International Journal for Multidisciplinary Research (IJFMR)

E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

A Review on Gibberellic Acid (GA) Mediated Regulation of Storage Mobilization in Cotyledons During Seed Germination

Lily Pal

Department of Botany, Durgapur Women's College, Durgapur, Paschim Bardhaman, 713209, West Bengal, India,

Abstract

Seed germination in higher plants is a tightly regulated developmental process that depends on the efficient mobilization of stored reserves to support early seedling growth. In dicotyledonous seeds, cotyledons function as the primary storage organs, accumulating proteins, carbohydrates, and lipids during seed maturation. Gibberellic acid (GA), a diterpenoid plant hormone, plays a central regulatory role in initiating and coordinating the mobilization of these reserves. Although GA-mediated reserve mobilization has been extensively characterized in cereal endosperm, its regulatory mechanisms in dicot cotyledons remain comparatively less understood. During germination, GA synthesized in the embryo acts as a key signaling molecule that stimulates the production and activation of hydrolytic enzymes responsible for degrading storage proteins, starch, and triacylglycerols. Protein mobilization involves the conversion of protein storage vacuoles into lytic compartments and the coordinated synthesis and trafficking of proteases. Carbohydrate degradation is mediated by amylases, phosphorylases, and sucrose-metabolizing enzymes, while lipid reserves are mobilized through lipase activity, β-oxidation, and the glyoxylate cycle. At the molecular level, GA perception via the GID1–DELLA signaling module activates GAMYB-dependent transcription of hydrolytic genes, integrating Ca^{2+} signaling and reactive oxygen species (ROS) pathways. Reserve mobilization in dicot seeds is further influenced by source–sink interactions between the embryonic axis and cotyledons. This review highlights the biochemical and physiological mechanisms by which GA facilitates the mobilization of storage reserves such as starch, proteins, and lipids in cotyledonary tissues. Emphasis is placed on the hormonal regulation of hydrolytic enzymes, particularly α-amylase and proteases, and the signal transduction pathways activated during germination.

Keywords: Gibberellic acid (GA), α-amylase, storage mobilization, cotyledon, aleurone layer

Introduction:

Seed germination is a critical phase in the life cycle of higher plants. For dicotyledonous seeds, cotyledons serve as primary storage organs, containing reserves required for the growth of the embryonic axis. Mobilization of these reserves is hormonally regulated, with gibberellic acid (GA) playing a central role. While GA's role in cereal endosperm has been extensively studied, its function in cotyledon-mediated storage mobilization in dicots warrants focused attention.

Overview of Gibberellic Acid (GA):

Gibberellins (GAs), diterpenoid carboxylic acids, are a class of plant hormone occurring in microbes to higher plants, acting as endogenous growth regulators and involved in various growth processes including seed germination, stem elongation, and flowering. The most bioactive form of GAs (e.g. GA_1, GA_4) play important roles in different physiological processes including breaking of seed dormancy, seed germination, stem elongation, induction of flowering and delay of senescence (Hedden and Thomas, 2012). GAs are usually available in very low amount (generally pg/g FW) in most of the plant tissues. However, reproductive organs like seeds and flowers have three orders of magnitude higher levels (ng/g FW) than what is present in the vegetative tissues like stems, roots and leaves (pg/g FW) (Tarkowská et al., 2014). As specific responses, GAs induce altered gene expression during enhanced stem elongation (Chory et al., 1987) and mobilization associated with seed germination (Baulcombe and Buffard, 1983). However, such a role of GAs in inducing expression of senescence-associated gene is not yet established (Okello et al., 2015).

During germination, GA is synthesized de novo in the embryo and acts as a signaling molecule to initiate the mobilization of stored food materials by upregulating hydrolytic enzymes. Practically, embryo is heterotrophic at seed germination stage, being dependent on nutritional support for maintaining growth unless the seedling becomes photosynthetically self-sufficient. Such support, in case of endospermic seeds, comes from the endosperm containing carbohydrate (mainly starch), as well as protein, lipid and minerals sufficient for supplying substrates to the growing seedling for several days (Ritchie et al., 2000). In general, GAs that come from the growing embryo regulate the process of storage mobilization in the endosperm of cereals (Kirmizi and Güleryüz, 2006). However, the role of GAs as regard to protein mobilization may depend on the species and cultivars. Further, in case of dicot seeds, a direct role of GAs in the regulation of proteinase expression during mobilization of reserves has not been confirmed (Taneyama et al., 2001).

In limited cases GAs have been found to retard leaf senescence as was reported in *Taraxacum officinale* (Fletcher and Osborne, 1965). Retardation of leaf senescence by GAs was marked by maintenance of chlorophyll and RNA levels along with blockade of further degradation. Interestingly, in case of juvenile ivy leaves senescence is delayed by GA (Horton and Bourguoin, 1992) while GA application can bring back the juvenility in case of adult ivy plants (Frydman and Wareing, 1974). GAs are well known for promotion of stem and leaf growth (Schippers et al., 2007) and are transported acropetally from root to shoot as reported in *Arabidopsis* (Saibo et al., 2003) and pea (Smith, 1993). GAs are reported to use both xylem and phloem as its transport tissues (Metzger and Zeevaart, 1980; Oden et al., 1995). Treatment with uniconazole, an inhibitor of GA biosynthesis, was shown to retard embryonic axis elongation by affecting specifically the cell elongation instead of cell division. Stem elongation induced by GAs mainly involve cell elongation (Hays et al., 2002). Paclobutrazol, another inhibitor of GA biosynthesis, was found to retard both the cell elongation and cell division in stems of safflower (Potter et al., 1993), while cell elongation only but not the cell division was affected by paclobutrazol in case of wheat leaves (Tonkison et al., 1995). Thus, triazole inhibitors appear to have differential responses perhaps depending on the species or organ (Hays et al., 2002).

Cotyledon Structure and Storage Components:

Cotyledons represent the initial leaves, also known as seed leaves, that emerge during the germination process of a seed. They constitute a part of the seed's embryo and are essential for providing nourishment to the developing seedling, either through food storage or facilitating nutrient absorption. Cotyledons function as storage organs, particularly in dicot seeds, where they reserve nutrients in various forms: carbohydrates (present in dicots like beans, lentils, and peas), proteins (found in legumes such as soybeans), and lipids (fats and oils) (notably in oil-rich seeds like sunflower, castor, and peanut). In non-endospermic seeds, such as beans, cotyledons retain all the essential nutrients required for germination. Conversely, in monocots like maize, the cotyledon, referred to as a scutellum, does not serve as a food storage organ but instead absorbs nutrients from the endosperm and transports them to the embryo. In endospermic seeds, also known as albuminous seeds, the endosperm acts as the primary storage tissue that supports the developing embryo, with cotyledons playing secondary roles (examples include maize, wheat, and barley).

GA-Induced Mobilization Mechanisms:

In addition to serving as mechanisms for dispersal and propagation, seeds represent the primary source of food for human consumption. As a significant source of protein, seeds exhibit a diverse range of protein content (10-40%), with cereals at the lower end and pulses at the higher end. The specific protein storage within seeds varies by plant species, and upon hydrolysis, it yields soluble amino acids essential for seed germination and the growth of seedlings (Shewry et al., 1995). Moreover, plants can be categorized according to their establishment strategies, which are based on the relative significance of storage mobilization and the photosynthetic roles of cotyledons during seedling development (Lovell & Moore, 1970; Ampofo et al., 1976; Ibarra-Manriquez et al., 2001; Kitajima, 2003). For instance, in seeds that exhibit hypogeal germination, cotyledons primarily fulfill a storage function, whereas in seeds that undergo epigeal germination, cotyledons not only contribute to storage mobilization but also play a crucial role in photosynthesis by adopting a leafy structure (Zhang et al., 2018).

The processes of seed germination and the mobilization of storage reserves are regulated by both developmental and environmental factors, such as light and temperature. Seeds store compounds including carbohydrates, proteins, and lipids, which are broken down into simpler soluble products, such as sucrose and amino acids, that are then transported to the developing seedling during the post-germinative phase. During seed development and maturation, storage compounds, especially proteins, are accumulated within the cells of the seed's storage organs; upon germination, these proteins are hydrolyzed and mobilized to provide the necessary energy and building blocks for seed germination and the initial growth of the seedling (Bewley and Black, 1994). In monocot species, these reserve proteins are stored in the endosperm, serving as a substrate source to facilitate embryonic growth (de Barros and Larkins, 1990; Koehler and Ho, 1990a; Washio and Ishikawa, 1992; Wrobel and Jones, 1992). Additionally, the proteolytic enzymes responsible for protein hydrolysis are primarily produced in the aleurone layer of grains, where their synthesis is up-regulated by gibberellins (GA) and down-regulated by abscisic acid (ABA) (Rogers et al., 1985; Koehler and Ho, 1990b; Watanabe et al., 1991; Cejudo et al., 1992; Marttila et al., 1993).

The cotyledons of dicotyledonous species exhibit a unique developmental process that enables them to store reserve nutrients within their cells. These cells shift from operating as vegetative structures to evolving into senescent organs, progressively exhausting their reserves throughout the processes of seed germination and seedling development. In contrast to monocot seeds, the majority of dicotyledonous legume seeds are non-endospermic, with cotyledons serving as storage organs that contain storage proteins. These proteins are hydrolyzed by proteinases, and the resulting soluble products, such as amino acids or amines, are transported to the embryonic axis to provide substrates for seedling growth (Bewley and Black, 1994; Vitale and Bollini, 1995). Typically, in these storage cotyledons, protein reserves are organized into protein bodies, which are spherical organelles ranging from 2 to 10 µm in diameter and are surrounded by a single membrane, found in specialized tissues. In addition to reserve proteins, other compounds such as phytin, lectins, and enzymes like acid hydrolases are also present. These protein bodies are formed and developed within the cotyledons of legume seeds during the maturation process (Van der Wilden et al., 1980). The protein levels in cotyledons start to diminish concurrently with the rise in proteolytic activity during germination, as noted in lima bean (Heywood and Gainer, 1974), horse gram (Karunagaran and Ramakrishna Rao, 1990; Rajeswari and Ramakrishna Rao, 2002), lupine (Nandi et al., 1995), *Phaseolus vulgaris* (Senyuk et al., 1998), *Vigna mungo* (Taneyama et al., 1996), and *Vicia sativa* (Misra and Kar, 1990). Newly formed storage proteins are directed to a specialized compartment known as the protein storage vacuole (PSV), which contains α- and δ-tonoplast intrinsic proteins (TIP) in their tonoplasts (Jiang et al., 2000). Various mechanisms for protein mobilization in germinating seeds have been proposed: (i) despite the arrival of storage proteins and proteases at the same PSVs during seed development, proteolysis does not occur due to the presence of certain protease repressing factors; the removal of these factors during seed germination triggers subsequent protein degradation within PSVs (Muntz et al., 2001), (ii) proteases in lytic compartments are physically separated from storage proteins in the PSV (Jiang et al., 2001), and protein breakdown occurs when proteases are released into the matrix to hydrolyze the storage proteins during seed germination, and (iii) proteins within the PSVs are degraded by specific proteases that are newly synthesized and transported to PSVs during germination (Wang et al., 2007). Thus, in the case of most dicotyledonous seeds, proteases (endopeptidases) are synthesized in the endoplasmic reticulum (ER) and stored in vesicles through the ER lumen. These vesicles are then transported to the protein bodies, where proteolytic enzymes are released into the protein bodies after the fusion of these vesicles, allowing them to interact with the storage proteins (Bewley, 2001). As protein degradation continues, large vacuoles are formed by the fusion of empty protein bodies that accumulate various hydrolases, thereby transforming these vacuoles into autophagic vesicles that are active in the complete degeneration of cotyledons during their senescence (Her-man et al., 1981).

In the cotyledons of *Vigna mungo* seeds, KDEL-tailed vesicles (KV) that contain sulfhydryl-endopeptidase (SH-EP), a type of cysteine protease, are newly synthesized and packaged as the proenzyme proSH-EP. These vesicles are then transported and fused with the protein storage vacuole (PSV) for the purpose of protein hydrolysis (Toyooka et al., 2000). During the process of seed germination, it has been established that physiologically, PSVs, which are acidic compartments containing active proteases, transform into lytic vacuoles (Bewley and Black, 1994). A specific protein family known as vacuolar sorting receptors (VSRs) is responsible for mediating the transport of soluble proteins to these lytic vacuoles (Neuhaus and Rogers, 1998; Neuhaus and Paris, 2005). Furthermore, the cotyledons of germinating seeds from both leguminous and non-leguminous species develop various types of proteases, including acid, neutral, and alkaline proteases (Misra and Kar, 1990; Shastry and John, 1991). The regulatory mechanisms governing storage mobilization in dicot seeds have been the subject of study, leading to the proposal of two hypotheses regarding the role of the axis in this process. According to one hypothesis, the growing axis acts as a sink that draws the breakdown products of storage, which could otherwise inhibit the synthesis of enzymes and/or their activities. The other hypothesis suggests that plant hormones produced by the growing axis stimulate the synthesis of hydrolytic enzymes that act on the storage reserves within the cotyledons (Davies and Slack 1981; Bewley and Black 1994; Nandi et al., 1995). Taking these hypotheses into account, it appears that both source-sink dynamics and hormonal stimuli may exert regulatory influences on storage mobilization and seedling growth, processes that seem to be synchronized. Nevertheless, despite these propositions, the precise role or involvement of the embryonic axis in regulating storage mobilization in dicotyledonous seeds remains to be thoroughly investigated for a clearer understanding (Ramakrishna and Rao, 2005).

In general, legume seeds contain starch, sugars (including monosaccharides, disaccharides, and oligosaccharides), other polysaccharides found in cell walls, and proteins. In the case of mung bean seeds, starch constitutes the primary storage carbohydrate, accounting for 45%, while sucrose and other carbohydrates are present in lower concentrations (Hedley, 2001). These storage compounds serve as substrates for energy through hydrolysis, which is essential for seed germination and the subsequent growth of seedlings. A cytoplasmic enzyme has been identified as responsible for the breakdown of starch in the cotyledons of mung beans (Harris, 1976). Furthermore, various other enzymes that degrade starch have been detected, including starch phosphorylase, beta amylase, enzymes that degrade sucrose, invertase, and sucrose synthase, along with several glycolytic enzymes such as fructose bisphosphate aldolase, enolase, and glyceraldehyde 3-phosphate dehydrogenase. During the initial phase of germination, starch hydrolysis is predominantly facilitated by beta amylase, which is present in high concentrations at this stage, while starch phosphorylase takes over in the later stages. The degradation of starch mediated by both amylase and phosphorylase has also been observed in germinating pea seeds (Juliano and Varner, 1969). The breakdown of sucrose, a product of starch catalyzed by starch phosphorylase, is subsequently converted into glucose and fructose through the action of invertase. However, sucrose synthase may also play a role in both the degradation and synthesis of sucrose, which is then transported to the growing axis via the phloem (Geigenberger and Stitt, 1991). In certain instances, such as the germination of lima beans, both pathways for sucrose degradation are active (Xu et al., 1989), whereas in the case of mung beans, invertase is responsible for catalyzing sucrose degradation. The reducing sugars that result from the breakdown of starch, facilitated by the actions of amylase and phosphorylase, are metabolized through glycolysis during the germination of mung bean seeds. Conversely, the storage proteins found within the protein bodies are broken down by both the hydrolytic enzymes that are stored and those synthesized anew in the cotyledons of mung beans (Ghosh and Pal, 2012).

In the context of oilseeds, triacylglycerol (TAG) serves as the primary storage form of lipids (Bewley and Black, 1994). Lipases cleave TAG to release fatty acids, which are subsequently further degraded via β-oxidation and the glyoxylate cycle (Kornberg and Beevers, 1957; Beevers, 1961). The expression levels of the genes encoding the pertinent enzymes exhibit a stage-dependent variation, peaking during imbibition and then gradually declining (Comai et al., 1989; Graham et al., 1990; Eastmond and Graham, 2000; Eastmond et al., 2000; Germain et al., 2001). The metabolism of TAG takes place in three distinct organelles within the oil-storing cells: the TAG-storing oil body, where the hydrolysis of TAG into free fatty acids (FFAs) and glycerol begins; the glyoxysome, where the oxidation of FFAs occurs, followed by the synthesis of succinate through the glyoxylate cycle; and the mitochondrion, where succinate is ultimately converted into malate or oxaloacetate for the production of sucrose, with the latter two compounds being further processed in the cytosol (Bewley, 2001).

In contrast to endosperm cells, cotyledonary cells remain viable while executing the systematic process of storage mobilization throughout seed germination. Notably, the depletion of storage begins in the cotyledon cells that are furthest from the vascular bundle (VB) and advances towards the VB, a pattern observed in the cotyledons of kidney beans and mung beans, contingent upon the rate of germination. Conversely, in soybean and fava bean, the degradation of storage commences in the cotyledon cells closest to the VB, subsequently progressing towards the outer cotyledon cells (Bewley and Black, 1978).

Integration of ROS with calcium (Ca^{2+})

It has been observed in barley aleurone protoplasts that the influx of Ca^{2+} is triggered by GA before the synthesis and secretion of α-amylase, with cytosolic Ca^{2+} levels being higher at the edges of aleurone cells (Gilroy and Jones, 1992). Moreover, the signaling induced by GA relies on localized fluctuations in cytosolic Ca^{2+} (Hooley, 1994), which may be involved in signaling through the formation of a Ca^{2+}/calmodulin (or calmodulin-like protein) complex. While Ca^{2+} is crucial in the α-amylase mediated degradation of storage compounds, further experimental evidence is required to clarify its role in senescence, especially in relation to cotyledon senescence.

Enzymatic Activation:

The synthesis of hydrolytic enzymes by GA involves the degradation of DELLA repressors, the activation of GAMYB transcription factors, and the Ca^{2+}-dependent secretion of enzymes like α-amylase into the endosperm. Gibberellic Acid (GA) is perceived by the GID1 receptor in the aleurone layer. The GA-GID1 complex binds to DELLA proteins (growth repressors), targeting them for polyubiquitination and subsequent degradation by the 26S proteasome (Ueguchi-Tanaka et al., 2005; Nakajima et al., 2006; Griffiths et al., 2007). The removal of DELLA proteins releases the inhibition of transcription factors, specifically GAMYB. This factor binds to the Gibberellin Response Element (GARE) located in the promoter regions of hydrolytic genes, initiating the transcription of mRNA for α-amylase, proteases, and β-glucanases. The mRNA is translated on the Rough Endoplasmic Reticulum (RER). High levels of cytosolic calcium ($[Ca^{2+}]_{cyt}$) are essential at this stage; Ca^{2+} acts as a signaling messenger to sustain transcription and as a structural cofactor required for the proper folding and stability of the α-amylase protein. The synthesized enzymes are transported through the Golgi apparatus and secreted into the starchy endosperm. This process is synchronized with Reactive Oxygen Species (ROS) production, which triggers programmed cell death (PCD) in aleurone cells, ensuring the total release of enzymes to mobilize nutrient reserves for the embryo. The production of hydrolytic enzymes by Gibberellic Acid (GA) entails the breakdown of DELLA repressors, the activation of GAMYB transcription factors, and the Ca^{2+}-dependent release of enzymes such as α-amylase into the endosperm (Hartweck and Olszewski, 2006).

Conclusion:

Seed germination in dicotyledonous plants is a highly coordinated physiological and biochemical process in which cotyledons function as central storage and regulatory organs. Although the role of gibberellic acid (GA) has been extensively characterized in cereal endosperm, accumulating evidence highlights its significant yet still incompletely understood contribution to storage mobilization in dicot cotyledons. GA synthesized in the embryo acts as a pivotal signaling molecule, promoting the expression and activation of hydrolytic enzymes responsible for the breakdown of proteins, carbohydrates, and lipids stored within cotyledonary tissues. The mobilization of these reserves involves intricate cellular mechanisms, including the transformation of protein storage vacuoles into lytic compartments, the targeted synthesis and trafficking of proteases, and the coordinated degradation of starch and triacylglycerols through organelle-specific pathways. These events are tightly regulated by hormonal interactions, source-sink relationships between the embryonic axis and cotyledons, and signaling networks involving Ca^{2+} and reactive oxygen species (ROS). The GA-GID1-DELLA signaling module further exemplifies how transcriptional regulation integrates hormonal perception with enzymatic activation, ensuring that nutrient release is synchronized with embryonic growth demands. Despite substantial progress in understanding reserve mobilization, important gaps remain-particularly regarding the direct regulatory role of GA in dicot cotyledons and its interaction with other signaling pathways during senescence and programmed cell death. A clearer elucidation of these mechanisms will not only advance fundamental plant developmental biology but may also have practical implications for agriculture, seed technology, and crop improvement. Overall, GA-mediated storage mobilization represents a finely tuned developmental program essential for successful seedling establishment and early plant survival.

References:

1. **Ampofo, S.T., Moore, K.G., Lovell, P.H. 1976.** Cotyledon photosynthesis during seedling development in *Acer*. New Phytol., 76: 41-52.
2. **Baulcombe, D.C., Buffard, D. 1983.** Gibberellic acid-regulated expression of α-amylase and six other genes in wheat aleurone layers. Planta, 157: 493-501.
3. **Beevers, H. 1961.** Metabolic production of sucrose from fat. Nature, 191: 433-436.
4. **Bewley, J.D. 2001.** Seed Germination and Reserve Mobilization. Encycl. Life Sci., 1-7.
5. **Bewley, J.D., Black, M. 1978.** Physiology and Biochemistry of Seeds. Volume 1, Springer-Verlag, Berlin, Heidelberg, New York, pp. 229-241.
6. **Bewley, J.D., Black, M. 1994.** Seeds: Physiology of Development and Germination. Plenum Press, New York. pp. 293-310.
7. **Cejudo, F.C., Murphy, G., Chinoy, C., Baulcombe, C. 1992.** A gibberellin-regulated gene from wheat with sequence homology to cathepsin B of mammalian cells. Plant J., 2: 937-978.
8. **Chory, J., Voytas, D.F., Olszewski, N.E., Ausubel, F.M. 1987.** Gibberellin-induced changes in the populations of translatable mRNAs and accumulated polypeptides in dwarfs of maize and pea. Plant Physiol., 83: 15-23.
9. **Comai, L., Dietrich, R.A., Maslyar, D.J., Baden, C.S., Harada, J.J. 1989.** Co-ordinate expression of transcriptionally regulated isocitrate lyase and malate synthase genes in *Brassica napus* L. The Plant Cell., 1: 293-300.
10. **Davies, H.V., Slack, P.T. 1981.** The control of food mobilization in the seeds of dicotyledonous plants. New Phytol., 88: 41-51.
11. **de Barros, E.G., Larkins, B.A. 1990.** Purification and characterization of zein-degrading proteases from endosperm of germinating maize seeds. Plant Physiol., 94(1): 297-303.
12. **Eastmond, P.J., Graham, I.A. 2000.** The multifunctional protein AtMFP2 is coordinately expressed with other genes of fatty acid β-oxidation during seed germination in *Arabidopsis thaliana*. Biochem. Soc. Trans., 28: 95-99.
13. **Eastmond, P.J., Hooks, M.A., Williams, D., Lange, P., Bechtold, N., Sarrobert, C., Nussaume, L., Graham, I.A. 2000.** Promoter trapping of a novel medium-chain acyl-CoA oxidase, which is induced transcriptionally during *Arabidopsis* seed germination. J. Biol. Chem., 275: 34375-34381.

14. **Fletcher, R.A., Osborne, D.J. 1965.** Regulation of protein and nucleic acid synthesis by gibberellin during leaf senescence. Nature, 207: 1176-1177.
15. **Frydman, V.M., Wareing, P.F. 1974.** Phase change in *Hedera helix* L.: III. The effects of gibberellins, abscisic acid and growth retardants on juvenile and adult ivy. J. Exp. Bot., 25: 420-429.
16. **Geigenberger, P., Stitt, M. 1991.** A ‘‘futile’’ cycle of sucrose synthesis and degradation is involved in regulating partitioning between sucrose, starch and respiration in cotyledons of germinating *Ricinus communis* L. seedlings when phloem transport is inhibited. Planta, 185 (1): 81-90.
17. **Germain, V., Rylott, E.L., Larson, T.R., Sherson, S.M., Bechtold, N., Carde, J.P., Bryce, J.H., Graham, I.A., Smith, S.M. 2001.** Requirement for 3-ketoacyl-CoA thiolase-2 in peroxisome development, fatty acid beta-oxidation and breakdown of triacylglycerol in lipid bodies of *Arabidopsis* seedlings. Plant J., 28(1): 1-12.
18. **Ghosh, S., Pal, A. 2012.** Identification of differential proteins of mung bean cotyledons during seed germination: A proteomic approach. Acta Physiol. Plant., 34(6): 2379-2391.
19. **Gilroy, S., Jones, R.L. 1992.** Gibberellic acid and abscisic acid coordinately regulate cytoplasmic calcium and secretory activity in barley aleurone protoplasts. Proc. Natl. Acad. Sci. U.S.A., 89: 3591-3595.
20. **Graham, I.A., Smith, L.M., Leaver, C.J., Smith, S.M. 1990.** Developmental regulation of expression of the malate synthase genes in transgenic plants. Plant Mol. Biol., 15: 539-549.
21. **Griffiths, J., Murase, K., Rieu, I., Zentella, R., Zhang, Z.L., Powers, S.J., Gong, F., Phillips, A.L., Hedden, P., Sun, T.P. 2007.** Genetic characterization and functional analysis of the GID1 gibberellin receptors in Arabidopsis. Plant Cell., 18: 3399-3414.
22. **Harris, N. 1976.** Starch grain breakdown in cotyledon cells of germinating mung bean seeds. Planta, 129: 271-272.
23. **Hartweck, L.M., Olszewski, N.E. 2006.** GIBBERELLIN INSENSITIVE DWARF1 is a gibberellin receptor that illuminates and raises questions about GA signaling. Plant Cell., 18: 278-282.
24. **Hays, D.B., Yeung, E.C., Pharis, R.P. 2002.** The role of gibberellins in embryo axis development. J. Exp. Bot., 53(375): 1747-1751.
25. **Hedden, P., Thomas, S.G. 2012.** Gibberellin biosynthesis and its regulation. Biochem. J., 444: 11-25.
26. **Hedley, C.L. 2001.** Grain legume carbohydrates. In: Hedley, C.L. (Ed.), Carbohydrates in grain legume seeds: improving nutritional quality and agronomic characteristics. CABI Publishing, New York, pp. 2-14.
27. **Herman, E.M., Baumgartner, B., Chrispeels, M.J. 1981.** Uptake and apparent digestion of cytoplasmic organelles by protein bodies (protein storage vacuoles) in mung bean cotyledons. Eur. J. Cell Biol., 24: 226-235.
28. **Heywood, D.A., Gainer, T.P. 1974.** Effects of germination of cotyledon nitrogen, starch, reducing sugar and growth of lime seedlings. Agron. J., 66: 360-363.
29. **Hooley, R. 1994.** Gibberellins: perception, transduction and responses. Plant Mol. Biol., 265: 1529-1555.
30. **Horton, R.F., Bourguoin, N. 1992.** Leaf senescence in juvenile ivy. Plant Physiol. Biochem., 30: 119-122.

31. **Jiang, L., Phillips, T.E., Hamm, C.A., Drozdowicz, Y.M., Rea, P.A., Maeshima, M., Rogers, S.W., Rogers, J.C. 2001.** The protein storage vacuole: a unique compound organelle. J. Cell Biol., 155: 991-1002.
32. **Jiang, L., Phillips, T.E., Rogers, S.W., Rogers, J.C. 2000.** Biogenesis of the protein storage vacuole crystalloid. J. Cell Biol., 150: 755-770.
33. **Juliano, B.O., Varner, J.E. 1969.** Enzymic degradation of starch granules in the cotyledons of germinating peas. Plant Physiol., 44: 886-892.
34. **Karunagaran, D., Ramakrishna Rao, P. 1990.** Axial control of protease development in the cotyledons of horse gram (*Macrotyloma uniflorum* Lam.) seeds during germination. Indian J. Plant Physiol., 33: 232-238.
35. **Kirmizi, S., Güleryüz, G. 2006.** Protein mobilization and proteolytic enzyme activities during seed germination of broad bean (*Vicia faba* L.). J. Biosci., 61: 222-226.
36. **Kitajima, K. 2003.** Impact of cotyledon and leaf removal on seedling survival in three tree species with contrasting cotyledon functions. Biotropica, 35: 429-434.
37. **Koehler, S.M., Ho, T.D. 1990a.** A major gibberellic acid-induced barley aleurone cysteine proteinase which digests hordein. Plant Physiol., 94(1): 251-258.
38. **Koehler, S.M., Ho, T.D. 1990b.** Hormonal regulation, processing, and secretion of cysteine proteinases in barley aleurone layers. Plant Cell, 2: 769-783.
39. **Kornberg, H.L., Beevers, H. 1957.** A mechanism of conversion of fat to carbohydrate in castor beans. Nature, 180(4575): 35-36.
40. **Lovell, P.H., Moore, K.G. 1970.** A comparative study of cotyledons as assimilatory organs. J. Exp. Bot., 21: 1017-1030.
41. **Marttila, S., Porali, I., Ho, T.H.D., Mikkonen, A. 1993.** Expression of the 30 kD cysteine endoprotease B in germinating barley seeds. Cell Biol. Int., 17: 205-212.
42. **Metzger, J.D., Zeevaart, J.A.D. 1980.** Comparison of the levels of 6 endogenous gibberellins in roots and shoots of spinach in relation to photoperiod. Plant Physiol., 66: 679-683.
43. **Misra, N.M., Kar, R.K. 1990.** Regulation of storage protein degradation in cotyledons of germinating cow pea. Ind. J. Plant Physiol., 4: 333-339.
44. **Muntz, K., Belozersky, M.A., Dunaevsky, Y.E., Schlereth, A., Tiedemann, J. 2001.** Stored proteinases and the initiation of storage protein mobilization in seeds during germination and seedling growth. J. Exp. Bot., 52: 1741-1752.
45. **Nakajima, K., Kawamura, T., Hashimoto, T. 2006.** Role of the *SPIRAL1* gene family in anisotropic growth of *Arabidopsis thaliana*. Plant Cell Physiol., 47: 513-522.
46. **Nandi, S.K., Palni, L.M.S., De Klerk, G.J.M. 1995.** The influence of the embryonic axis and cytokinin on reserve mobilization in germinating lupin seeds. J. Exp. Bot., 46: 329-336.
47. **Neuhaus, J.M., Paris, N. 2005.** Plant vacuoles: from biogenesis to function. Plant Cell Monogr., 1: 63-82.
48. **Neuhaus, J.M., Rogers, J.C. 1998.** Sorting of proteins to vacuoles in plant cells. Plant Mol. Biol., 38: 127-144.
49. **Oden, P.C., Wang, Q., Hogberg, K.A., Werner, M. 1995.** Transport and metabolism of gibberellins in relation to flower bud differentiation in Norway spruce (*Picea abies*). Tree Physiol., 15: 451-456.

50. **Okello, R.C.O., de Visser, P.H.B., Heuvelink, E., Marcelis, L.F.M., Struik, P.C. 2015.** Light mediated regulation of cell division, endoreduplication and cell expansion. Environ. Exp. Bot., http://dx.doi.org/10.1016/j.envexpbot.2015.04.003
51. **Potter, T.I., Zanewich, K.P., Rood, S.B. 1993.** Gibberellin physiology of safflower: Endogenous gibberellins and response to gibberellic acid. Plant Growth Regul., 12: 133-140.
52. **Rajeswari, J., Ramakrishna Rao, P. 2002.** Storage protein degradation in germinating horse gram seeds. Indian J. Plant Physiol., 7: 314-320.
53. **Ramakrishna, V., Rao, P.R. 2005.** Axial control of protein reserve mobilization during germination of indian bean (*Dolichos lablab* L.) seeds. Acta Biol. Szeged., 49(3-4): 23-27.
54. **Ritchie, S., Swanson, S.J., Gilroy, S. 2000.** Physiology of the aleurone layer and starchy endosperm during grain development and early seedling growth : new insights from cell and molecular biology. Seed Sci. Res., 10: 193-212.
55. **Rogers, J.C., Dean, D., Heck, G.R. 1985.** Aleurain: a barley thiol protease closely related to mammalian cathepsin H. Proc. Natl. Acad. Sci. U.S.A., 82: 6512-6516.
56. **Saibo, N.J.M., Vriezen, W.H., Beemster, G.T.S., Van Der Straeten, D. 2003.** Growth and stomata development of *Arabidopsis* hypocotyls are controlled by gibberellins and modulated by ethylene and auxins. Plant J., 33: 989-1000.
57. **Schippers, J.H.M., Jing, H.C., Hille, J., Dijkwel, P.P. 2007.** Developmental and hormonal control of leaf senescence. In: Gan, S. (Ed.), Senescence Processes in Plants. Blackwell Publ., pp. 145-170.
58. **Senyuk, V., Rotari, V., Becker, C., Zakharov, A., Horstmann, C., Muntz, K., Vaintraub, I. 1998.** Does an asparaginyl-specific cysteine endopeptidase trigger phaseolin degradation in cotyledons of kidney bean seedlings? Eur. J. Biochem., 258: 546-548.
59. **Shastry, M., John, E. 1991.** Biochemical changes and in vitro protein digestibility of the endosperm of germinating *Dolichos lablab*. J. Sci. Food Agric., 55: 529-538.
60. **Shewry, P.R., Napier, J.A., Tatham, A.S. 1995.** Seed storage proteins : Structures and biosynthesis. Plant Cell, 7: 945-956.
61. **Smith, V.A. 1993.** Gibberellin translocation in *Pisum sativum* L. III. Biological and biochemical consequences of the *le* mutation. Planta, 191: 158-165.
62. **Taneyama, M., Okamoto, T., Yamane, H., Minamikawa, T. 2001.** Involvement of gibberellins in expression of a cysteine proteinase (SH-EP) in cotyledons of *Vigna mungo* seedlings. Plant Cell Physiol., 42(11): 1290-1293.
63. **Taneyama, M., Okamoto, T., Yamauchi, D., Minamikawa, T. 1996.** Development of endopeptidase activity in cotyledons of *Vigna mungo* seedling: Effects of exogenously applied end products and plant hormones. Plant Cell Physiol., 37: 19-26.
64. **Tarkowská, D., Novák, O., Floková, K., Tarkowski, P., Turečková, V., Grúz, J., Rolčík, J., Strnad, M. 2014.** Quo vadis plant hormone analysis? Planta, 240(1): 55-76.
65. **Toyooka, K., Okamoto, T., Minamikawa, T. 2000.** Mass transport of proform of a KDEL-tailed cysteine proteinase (SH-EP) to protein storage vacuoles by endoplasmic reticulum-derived vesicle is involved in protein mobilization in germinating seeds. J. Cell Biol., 148: 453-464.
66. **Ueguchi-Tanaka, M., Ashikari, M., Nakajima, M., Itoh, H., Katoh, E., Kobayashi, M., Chow, T., Hsing, Y.C., Kitano, H., Yamaguchi, I., Matsuoka, M. 2005.** GIBBERELLIN INSENSITIVE DWARF1 encodes a soluble receptor for gibberellin. Nature., 437: 693-698. doi:10.1038/nature04028.

67. **Van der Wilden, W., Herman, E.M., Chrispeels, M.J. 1980.** Protein bodies of mung bean cotyledons as autophagic organelles. Proc. Natl. Acad. Sci. U.S.A., 77(1): 428-432.
68. **Vitale, A., Bollini, R. 1995.** Legume storage proteins. In: Kigel, J., Galili, J. (Eds.), Seed Development and Germination. Marcel Dekker, Inc., New York, pp. 103-138.
69. **Wang, J., Li, Y., Lo, S.W., Hillmer, S., Sun, S.S.M., Robinson, D.G., Jiang, L. 2007.** Protein mobilization in germinating mung bean seeds involves vacuolar sorting Receptors and multivesicular bodies. Plant Physiol., 143: 1628-1639.
70. **Washio, K., Ishikawa, K. 1992.** Structure and expression during the germination of rice seeds of the gene for a carboxypeptidase. Plant Mol. Biol., 19: 631-640.
71. **Watanabe, H., Abe, K., Emori, Y., Hosoyama, H., Arai, S. 1991.** Molecular cloning and gibberellin-induced expression of multiple cysteine proteinase of rice seeds (oryzains). J. Biol. Chem., 226: 16897-16902.
72. **Weiss, D., Ori, N. 2007.** Mechanisms of Cross Talk between Gibberellin and Other Hormones. Plant Physiol., 144(3):1240-1246. doi: 10.1104/pp.107.100370
73. **Wrobel, R., Jones, B.L. 1992.** Appearance of endoproteolytic enzymes during the germination of barley. Plant Physiol., 100: 1508-1516.
74. **Xu, D.P., Sung, S.J.S., Black, C.C. 1989.** Sucrose metabolism in lima bean seeds. Plant Physiol., 89: 1106-1116.
75. **Zhang, H., Wu, Y., Matthew, C., Zhou, D., Wang, P., Matthew, C. 2018.** Contribution of cotyledons to seedling dry weight and development in *Medicago falcata* L. New Zeal. J. Agric. Res., 51(2): 107-114.