Chapter 8

Metabolic Processes During Seed Germination

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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), dienelactone 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 l3S 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 C_1 and C_3 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].

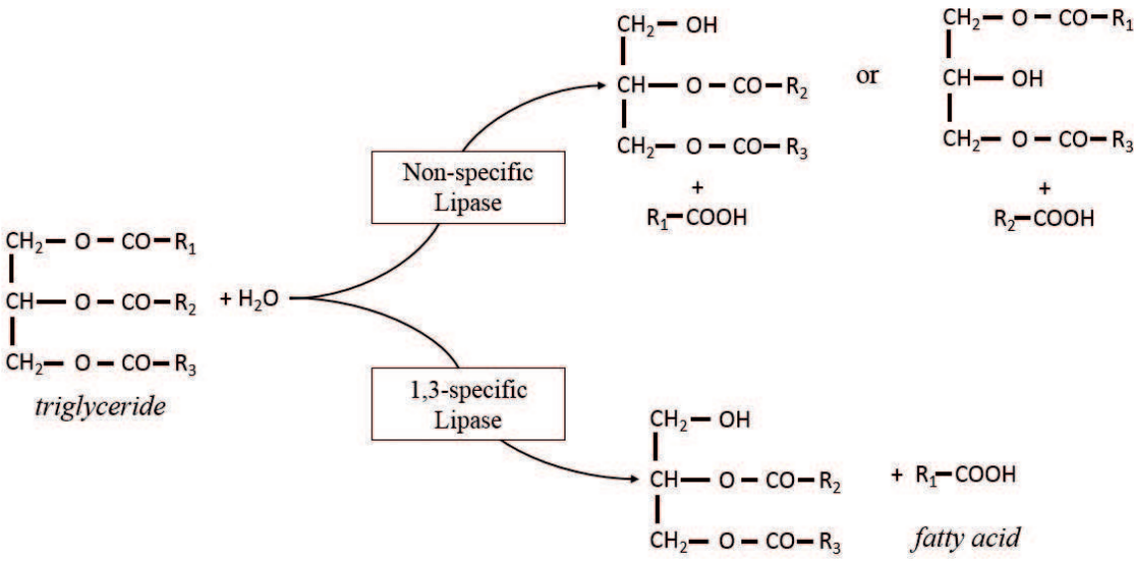

Figure 1. Regioselective: non-specific and 1,3 specific lipases catalyze the hydrolysis of triglycerides in different manners with the production of fatty acids.

The induction of lipase activity during germination might be dependent on factors from embryo [65]. Early study of Shoshi and Reevers [66] showed the presence of two lipases in the endosperm of Castor been 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 crosstalk 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 GA_3 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 matric 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, GA_3 seed treatment has not affect seed germination substantially. It is documented that GA_3 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 H_2O_2 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], H_2S [149], ·OH [150] and H_2O_2 [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 H_2O_2 in radish plants [153] and *Arabidopsis* [154]. On the other hand, exogenous application of H_2O_2 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 H_2O_2 diminished the inhibitory effects of ABA on endosperm damage. Müller et al. [155] showed that H_2O_2 abolishes inhibitory effects of ABA on endosperm rupture. As suggested previously by Lariguet et al. [154], H_2O_2 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 dehyrogenase, 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 pretreatment 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.

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