Course : BB 404

Seed Gemination

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. To sustain a good seedling development seed stores a food reserve mainly as proteins, lipids and carbohydrates. Protein and oil bodies are the major reserve in oilseed which represent a source for each of energy, carbon, and nitrogen during seedling establishment.Enzymatic hydrolysis of protein, lipid and carbohydrate, and transportation of metabolites is dependent mainly on water availability.

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.

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

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. Germination is considered a response includes bidirectional interactions between the embryo and endosperm since the endosperm can secrete signals to control embryo growth.

Phytohormones have essential role in inducing plant acclimatization to change in environmental conditions by mediating growth, development, source/sink transitions, and nutrient allocation. Phytohormones are considered the most important endogenous substances for modulating physiological and molecular responses. 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. 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. Wheat seeds were priming to increase germination characteristics and stress tolerance. As seeds imbibe water, metabolic processes initiate with an increase in respiration rate. Early developmental stages of seedling require fueling energy before it becomes autotrophic. 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%).

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. 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.

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. Hydrolysis of stored proteins produced free amino acids, which support protein synthesis in endosperm and embryo and so proceeding of germination process. A disulfide proteome technique was developed 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

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.

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 has been investigated during seed germination where it is maximum value. 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.

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. 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.

Effect of abiotic stress on metabolic activities during seed germination

Seed germination is particularly vulnerable to environmental stress encountered conditions, specifically salt and water which are widespread problem around the world. 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. Water and salt stress conditions affect seed germination with reducing germination rate and delay in the initiation of germination.

Under water stress, enzymes activity such as α-amylase in *Cicer arietinum* cotyledons or α- and β-amylase in *Medicago sativa* germinating seeds were **reduced**. In contrast, water stress conditions led to an **increase** in the activity of α-amylase in *Hordeum vulgare* seedlings, β-amylase in *Cucumis sativus* cotyledons, cytosolic glyceraldehyde-3-phosphate dehydrogenase in *Craterostigma plantagineum* plants and protease in *Oryza sativa* seedlings.

Salt stress causes ion toxicity, osmotic stress and reactive oxygen species (ROS) stress. ROS reacts with cell macromolecules and lipids, and disrupt diverse physiological and biochemical processes, such as hormonal imbalance and reduced use of reserves. Plants develop ROS-scavenging mechanisms include enzymatic and non-enzymatic antioxidant systems that protect plants against oxidative damage. Therefore, improvement the activity of antioxidant enzymes in plants organs is necessary for increasing plant's salt tolerance. 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.

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.

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. Both JA and SA were shown to act as negative regulators of seed germination. Auxins are considered to be regulators of the seed germination process in a crosstalk with GAs, ABA, and ET. The brassinosteroids signal could stimulate germination by decreasing the sensitivity to ABA. 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. 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, β-1,4 mannan endohydrolase which hydrolyze the endosperm and alleviate the inhibitory effects of ABA on embryo growth potential. 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 favourable environmental conditions and a reverse ration under unfavourable 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. A rapid decrease of ABA endogenous content during Phase II is one of many factors that influence the successful completion of germination

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. 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.

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).