumulation of double-strand breaks in genome and deleterious effects on cell viability [102]. DNA oxidation by ROS is considered a main source of DNA damage during seed storage and germination.

Kong and Lin [103] have shown that mRNA is much more sensitive to oxidative damage than DNA, mainly due to its cellular localization, single stranded structure and lack of repair mechanisms. Guanine is the most frequently oxidized base in RNA leads to the accumulation of 8-hydroxyguanosine (8-OHG). Oxidative damage to mRNA results in the inhibition of protein synthesis and in protein degradation [104]. Oxidation of protein by ROS result in alteration of protein functions due to enzymatic and binding properties modifications [105]. H_2O_2 accumulation and associated oxidative damages together with a decline in antioxidant mechanisms can be regarded as a source of stress that may suppress germination. On the other hand, Barba-Espin et al. [106] reported that the selective oxidation of proteins and mRNAs can act as a positive regulator of seed germination.

Using of calcium sensors called Ca^{2+} binding proteins revealed an increase in intracellular calcium concentration under abiotic-stress conditions [107]. This is accompanied with enhancement of calcium-dependent protein kinases (CDPKs), calcium/calmodulin-dependent protein kinases (CCaMKs) or phosphatases which stimulate the phosphorylation/or dephosphorylation of specific transcription factors, resulting in an increase of stress-responsive genes expression [108]. However, activated Ca^{2+} sensors regulate stress-responsive genes either by binding to cis-elements in the promoters or by interacting with DNA-binding proteins of genes that led to gene activation or suppression.

Stressed-germinating wheat seeds develop a powerful regulator mechanism in response to stresses which is calreticulin-like protein (M16 and M13) and abundant Ca^{2+} -binding protein predominantly located in the endoplasmic reticulum (ER) of higher plants [109]. Its expression trend was mainly up-regulated, especially in the last period of germination which hints that wheat seed may encounter stress in late germination [110]. Another regulator mechanism with peptidyl-prolyl cis-trans isomerase activity which involved in signal transduction, cell apoptosis, and protein folding called cyclophilin (M51) was detected in stressed germinating wheat seeds [111]. Because of the cellular structure is not complete in early germination, M51 increased slowly in first three germination stages but increased sharply in the last stage [109].

One of the most effective factors on seed imbibition and germination is the temperature. It affects water uptake and reactivation of metabolic processes [7]. Many physiological, biochemical and molecular disturbance will occur with temperature deviation away from optimal to sustain cellular homeostasis [112].

4. The role of phytohormones during germination

Plants are characterized by producing various types of growth regulators that differed in their chemical structure and physiological action. They include auxins, cytokinins (CK), gibberellins (GA), abscisic acid (ABA), ethylene (ET), salicylic acid (SA), jasmonates (JA), brassinosteroids (BR) and strigolactones. Each of ABA, SA, JA and ET is found to play an essential role in mediating plant defense response against stresses [113]. During the early phase of seed germination, a decrease in JA and SA contents and an increased level of auxins were recorded

in *Arabidopsis* seeds [114]. Both JA and SA were shown to act as negative regulators of seed germination [115]. Auxins are considered to be regulators of the seed germination process in a crosstalk with GAs, ABA, and ET [116]. The brassinosteroids signal could stimulate germination by decreasing the sensitivity to ABA [117].

A variety of cellular processes in plants are under control of phytohormones which play key roles and coordinate various signal transduction pathways during abiotic-stress response [118]. Seed imbibitions resulted in an activation of GA biosynthesis and response pathways with the production of the bioactive GAs. Then, GAs stimulated the genes encoding for enzymes such as endo- β -1,3 glucanase [119], β -1,4 mannan endohydrolase [120] which hydrolyze the endosperm and alleviate the inhibitory effects of ABA on embryo growth potential [121]. These results are indicated the antagonistic relation between each of ABA and GA which interpret the presence of high GA and low ABA levels in seeds under favorable environmental conditions and a reverse ration under unfavorable conditions. Thus, the cross-talk relation between seed dormancy and germination is balanced by GA-ABA ration, a key mechanism for cope early abiotic-stress conditions.

ABA inhibits water uptake by preventing cell wall loosening of the embryo and thereby reduces embryo growth potential [122]. GAs are involved in direct enhancement the growth of the embryo during late phase [123]. GAs repressive the ABA effect during the early and the late phases of germination through stimulation of genes expression encoding cell wall loosening that result in remodeling enzymes such as α -expansins in early phase of germination. Light and cold act together to break dormancy of imbibed seeds and to promote seed germination by increasing GAs levels. A rapid decrease of ABA endogenous content during Phase II is one of many factors that influence the successful completion of germination [124]. Highly leakage of cellular solutes due to initial imbibition indicates cellular membranes damage caused by rehydration. In addition, drying and rapid seed dehydration processes influence DNA integrity [125]. Seeds have developed a number of repair mechanisms during seed germination, including the repair of membranes, as well as proteins and DNA [126].

Under stress conditions, phytohormones play a crucial role via responsive protein mediated stress. It was found C1-(cysteine rich protein family) domain containing proteins that play a part in plant hormone-mediated stress responses [127]. In addition, 72 responsive proteins mediated stress are identified in *Arabidopsis* that contained all three unique signature domains. Many hydrolytic enzymes biosynthesis and activity are influenced by GA₃ in wheat and barley. Catalase and ascorbate peroxidase activity showed a significant improvement in wheat SA- and GA-primed wheat seeds compared to the unprimed [128, 129].

5. Priming strategy to improve seed germination under stressful or non-stressful conditions

Under various conditions, the potential of seeds for rapid uniform emergence and development under various conditions is determined mostly by seed vigor trait [130]. Recent strategies for improvement seed quality involved classical genetic, molecular biology and

invigoration treatments known as priming treatments. Seed priming was aimed primarily to control seed hydration by lowering external water potential, or shortening the hydration period, because of most seeds are partially hydrated after priming process and reach a pre-germinate stage without radicle protrusion [131]. It was reported that primed seeds showed improved germination rate and uniformity under both optimal and adverse environments in wheat [132]. The cellular mechanism of priming as it relates to improved stress tolerance in germinating seeds is still required more study.

Currently seed priming techniques include osmopriming (soaking seeds in osmotic solutions as PEG or in salt solutions), hydropriming (soaking seeds in predetermined amounts of distilled water or limiting imbibition periods), and hormone priming (seed are treated with plant growth regulators) which are more commonly studied in laboratory conditions, and thermopriming (it is a physical treatment achieved by pre-sowing of seeds at different temperature that improve germination vigor under adverse environmental conditions) and matrix priming (mixing seeds with organic or inorganic solid materials and water in definite proportions and in some cases adding chemical or biological agents) [130, 133]. Hydropriming and osmopriming with large-sized priming molecules cannot permeate cell wall/membrane so water influx would be the only external factor affecting priming. The determination of suitable priming technique is dependent mainly on plant species, seed morphology and physiology. On the other hand, salts and hormone priming affect not only the seed hydration but also other germination-related processes due to absorption of exogenous ions/hormones, consequently confusing the effects of imbibition *versus* that of ions/hormones.

Improvement germination performance of primed seeds may be considered a result of advanced metabolism processes [134] including enhancement each of the efficiency of respiration [135] and antioxidant activity [136], initiation of repairing processes [137] and alteration phytohormonal balance [138]. Also, improvement of germination performance may be linked to higher expressions of gene sand proteins involved in water transport, cell wall modification, cytoskeletal organization, and cell division and increases in protein synthesis potential, post-translational processing capacity, and targeted proteolysis have been linked to the advanced germination of primed seeds [139].

Seed germination process is regulated by a network of transcription factors that have both confused and separate functions. In order to maintain or break the period of arrested germination and to complete germination under stress conditions, different metabolic pathways including phytohormones biosynthesis and signal transduction pathways, chromatin modifications, and microRNA post transcriptional regulation, are involved [140].

Many effects on metabolic processes, germination performance and seedling establishment due to seed priming with H_2O_2 were observed although seed soaking followed by dehydration have an important role in controlling gene expression and biosynthesis of proteins [141].

Seed priming with auxin, cytokinin, GA, and ethylene (ET) resulted in improvement of germination of pigeon pea seeds under both control and Cd-stress conditions [142]. ABA pretreated seeds showed a reduction in germination that may be attributed to metabolic deviation, limiting the available energy and changes in metabolomics or may be attributed to modulate the

endogenous ABA level [143]. On contrary, GA₃ seed treatment has not affect seed germination substantially. It is documented that GA₃ have a stimulatory effects on germination and associated enzymes [144]. Also, auxin namely IAA is documented to regulate seed dormancy and plant shade avoidance syndrome that adversely affects seedling development and crop yield [145]. Cytokinin pretreatment may act as auxins in promoting seed germination by antagonizing the inhibitory effect of ABA on germination process. However, it was found that cytokinin antagonize the inhibitory effect of ABA on post-germinating growth of *Arabidopsis* through the stimulation of ABI5 protein degradation [146].

Recently published data support the existence of interactions between ROS and phytohormone signaling networks that modulate gene expression and cellular redox status [147]. Interaction between phytohormones and H₂O₂ can be antagonistic or synergistic. Signaling processes trigger interactions are not developed only between particular phytohormones but also between phytohormones and other signaling molecules such as NO [148], H₂S [149], ·OH [150] and H₂O₂ [151], which is believed to play a central role in signaling processes during plant development and stress responses [152]. GA treatment enhanced ROS production namely superoxide and H₂O₂ in radish plants [153] and *Arabidopsis* [154]. On the other hand, exogenous application of H₂O₂ does not influence ABA biosynthesis and signaling but it has a more pronounced effect on GA signaling, resulting in the modulation of hormonal balance and in subsequent germination initiation [154]. It was showed that H₂O₂ diminished the inhibitory effects of ABA on endosperm damage. Müller et al. [155] showed that H₂O₂ abolishes inhibitory effects of ABA on endosperm rupture. As suggested previously by Lariguet et al. [154], H₂O₂ regulates the expression of gene encoding enzyme hydrolyzing the testa and endosperm, which facilitate *Arabidopsis* germination by releasing the embryo from the control of the seed envelope.

6. The respiratory reactivation during seed germination

The initial liberation of seed stored food at the beginning of germination is mainly by anaerobic respiration. Anaerobic respiration is catalyzed by the activity of enzymes which are not required aerobic conditions such as dehydrogenases [156]. Dehydrogenase facilitating the transport of electrons from substrates to oxygen through electron transport chain using nicotinamide adenine dinucleotide (NAD⁺), nicotinamide adenine dinucleotide phosphate (NADP⁺) or riboflavin as cofactor [157]. Activities of dehydrogenases have been shown to involve the activities of alcohol dehydrogenase, lactate dehydrogenase and succinate dehydrogenase [158] which mediated the conversion of storage lipid and carbohydrates through the anaerobic respiration. Succinate dehydrogenase, a complex enzyme tightly bound to the inner mitochondrial membrane oxidizes succinate to fumarate [159]. Lactate dehydrogenase catalyzes the reversible oxidation of lactate to pyruvate using NAD⁺ as a co-enzyme. Anaerobic respiration was recorded to take place during resting stages of seeds and the initial stages of seed germination [160]. It was showed that the reactivity of dehydrogenases covered the first 3 days of cowpea seed germinations [161].

The increase in respiratory rate in germinating seeds is associated with the increase in glycolytic activity. The intermediates of glycolysis are transferred to the OPPP pathway which feeds its products

back into glycolysis, so the activity of this pathway is also important in determining the flux through glycolysis [162]. During germination, seeds use sugars and other molecules as a substrate for respiration. α -amylase and β -amylase are involved in degradation of endosperm starch. Starch hydrolysis into glucose is catalyzed by action of α - and β -amylases, debranching enzyme and α -glucosidases (maltase) [163]. So, importance of amylases is related to their ability to provide growing embryo with respiratory substrates for producing energy and carbon source until the established seedling can photosynthesize. In addition, embryo growth from quiescent stage to active phase depending mainly on the utilization of stored ATP and storage lipid breakdown products [164].

Seed germination represents a good period for mitochondria development study. Results obtained from previous transcriptome studies recorded a substantial increase in mitochondrial transcripts encoding proteins and protein content accompanied with changes in their functions during early 3 h of seed imbibitions [165]. During the first 48 h of seed imbibitions, 56 differentially expressed proteins were detected which include the outer membrane channel TOM40 and the inner membrane TIM17/22/23 families, compared to dry seed.

The interpretation of suggestion that import pathway capacity is absolutely dependent on the presence of oxygen (aerobic respiration) is related to the significant decrease in capacity of the general import pathway in mitochondria under anaerobic conditions, compared to under aerobic conditions. In supporting for this suggestion, three proteins from the TIM17/22/23 family were found to be 6–14 folds up-regulated under anaerobic conditions [166] and a decline in proteins involving import apparatus was detected in the mature mitochondria that might be suggested that the accumulation of these import proteins in the dry seed could operate functions after 2 h imbibition, and then serve as donors of TCA cycle and electron transport chain components [167].

7. The role of glyoxylate cycle in oilseed germination

Glyoxylate cycle has been known to play a crucial role in lipid degradation in oilseeds, whereas stored lipid is converted into glucose the main respiratory substrate during germination and hence seedling establishment [168]. Seed imbibition triggers highly increase in oxygen consumption which reflects the enhancement of oxidation of produced carbohydrates from the glyoxylate cycle [169]. Alongside to glyoxylate cycle, the OPPP operates where a number of enzymes and intermediates participate the two pathways [170]. It functions to provide the cell with NADPH for biosynthetic reactions and appears to be important in the regulation of germination [171].

The action of the two glyoxylate cycle enzymes isocitrate lyase (ICL) and malate synthase (MS) that by pass the decarboxylation steps of the TCA cycle are essential in oilseed germination. Whereas, two moles of acetyl-CoA are introduced with each turn of the cycle, resulting in the synthesis of one mole of the four-carbon compound succinate that are transported from the glyoxysome into the mitochondrion and converted into malate via TCA cycle. This malate is then exported to cytosol in exchange for succinate and is converted to oxalacetate. PEP-CK catalyzes the conversion of oxaloacetate to phosphoenolpyruvate and this fuels the synthesis of soluble carbohydrates necessary to germination [169].

8. Conclusion

Under stressful conditions, oxidative damage to mRNA results in the inhibition of protein synthesis and in protein degradation which caused disturbance in protein functions due to enzymatic and binding properties modification. Consequently; seed germination may delay or suppress. The priming techniques improve stress acclimation mechanisms during germination but the cellular mechanism of priming is still requires more studying. In response to abiotic stresses, activity of acid phosphatases increased to match a definite level of inorganic phosphate which can be co-transported with H^+ down proton motive force gradient. The signaling interactions among multiple phytohormones are rather common in controlling various growth and developmental processes. Hormonal signaling coordination may be regulated through controlling biosynthesis of certain phytohormone, by modifying the available pool of hormone molecules or by elaborate regulation of the signaling process. However; seed pre-treatment with each of GAs, auxins or cytokinin promote seed germination not only through stimulation of hydrolyzing enzymes but also by antagonizing the inhibitory effect of ABA on germination process. Phytohormone signal crosstalk will present valuable new avenues for genetic improvement of crop plants needed to meet the future food production targets in the face of global climate change. Surprising; seed priming with H_2O_2 resulted in improvement germination process and seedling establishment. This may be resulted from its effect on GA signaling and modulation of hormonal balance that promote initiation of seed germination. In addition; H_2O_2 diminished the inhibitory effects of ABA on endosperm damage through expression of gene encoding enzyme hydrolyzing the testa and endosperm with the releasing of embryo.

Authors details

Awatif S. Ali^{1*} and Alaaeldin A. Elozeiri²

Address all correspondence to: awatifali95@yahoo.com

¹ Botany Department, Faculty of Science, Kafr Elsheikh University, Kafr Elsheikh, Egypt

² Environmental Engineering Department, Zewail City of Science and Technology, Cairo, Egypt

References

- [1] Hasanuzzaman M, Nahar K, Alam MM, Roychowdhury R, Fujita M. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *International Journal of Molecular Sciences*. 2013;**14**:9643-9684
- [2] Borgheretti F, Ferreira AG. *Germinação do básico ao aplicado*. Porto Alegre: Artmed; 2000. 222 p

- [3] Zienkiewicz Z, Zienkiewicz AK, Rejon JD, Alche JD, Castro AJ, Rodringuez-Garcia MI. Olive seed protein bodies store degrading enzymes involved in mobilization of oil bodies. *Journal of Experimental Botany*. 2014; **65**:103-115. DOI: 10.1093/jxb/ert355
- [4] Gonçalves JFC, et al. Aspectos fisiológicos bioquímicos de plantas da Amazônia. Projeto Jacaranda Fase II: Pesquisas Florestais na Amazônia Central. Manaus: INPA; 2003. p. 89-101
- [5] Bewley JD, Black M. *Physiology of Development and Germination*. 2nd ed. New York: Plenum Press; 1994 445 p
- [6] Bewley JD. Seed germination and dormancy. *The Plant Cell*. 1997;**9**:1055-1066
- [7] Bewley JD, Bradford K, Hilhorst H, Nonogaki H. *Seeds: Physiology of Development, Germination and Dormancy*. 3rd ed. New York: Springer; 2013
- [8] Jacobsen JV, Chandler PM. Gibberellin and abscisic acid in germinating cereals. In: Davies PJ, editor. *Plant Hormones: Physiology, Biochemistry and Molecular Biology*. Boston, MA: Kluwer; 1995. p. 164-193
- [9] Bethke PC, Schuurink R, Jones RL. Hormonal signaling in cereal aleurone. *Journal of Experimental Botany*. 1997;**48**:1337-1356. DOI: 10.1093/jxb/48.7.1337
- [10] Lee SJ, Kang JY, Park HJ, Kim MD, Bae MS, Choi HI, et al. DREB2C interacts with ABF2, a bZIP protein regulating abscisic acid-responsive gene expression, and its overexpression affects abscisic acid sensitivity. *Plant Physiology*. 2010;**153**(2):716-727
- [11] Firenzuoli AM, Vanni P, Ramponi G, Baccari V. Changes in enzyme levels during germination of seeds of *Triticum durum*. *Plant Physiology*. 1968;**43**:260-264. DOI: 10.1104/pp.43.2.260
- [12] Carter LM, Chesson JH. Two USDA researchers develop a moisture seeking attachment for crop seeders that is designed to help grower's plant seed in soil sufficiently moist for germination. *Seed World*. 1996;**134**:14-15
- [13] Munns R. Comparative physiology of salt and water stress. *Plant, Cell & Environment*. 2002;**25**:239-250
- [14] Singh J, Sastry EVD, Singh V. Effect of salinity on tomato (*Lycopersicon esculentum* mill.) during seed germination stage. *Physiology and Molecular Biology of Plants*. 2012;**18**:45-50
- [15] Kaur S, Gupta AK, Kaur N. Effect of GA₃, kinetin and indole acetic acid on carbohydrate metabolism in chickpea seedlings germinating under water stress. *Plant Growth Regulation*. 2000;**30**:61-70
- [16] Zeid IM, Shedeed ZA. Response of alfalfa to putrescine treatment under drought stress. *Biologia Plantarum*. 2006;**50**:635-640
- [17] Jacobsen JV, Hanson AD, Chandlor PC. Water stress enhances expression of α -amylase gene in barley leaves. *Plant Physiology*. 1986;**80**:350-359
- [18] Todaka D, Matsushima H, Morhashi Y. Water stress enhances α -amylase activity in cucumber cotyledons. *Journal of Experimental Botany*. 2000;**51**:739-745

- [19] Velasco R, Salamini F, Bartels D. Dehydration and ABA increase mRNA levels and enzyme activity of cytosolic GAPDH in the resurrection plant *Craterostigma plantagineum*. *Plant Molecular Biology*. 1994;**26**:541-546
- [20] Pandey R, Agarwal RM, Jeevaratnam K, Sharma GL. Osmotic stress-induced alterations in rice (*Oryza sativa* L.) and recovery on stress release. *Plant Growth Regulation*. 2004;**42**:79-87
- [21] Baatour O, Kaddour R, Wannes WA, Lachaâl M, Marzouk B. Salt effects on the growth, mineral nutrition, essential oil yield and composition of marjoram (*Origanum majorana*). *Acta Physiologiae Plantarum*. 2010;**32**:45-51
- [22] Job C, Rajjou L, Lovigny Y, Belghazi M, Job D. Patterns of protein oxidation in *Arabidopsis* seeds and during germination. *Plant Physiology*. 2005;**138**:790-802. DOI: 10.1104/pp.105.062778
- [23] Garg N, Manchanda G. ROS generation in plants: Boon or bane? *Plant Biosystems*. 2009;**143**:81-96
- [24] Yacoubi R, Job C, Belghazi M, Job D. Proteomic analysis of the enhancement of seed vigour in osmoprimed alfalfa seeds germinated under salinity stress. *Seed Science Research*. 2013;**23**(2):99-110
- [25] Yin H, Chen Q, Yi M. Effects of short-term heat stress on oxidative damage and responses of antioxidant system in *Lilium longiflorum*. *Plant Growth Regulators*. 2008;**54**:45-54
- [26] Zhang Q, Li JJ, Zhang WJ, Yan SN, Wang R, Zhao JF, Li YJ, Qi ZG, Sun ZX, Zhu ZG. The putative auxin efflux carrier OsPIN3t is involved in the drought stress response and drought tolerance. *The Plant Journal*. 2012;**72**:805-816
- [27] Das AB. Bio prospecting and genetic engineering of mangrove genes to enhance salinity tolerance in crop plants. In: Jain SM, Gupta SD, editors. *Biotechnology of Neglected and Underutilized Crops*. New York: Springer; 2013. p. 385-456
- [28] Fahad S, Nie L, Chen Y, Wu C, Xiong D, Saud S, Hongyan L, Cui K, Huang L. Crop plant hormones and environmental stress. *Sustain Agriculture Reviews*. 2015;**15**:371-400
- [29] Koornneef M, Bentsink L, Hilhorst H. Seed dormancy and germination. *Current Opinion in Plant Biology*. 2002;**5**:33-36
- [30] Ansari O, Choghazardi HR, Sharif Zadeh F, Nazarli H. Seed reserve utilization and seedling growth of treated seeds of mountain ray (*Secale montanum*) as affected by drought stress. *Cercetări Agronomice în Moldova*. 2012;**2**(150):43-48
- [31] Patade VY, Maya K, Zakwan A. Seed priming mediated germination improvement and tolerance to subsequent exposure to cold and salt stress in capsicum. *Research Journal of Seed Science*. 2011;**4**(3):125-136
- [32] Mayer AM, Shain Y. Control of seed germination. *Annual Review of Plant Physiology*. 1974;**25**:167-193
- [33] Bewley JD, Black M. *Seeds: Physiology of Development and Germination*. New York: Plenum Press; 1985

- [34] Mayer AM, Poljakoff-Mayber A. The Germination of Seeds. 4th ed. Oxford: Pergamon Press; 1989
- [35] Salisbury FB, Ross CW. Plant Physiology. 4th ed. Belmont, California, USA: Wadsworth Publishing Company; 1991
- [36] Shutov AD, Vaintraub IA. Degradation of storage proteins in germinating seeds. *Phytochemistry*. 1987;**26**:1557-1566
- [37] Gepstein S, Han I. Evidence for the involvement of cytokinin in the regulation of proteolytic activity in cotyledons of germinating beans. *Plant and Cell Physiology*. 1980;**21**(1):57-63
- [38] Shivaraj B, Pattabiraman TN. Natural plant enzyme inhibitors, part VIII. *Indian Journal of Biochemistry & Biophysics*. 1980;**17**:181-185
- [39] Veerabhadrappe PS, Manjunath NH, Virupaksha TK. Proteinase inhibitors of finger millet (*Eleusine coracana* Gaertn.). *Journal of the Science of Food and Agriculture*. 1978;**29**(4):353-358
- [40] Tully RE, Beevers H. Protease and peptidases of castor bean endosperm. Enzyme characterization and changes during germination. *Plant Physiology*. 1978;**62**:726-750
- [41] Schlereth A, Becker C, Horstmann C, Tiedemann J, Müntz K. Comparison of globulin mobilization and cysteine proteinases in embryonic axes and cotyledons during germination and seedling growth of vetch (*Vicia sativa* L.). *Journal of Experimental Botany*. 2000;**51**:1423-1433
- [42] Yano H, Wong JH, Lee YM, Cho MJ, Buchanan BB. A strategy for the identification of proteins targeted by thioredoxin. *Proceedings of the National Academy of Sciences USA*. 2001;**98**:4794-4799. DOI: 10.1073/pnas.071041998
- [43] Yano H, Masaharu KM. Disulfide proteome yields a detailed understanding of redox regulations: A model study of thioredoxin-linked reactions in seed germination. *Proteomics*. 2006;**6**:294-300. DOI: 10.1002/pmic.200402033
- [44] Dunaevsky YE, Belozersky MA. Proteolysis of the main storage protein of buckwheat seeds at the early stage of germination. *Physiologia Plantarum*. 1989;**75**:424-428
- [45] Belozersky MA, Dunaevsky YE, Voskoboynikova NE. Isolation and properties of a metalloproteinase from buckwheat seeds. *The Biochemical Journal*. 1990;**272**:677-682
- [46] Elpidina EN, Dunaevsky YE, Belozersky MA. Protein bodies from buckwheat seed cotyledons: Isolation and characteristics. *Journal of Experimental Botany*. 1990;**41**:969-977
- [47] Dunaevsky YE, Belozersky MA. The role of cysteine proteinase and carboxypeptidase in the breakdown of storage proteins in buckwheat seeds. *Planta*. 1989;**179**:316-322
- [48] Paleg LG. Physiological effects of gibberellic acid II. On starch hydrolysis enzymes of barley endosperm. *Plant Physiology*. 1960;**35**:902-906
- [49] Chrispeels MJ, Varner JE. Gibberellic acid enhanced synthesis and release of α -amylase and ribonuclease by isolated barley aleurone layers. *Plant Physiology*. 1967;**42**:398-406

- [50] Jones RL, Jacobsen JV. Regulation of synthesis and transport of secreted proteins in cereal aleurone. *International Review of Cytology*. 1991;**126**:49-88
- [51] Filner P, Varner JE. A simple and unequivocal test for *de novo* synthesis of enzymes: Density labeling of barley α -amylase with H_2O^{18} . *Proceedings of the National Academy of Sciences USA*. 1967;**58**:1520-1526
- [52] Parys E, Romanowska E, Poskuta J. Amylase activities in attached and excised cotyledons and in embryonic axes of *Pisum sativum* L. *Plant and Cell Physiology*. 1983;**21**:181-188
- [53] Wang Y, Wang DM, Liang HG. Effect on plumular axis on the amylase activity in cotyledons of germinating pea seeds. *Acta Phytophysiology Sinica*. 1988;**14**(3):244-249
- [54] Kępczyński J. Ethylene-dependent action of gibberellins in seed germination of *Amaranthus caudatus*. *Physiologia Plantarum*. 1986;**67**:584-587
- [55] Wilson AM. Amylase synthesis and stability in crested wheatgrass seeds at low water potentials. *Plant Physiology*. 1971;**48**:541-546
- [56] Hellyer SA, Chandler IC, Bosley JA. Can the fatty acid selectivity of plant lipases be predicted from the composition of the seed triglyceride? *Biochimica Biophysica Acta*. 1999;**1440**(2-3):215
- [57] Paques FW, Macedo GA. Lipases de Látex Vegetais: Propriedades e Aplicações Industriais: A Review. *Química Nova*. 2006;**29**(1):93
- [58] Quettier AL, Eastmond PJ. Storage oil hydrolysis during early seedling growth. *Plant Physiology and Biochemistry*. 2009;**47**:485
- [59] Hutton D, Stumpf PK. Fat metabolism in higher plant. Characterisation of β -oxidation system from maturing and germinating castor bean seeds. *Plant Physiology*. 1969;**44**:508-516
- [60] Pereira EP, Zanin GM, Castro HF. Immobilization and catalytic properties of lipase on chitosan for hydrolysis and etherification reactions. *Brazilian Journal of Chemical Engineering*. 2003;**20**(4):343
- [61] Leal MCM, Cammarota MC, Freire DMG, Sant'Anna JGL. Hydrolytic enzymes as coadjuvants in the anaerobic treatment of dairy waste waters. *Brazilian Journal of Chemical Engineering*. 2002;**19**(2):175
- [62] Freire GDM, Castilho FL. Lipases em Biocatálise. In: Bon et al. (org), editor. *Enzimas em biotecnologia: Produção, Aplicação e Mercado*. Rio de Janeiro: Interciência; 2008
- [63] Villeneuve P. Plant lipases and their applications in oils and fats modification. *European Journal of Lipid Science and Technology*. 2003;**105**(6):308
- [64] Castro HF, Anderson WA. Fine chemicals by biotransformation using lipases. *Química Nova*. 1995;**18**(6):544-554
- [65] Tavener RJA, Laidman DL. The induction of triglyceride metabolism in the germinating wheat grain. *Phytochemistry*. 1972;**11**:981-987

- [66] Shoshii M, Reeves H. Lipase activity in castor bean endosperm during germination. *Plant Physiology*. 1974;**54**:23-28
- [67] Anthony HC, Huang AHC, Moreau RA. Lipases in the storage tissues of peanut and other oil seeds during germination. *Planta*. 1978;**141**:111-116
- [68] Baud S, Dubreucq B, Miquel M, Rochat C, Lepiniec L. Storage reserve accumulation in *Arabidopsis*: Metabolic and developmental control of seed filling. *Arabidopsis Book* American Society of Plant Biology. 2008;**6**: e0113. DOI: 10.1199/tab.0113
- [69] Ratajczak W. Asparagine metabolism in developing seeds of *Lupinus luteus* L. *Biochemie und Physiologie der Pflanzen*. 1986;**181**:17-22
- [70] Lehmann T, Ratajczak L. The pivotal role of glutamate dehydrogenase (GDH) in the mobilization of N and C from storage material to asparagine in germinating seeds of yellow lupine. *Journal of Plant Physiology*. 2008;**165**:149-158. DOI: 10.1016/j.jplph.2006.12.010
- [71] Borek S, Galor A, Paluch E. Asparagine enhances starch accumulation in developing and germinating lupin seeds. *Journal of Plant Growth Regulation*. 2013;**32**:471-482
- [72] Ratajczak W, Gwóźdz' EA, Miądowicz M. Effects of nitrogen nutrition on storage protein composition yellow lupin cotyledons cultured in vitro. *Acta Physiologiae Plantarum*. 1996;**18**:295-304
- [73] Vidal EA, Gutiérrez RA. A systems view of nitrogen nutrient and metabolite responses in *Arabidopsis*. *Current Opinion in Plant Biology*. 2008;**11**:521-529
- [74] Pracharoenwattana I, Smith SM. When is a peroxisome not a peroxisome? *Trends in Plant Science*. 2008;**13**:522-525
- [75] Borek S, Ratajczak L. Storage lipids as a source of carbon skeletons for asparagine synthesis in germinating seeds of yellow lupine (*Lupinus luteus* L.). *Journal of Plant Physiology*. 2010;**167**:717-724
- [76] Jacela JY, DeRouehey Tokach MD, Goodband RD, Nelssen JL, Renter D, Dritz SS. Feed additives for swine: Fact sheets-prebiotics and probiotics and phytochemicals. *Journal of Swine Health and Production*. 2010;**18**:87-91
- [77] Lott JNA, Greenwood JS, Batten GD. Mechanisms and regulation of mineral nutrient storage during seed development. In: Kigel J, Galili G, editors. *Seed Development and Germination*. New York: Marcel Dekker Inc; 1995. p. 215-235
- [78] Thompson LU, Zhang L. Phytic acid and minerals: Effect on early markers of risk for mammary and colon carcinogenesis. *Carcinogenesis*. 1991;**12**:2041-2045
- [79] Coelho SM, Taylor AR, Ryan KP, Sousa-Pinto I, Brown MT, Brownlee C. Spatiotemporal patterning of reactive oxygen production and Ca^{2+} wave propagation in *Fucus rhizoid* cells. *The Plant Cell*. 2002;**14**:2369-2381
- [80] Okazaki Y, Katayama T. Reassessment of the nutritional function of phytic acid, with special reference to myo-inositol function. *Journal of Japan Society of Nutrition and Food Sciences*. 2005;**58**:151-156

- [81] Shi H, Bressan R, Hasegawa PM, Zhu J-K. In: Broadlay M, White P, editors. Sodium in Plant Nutritional Genomics. London: Blackwell Publishing; 2005. p. 127-149
- [82] Buerkert A, Haake C, Ruckwied M, Marschner H. Phosphorus application affects the nutritional quality of millet grain in the sahel. *Field Crops Research*. 1998;**57**:223-235
- [83] Santos JCP. Estado nutricional do feijoeiro (*Phaseolus vulgaris* L.) e teores de nutrientes e fitatos nos grãos. Piracicaba: Universidade de São Paulo. Tese de doutorado; 1998
- [84] Raboy V, Noaman MM, Taylor GA, Pickett SG. Grain phytic acid and protein are highly correlated in winter wheat. *Crop Science*. 1991;**31**:631-635
- [85] Hubel F, Beck E. Maize root phytase. Purification, characterization, and localization of enzyme activity and its putative substrate. *Plant Physiology*. 1996;**112**:1429-1436
- [86] Mukherji S, Dey B, Paul AK, Sircar SM. Changes in phosphorus fractions and phytase activity of rice seeds during germination. *Physiologia Plantarum*. 1971;**25**:94-97
- [87] Silva LG, Trugo LC. Characterization of phytase activity in lupin seed. *Journal of Food Biochemistry*. 1996;**20**:329-340
- [88] Andriotis VME, Ross JD. Isolation and characterization of phytase from dormant *Corylus avellana* seeds. *Phytochemistry*. 2003;**64**:689-699
- [89] Azarkovich MI, Dmitrieva MI, Sobolev AM. Mobilization of protein and phytin in aleurone grains of germinating castor beans. *Russian Journal of Plant Physiology*. 1999;**46**:349-356
- [90] Ogawa M, Tanaka K, Kasai Z. Accumulation of phosphorus, magnesium, and potassium in developing rice grains: Followed by electron microprobe X-ray analysis focusing on the aleurone layer. *Plant and Cell Physiology*. 1979;**20**:19-27
- [91] Raboy V. Myo-Inositol-1,2,3,4,5,6-hexakisphosphate. *Phytochemistry*. 2003;**64**:1033-1043
- [92] Fincher GB. Molecular and cellular biology associated with endosperm mobilization in germinating cereal grains. *Annual Review of Plant Physiology*. 1989;**40**:305-346
- [93] Mihoub A, Chaoui A, El-Ferjani E. Biochemical changes associated with cadmium and copper stress in germinating pea seeds (*Pisum sativum* L.). *Comptes Rendus Biologies*. 2005;**328**:33-41
- [94] Olmos E, Hellin E. Cytochemical localization of ATPase plasma membrane and acid phosphatase by cerium based in a salt-adapted cell line of *Pisum sativum*. *Journal of Experimental Botany*. 1997;**48**:1529-1535
- [95] Bai B, Sikron S, Gendler T, Kazachkova Y, Barak S, Grafi G, Khozin-Goldberg I, Fait A. Ecotypic variability in the metabolic response of seeds to diurnal hydration-dehydration cycles and its relationship to seed vigor. *Plant and Cell Physiology*. 2011;**53**(1):38-52
- [96] Nonogaki H, Bassel GW, Bewley JD. Germination–stillamystery. *Plant Science*. 2010; **179**:574-581. DOI: 10.4161/psb.25504
- [97] El-Maarouf-Bouteau H, Bailly C. Oxidative signaling in seed germination and dormancy. *Plant Signaling & Behavior*. 2008;**3**:175-182. DOI: 10.4161/psb.3.3.5539

- [98] Kubala S, Wojtyła Ł, Quinet M, Lechowska K, Lutts S, Garnczarska M. Enhanced expression of the proline synthesis gene P5CSA in relations to seed osmopriming improvement of *Brassica napus* germination under salinity stress. *Journal of Plant Physiology*. 2015;**183**:1-12. DOI: 10.1016/j.jplph.2015.04.009
- [99] Bailly C, El-Maarouf-Bouteau H, Corbineau F. From intra cellular signaling networks to cell death: The dual role of reactive oxygen species in seed physiology. *Comptes Rendus Biologies*. 2008;**331**:806-814. DOI: 10.1016/j.crv.2008.07.022
- [100] Kumar SPJ, Prasad SR, Banerjee R, Thammineni C. Seed birth to death: Dual functions of reactive oxygen species in seed physiology. *Annals of Botany*. 2015;**116**:663-668. DOI: 10.1093/aob/mcv098
- [101] Møller IM, Jensen PE, Hansson A. Oxidative modifications to cellular components in plants. *Annual Review of Plant Biology*. 2007;**58**:459-481. DOI: 10.1146/annurev.arplant.58.032806.103946
- [102] Pommier Y, Redon C, Rao VA, Seiler JA, Sordet O, Takemura H, et al. Repair of and checkpoint response to topoisomerase I-mediated DNA damage. *Mutation Research*. 2003;**532**:173-203. DOI: 10.1016/j.mrfmmm.08.016
- [103] Kong QM, Lin CG. Oxidative damage to RNA: Mechanisms, consequences and diseases. *Cellular and Molecular Life Sciences*. 2010;**67**:1817-1829. DOI: 10.1007/s00018-010-0277-y
- [104] Chmielowska-Bkak J, Izbiańska K, Deckert J. Products of lipid, protein and mRNA oxidation as signals and regulators of gene expression. *Frontiers in Plant Science*. 2015;**6**:405. DOI: 10.3389/fpls.2015.00405
- [105] Davies MJ. The oxidative environment and protein damage. *Biochimica et Biophysica Acta*. 2005;**1703**:93-109. DOI: 10.1016/j.bbapap.2004.08.007
- [106] Barba-Espin G et al. Role of thioproline on seed germination: Interaction ROS-ABA and effects on antioxidative metabolism. *Plant Physiology and Biochemistry*. 2011;**59**:30-36. DOI: 10.1016/j.plaphy.2011.12.002.3
- [107] Kudla J, Batistic O, Hashimoto K. Calcium signals: The lead currency of plant information processing. *The Plant Cell*. 2010;**22**:541-563
- [108] Reddy AS, Ali GS, Celesnik H, Day IS. Coping with stresses: Roles of calcium- and calcium/calmodulin-regulated gene expression. *The Plant Cell*. 2011;**23**(6):2010-2032
- [109] Finnie C, Melchior S, Roepstorff P, Svensson B. Proteome analysis of grain filling and seed maturation in barley. *Plant Physiology*. 2002;**129**:1308-1319
- [110] Komatsu S, Yang G. Over-expression of calcium-dependent protein kinase 13 and calreticulin interacting protein 1 confers cold tolerance on rice plants. *Molecular Genetics and Genomics*. 2007;**277**:713-723
- [111] Galat A. Variations of sequences and amino acid composition of proteins that sustain their biological function: An analysis of the cyclophilin family of proteins. *Archives of Biochemistry and Biophysics*. 1999;**371**(2):149-162

- [112] Fitter AH, Hay RKM. Environmental Physiology of Plants. New York: Academic Press; 1981
- [113] Wani SH, Kumar V, Shriram V, Kumar S. Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. *The Crop Journal*. 2016;**4**:162-176
- [114] Preston J, Tatematsu K, Kanno Y, Hobo T, Kimura M, Jikumaru Y, et al. Temporal expression patterns of hormone metabolism genes during imbibition of *Arabidopsis thaliana* seeds: A comparative study on dormant and non dormant accessions. *Plant and Cell Physiology*. 2009;**50**:1786-1800
- [115] Dave A, ML H'n, He Z, Andriotis VM, Vaistij FE, Larson TR, et al. 12-Oxo-phytodienoic acid accumulation during seed development represses seed germination in *Arabidopsis*. *The Plant Cell*. 2011;**23**:583-599
- [116] Carrera E, Holman T, Medhurst A, Dietrich D, Footitt S, Theodoulou FL, et al. Seed after-ripening is a discrete developmental pathway associated with specific gene networks in *Arabidopsis*. *The Plant Journal*. 2008;**53**:214-224
- [117] Steber CM, McCourt P. A role for brassinosteroids in germination in *Arabidopsis*. *Plant Physiology*. 2001;**125**:763-769
- [118] Vob U, Bishopp A, Farcot A, Bennett MJ. Modelling hormonal response and development. *Trends in Plant Science*. 2014;**19**:311-319
- [119] Leubner-Metzger G, Fründt C, Vögeli-Lange R, Meins FJ. β -1, 3-glucanases in the endosperm of tobacco during germination. *Plant Physiology*. 1995;**109**:751-759
- [120] Nonogaki H, Gee OH, Bradford KJ. A germination-specific endo- β -mannanase gene is expressed in the micropylar endosperm cap of tomato seeds. *Plant Physiology*. 2000;**123**:1235-1245
- [121] Koornneef M, Karssen CM. Seed dormancy and germination. In: *Arabidopsis Book*/Cold Spring Harbor Monograph Archive. 1994;**27**:313-334
- [122] Schopfer P, Plachy C. Control of seed germination by abscisic acid. *Plant Physiology*. 1985;**77**(3):676-686
- [123] Debeaujon I, Koornneef M. Gibberellin requirement for *Arabidopsis* seed germination is determined both by testa characteristics and embryonic abscisic acid. *Plant Physiology*. 2000;**122**:415-424
- [124] Weitbrecht K, Müller K, Leubner-Metzger G. First off the mark: Early seed germination. *Journal of Experimental Botany*. 2011;**62**:3289-3309
- [125] Matilla A, Gallardo M, Puga-Hermida MI. Structural, physiological and molecular aspects of heterogeneity in seeds: A review. *Seed Science Research*. 2005;**15**:63-76
- [126] Oge L, Bourdais G, Bove J, Collet B, Godin B, Granier F, et al. Protein repair-L isoaspartyl methyltransferase 1 is involved in both seed longevity and germination vigor in *Arabidopsis*. *The Plant Cell*. 2008;**20**:3022-3037

- [127] Bhaskar RV, Mohanty B, Verma V, Wijaya E, Kumar PP. A hormone-responsive C1-domain-containing protein At5g17960 mediates stress response in *Arabidopsis thaliana*. PLoS. 2015;**10**(1):115-118. DOI: 10.1371/journal.pone.0115418
- [128] Hammerton RW, Ho T-HD. Hormonal regulation of the development of protease and carboxypeptidase activities in barley aleurone layers. Plant Physiology. 1986;**80**:692-697
- [129] Stuart IM, Loi L, Fincher GB. Development of (1-3, 1-4)- α -D-glucan endohydrolase iso-enzymes in isolated scutella and aleurone layers of barley (*Hordeum vulgare*). Plant Physiology. 1986;**80**:310-314
- [130] Paparella S, Araújo SS, Rossi G, Wijayasinghe M, Carbonera D, Balestrazzi A. Seed priming: State of the art and new perspectives. Plant Cell Reports. 2015;**34**:1281-1293. DOI: 10.1007/s00299-015-1784-y
- [131] Hilhorst HWM, Finch-Savage WE, Buitink J, Bolingue W, Leubner-Metzger G. Dormancy in plant seeds. In: Lubzens E, Cerdà J, Clarck M, editors. Dormancy and Resistance in Harsh Environments. Heidelberg: Springer-Verlag; 2010. p. 43-67
- [132] Zhuo J, Wang W, Lu Y, Sen W, Wang X. Osmopriming-regulated changes of plasma membrane composition and function were inhibited by phenylarsine oxide in soybean seeds. Journal of Integrative Plant Biology. 2009;**9**:858-867
- [133] Jisha KC, Vijayakumari KJT, Puthur JT. Seed priming for abiotic stress tolerance: An overview. Acta Physiologiae Plantarum. 2013;**3**:1381-1396. DOI: 10.1007/s11738-012-1186-5
- [134] Soeda Y, Konings MCJM, Vorst O, van Houwelingen AMML, Stoop GM, Maliepaard CA, Koddle J, Bino RJ, Groot SPC, van der Geest AHM. Gene expression programs during *Brassica oleracea* seed maturation, osmopriming, and germination are indicators of progression of the germination process and the stress tolerance level. Plant Physiology 2005;**137**:354-368
- [135] Sun J, Hutchins DA, Feng Y, Seubert EL, Caron DA, Fu F-X. Effects of changing $p\text{CO}_2$ and phosphate availability on domoic acid production and physiology of the marine harmful bloom diatom *Pseudo-nitzschia multiseries*. Limnology and Oceanography. 2011;**56**:829-840. DOI: 10.4319/lo.2011.56.3.0829
- [136] Chen K, Arora R. Dynamics of the antioxidant system during seed osmopriming, post-priming germination, and seedling establishment in spinach (*Spinacia oleracea*). Plant Science. 2011;**180**:212-220. DOI: 10.1016/j.plantsci.2010.08.007
- [137] Balestrazzi A, Macovei C, Carbonera D. Seed imbibition in *Medicago truncatula* Gaertn. Expression profiles of DNA repair genes in relation to PEG-mediated stress. Journal of Plant Physiology. 2011;**168**:706-713. DOI: 10.1016/j.jplph.2010.10.008
- [138] El-Araby MM, Moustafa SMA, Ismail AI, Hegazi AZA. Hormone and phenol levels during germination and osmopriming of tomato seeds, and associated variations in protein patterns and anatomical seed features. Acta Agronomica Hungarica. 2006;**54**:441-458. DOI: 10.1556/AAgr.54.2006.4.7

- [139] Kubala S, Garnczarska M, Wojtyła Ł, Clippe A, Kosmala A, Zmiénko A, et al. Deciphering priming-induced improvement of rape seed (*Brassica napus* L.) germination through an integrated transcriptomic and proteomic approach. *Plant Science*. 2015;**231**:94-113. DOI: 10.1016/j.plantsci.2014.11.008
- [140] Hilhorst HWM. Definition and hypotheses of seed dormancy. In: Bradford KJ, Nonogaki H, editors. *Seed Development, Dormancy and Germination*. Annual Plant Reviews. Vol. 27, Chap 4. Sheffield, UK: Blackwell Publishing; 2007. p. 50-71
- [141] Sneideris LC, Gavassi MA, Campos ML, D'Amico-Damião V, Carvalho RF. Effects of hormonal priming on seed germination of pigeon pea under cadmium stress. *Anais da Academia Brasileira de Ciências*. 2015;**87**:1847-1852. DOI: 10.1590/0001-3765201520140332
- [142] Sneideris AC, Gavassi MA, Campiao VDA, Carvalho RF. Effects of hormonal priming on seed germination of pigeon pea under cadmium stress. *Annals of the Brazilian Academy of Sciences*. 2015;**87**(3):1847-1852. DOI: 10.1590/0001-3765201520140332
- [143] Fincher GB. Molecular and cellular biology associated with endosperm mobilization in germinating cereal grains. *Annual Review of Plant Physiology and Plant Molecular Biology*. 1989;**40**:305-346
- [144] Liu W-Z, Kong D-D, Gu X-X, Gao HB, Wang J-Z, Xia M, et al. Cytokinins can act as suppressors of nitric oxide in *Arabidopsis*. *Proceedings of the National Academy of Sciences USA*. 2013;**110**:1548-1553. DOI: 10.1073/pnas.1213235110
- [145] Procko C, Crenshaw CM, Ljung K, Noel JP, Chory J. Cotyledon generated auxin is required for shade-induced hypocotyl growth in *Brassica rapa*. *Plant Physiology*. 2014;**165**:1285-1301. DOI: 10.1104/pp.114.241844
- [146] Guan C, Wang X, Feng J, Hong S, Liang Y, Ren B, et al. Cytokinin antagonizes abscisic acid-mediated inhibition of cotyledon greening by promoting the degradation of abscisic acid insensitive 5 protein in *Arabidopsis*. *Plant Physiology*. 2014;**164**:1515-1526. DOI: 10.1104/pp.113.234740
- [147] Xia X-J, Zhou Y-H, Shi K, Zhou J, Foyer CH, Hu J-Q. Inter play between reactive oxygen species and hormones in the control of plant development and stress tolerance. *Journal of Experimental Botany*. 2015;**66**:2839-2856. DOI: 10.1093/jxb/erv089
- [148] Sanz L, Albertos P, Mateos I, Sánchez-Vicente I, Lechón T, Fernández Marcos M, Lorenzo O. Nitric oxide (NO) and phytohormones crosstalk during early plant development. *Journal of Experimental Botany*. 2015;**66**:2857-2868
- [149] Jin Z, Pei Y. Physiological implications of hydrogen sulfide in plants: Pleasant exploration behind its unpleasant odour. *Oxidative Medicine and Cellular Longevity*. 2015;**397502**. DOI: 10.1155/2015/397502
- [150] Richards SL, Wilkins KA, Swarbreck SM, Anderson AA, Habib N, Smith AG, et al. The hydroxyl radical in plants: From seed to seed. *Journal of Experimental Botany*. 2015;**66**:37-46. DOI: 10.1093/jxb/eru 398

- [151] Diaz-Vivancos P, Barba-Espín G, Hernández JA. Elucidating hormonal/ROS networks during seed germination: Insights and perspectives. *Plant Cell Reports*. 2013;**32**:1491-1502. DOI: 10.1007/s00299-013-1473-7
- [152] Petrov VD, Van Breusegem F. Hydrogen peroxide—a central hub for information flow in plant cells. *AoB Plants*. 2012;**2012**:pls014. DOI: 10.1093/aobpla/pls014
- [153] Schopfer P, Plachy C, Frahry G. Release of reactive oxygen intermediates (superoxide radicals, hydrogen peroxide, and hydroxyl radicals) and peroxidase in germinating radish seeds controlled by light, gibberellin, and abscisic acid. *Plant Physiology*. 2001;**125**:1591-1602. DOI: 10.1104/pp.125.4.1591
- [154] Lariguet P, Ranocha P, DeMeyer M, Barbier O, Penel C, Dunand C. Identification of a hydrogen peroxide signaling pathway in the control of light-dependent germination in *Arabidopsis*. *Planta*. 2013;**238**:381-395. DOI: 10.1007/s00425-013-1901-5
- [155] Müller K, Hess B, Leubner-Metzger G. A role for reactive oxygen species in endosperm weakening. In: Adkins S, Shmore SA, Navie S, editors. *Seeds: Biology, Development and Ecology*. Wallingford: CAB International; 2007. p. 287-295
- [156] Turner JF, Turner DH. The regulation of carbohydrate metabolism. *Annual Review of Plant Physiology*. 1975;**26**:159-186
- [157] Robert KM, David AB, Katheleen MB, Peter JK, Victor WR, Weil P. A Harper's illustrated biochemistry. *Biologic Oxidation*. 2009;**12**:99-100
- [158] Oaikhena EE, Ajibade GA, Appah J, Bello M. Dehydrogenase enzyme activities in germinating cowpea (*Vigna unguiculata* (L) Walp). *Journal of Biology, Agriculture and Healthcare*. 2013;**3**:32-36
- [159] Devlin T. Text book of biochemistry with clinical correlations. *Bioenergetics mitochondria and oxidative. Metabolism*. 2011;**14**:554-555
- [160] Jones JD, Burneth P, Zollman P. The glyoxylate cycle does it function in the dormant or active seed. *Comparative Biochemistry and Physiology Part B: Biochemistrty and Molecular Biology*. 1999;**124**(2):177-179
- [161] Ebukanson GJ, Bassey ME. *About Seed Plants*. Kaduna: Baraka Press and Publishers Ltd; 1992. p. 36-39
- [162] Podestá FE, Plaxton WC. Regulation of cytosolic carbon metabolism in germinating *Ricinus communis* cotyledons. II. Properties of phosphoenolpyruvate carboxylase and cytosolic pyruvate kinase associated with the regulation of glycolysis and nitrogen assimilation. *Planta*. 1994;**194**:381-387
- [163] Andriotis VME, Saalbach G, Waugh R, Field RA, Smith AM. Maltase involved in starch metabolism in barley endosperm is encoded by a single gene. *PLoS One*. 2016;**11**(3):e0151642. DOI: 10.1371/journal.pone.0151642
- [164] Nandi S, Das G, Sen-Mendi S. β -amylase activity as an index for germination potential in rice. *Annals of Botany*. 1995;**75**:463-467

- [165] Howell KA, Narsair R, Carroll A, Ivanova A, Lohse M, Usadel B, et al. Mapping metabolic and transcript temporal switches during germination in rice high- lights specific transcription factors and the role of RNA in stability in the germination process. *Plant Physiology*. 2009;**149**:961-980. DOI: 10.1104/pp.108.129874
- [166] Howell KA, Cheng K, Murcha MW, Jenkin LE, Millar AH, Whelan J. Oxygen initiation of respiration and mitochondrial biogenesis in rice. *The Journal of Biological Chemistry*. 2007;**282**:15619-15619
- [167] Howell KA, Millar AH, Whelan J. Ordered assembly of mitochondria during rice germination begins with promitochondrial structures rich in component of the protein import apparatus. *Plant Molecular Biology*. 2006;**60**:201-223
- [168] Eastmond PJ, Germain V, Lange PR, Bryce JH, Smith SM, Graham IA. Post germinative growth and lipid catabolism in oilseeds lacking the glyoxylate cycle. *Proceedings of the National Academy of Sciences of the United States of America*. 2000;**97**:5669-5674
- [169] Muscolo A, Sidari M, Mallamaci C, Attinà E. Changes in germination and glyoxylate and respiratory enzymes of *Pinus pinea* seeds under various abiotic stresses. *Journal of Plant Interactions*. 2007;**2**(4):273-279. DOI: 10.1080/17429140701713795
- [170] ap Rees T. Integration of pathways of synthesis and degradation of hexose phosphates. In: Preiss J, editor. *The Biochemistry of Plants*. London: Academic Press. 1980; pp. 1-42.
- [171] Perino C, Come D. Physiological and metabolic study of the germination phases in apple embryo. *Seed Science and Technology*. 1991;**19**:1-14