REVIEW OF LITERATURE

2.1 *Arabidopsis (Arabidopsis thaliana)* as a model plant:

Arabidopsis thaliana is a small and simple angiosperm belonging to the *Brassicaceae* or mustard family. Although *Arabidopsis* does not cover a major agronomic background, but it has become a robust and renowned plant model system of choice for research in plant biology; because of its small size of genome (120Mbp), a rapid life cycle, availability of large number of seeds, easy cultivation in restricted space, efficient transformation methods, its broad geographic distribution which leads to its successful adaptation to stressful conditions to ensure sustainability even is adverse state. The 120-megabase genome of *Arabidopsis* is organized into five chromosomes and contains approximately 20,000 genes (Meinke *et al.*, 1998).

2.2 Classification:

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Dilleniidae
Order	Capparales
Family	Brassicaceae
Genus	*Arabidopsis*
Species	*Thaliana*

2.3 What is seed?

The seed is the principal stage in the life cycle of many higher plants. It originates from a double fertilization event, where one nucleus from pollen fertilizes the egg cell of the mega gametophyte producing the diploid embryo, and a second nucleus of the same pollen fertilizes the central diploid nucleus of megaspore, from which produces the triploid endosperm (Goldberg *et al.*, 1994; Reiser and Fischer, 1993; West and Harada, 1993). There are three compartments in seed: the embryo, the endosperm and the seed coat. The embryo is considered as the future adult plant. It preserves and encircles all the necessary elements and fundamental patterns for the new plant to grow and develop after germination. The endosperm acts as the reservoir of all the required nutrients that the embryo will use during

development and until the new plant becomes self sufficient. The seed coat arises from the integuments of the ovule and prevents the vital part of the seed from mechanical injury, drying out and from predators.

2.3.1 Seed development: associated key events

Seed development and physiology involves many events occurring in different cellular compartments, such as the embryo, endosperm, and seed coat. Therefore a mosaic of gene expression programme occurs in parallel among seed tissues during different developmental stages (Le *et al.*, 2007). Seed development is a complex and highly co-ordinated process that involves the integration of many genetic, physiological, metabolic and signaling pathways, which also are effected by endogenous and environmental signals and stimuli (Wasternack *et al.*, 2013). Seed development is an intricate process that can be roughly divided into proper embryogenesis (cell division and morphogenesis), followed by a maturation phase (Fig 2.2), characterised by storage compound accumulation, acquisition of desiccation tolerance, growth arrest and the entry into a dormancy period of variable length that is broken upon germination (Harada, 1997). Therefore embryogenesis, embryo maturation, dormancy and germination all are developmentally associated processes (Holdsworth *et al.*, 1999) occuring within seeds. If any deformity occurs at any developmental stages of seed, neither dormancy nor germination could happen properly.

2.3.1.1 Embryogenesis

Embryogenesis is the process by which the plant embryo is formed and developed starting from egg cell fertilization. This includes zygotic cell proliferation along with cell differentiation and patterning (**Fig 2.1**), specification of the apical-basal axis with the formation of the shoot and root meristems, and the formation of one (as in monocots) or two (as in dicots) cotyledons accommodate the nutrients for the developing embryo (and, in some species the germinating seedling). The next stages of embryo development basically involve the establishment of a dormant state that empower its prolonged survival until environmental conditions are favourable for the germination of the seed (Park and Harada, 2008). Briefly, embryogenesis begins with the zygotic single cell (**Fig 2.1**) and terminates with the formation of heart shape (**Fig 2.1**) complete embryo (Mayer *et al.*, 1991).

In higher plants there are two major phases of embryogenesis, one is morphogenesis and another is maturation. During morphogenesis the complete structure of an embryo is

generated, and the maturation phase includes cell expansion together with the food reserves within the macromolecules in order to make prepare the embryo (the future plant) for desiccation tolerance, germination and early seedling growth. The *Arabidopsis* mutants which have defects during their embryogenesis helps us to find out the queries about the factors that regulate the proper formation of embryo using genetics, molecular and biochemical analyses (Park and Harada, 2008).

Embryogenesis starts with the double fertilization event where one haploid nucleus from pollen merges with the egg cell nucleus and another haploid nucleus from the same pollen fuses with the central nucleus of the megaspore to create the zygote and endosperm mother cell, respectively. There after occur the asymmetrical zygotic division, that cause the apical cell within the embryo proper and a basal cell which produces the hypophysis and the suspensor (Park and Harada, 2008).The hypophysis further generates the root quiescent center and the the central root cap, while the suspensor being as a transient organ acts the significant role for the complete development of an embryo. At the time of the morphological development of an embryo, the apical cell goes through two longitudinal divisions followed by a transverse division to produce an eight- celled, octant stage proembryo. Each cell of the embryo proper at octant stage experiences a periclinal (parallel to the surface /or, axis) division thereby producing the protoderm or embryonic epidermis and a dermatogen-stage of globular embryo (Park and Harada, 2008). Localized cell divisions lead to the appearance of cotyledon lobes and a reallocation in the proper embryonic morphological symmetry to bilateral from radial with creation of the heart shape embryo. During the torpedo stage, almost every developmental tools of an embryo are there. The apical basal axis produces shoot apical meristem (SAM), root apical meristem (RAM), hypocotyls, cotyledons and radicle (**Fig 2.1**).The embryo includes three primary tissues along the radial axis, (i) outer protoderm, (ii) middle ground tissues and (iii) inner procambium tissues. Though the fundamental plant architecture is shaped through embryogenesis, but the proper formation of organ and tissues occur during post-embryonic development (Park and Harada, 2008).

2.3.1.1.1 Cell elongation phase

Growth depends upon cell division and its elongation. The sequence of processes that occur during cell division is referred to as the cell cycle, which is dependent on DNA synthesis and

replication, and which leads to specific patterns of organogenesis and morphogenesis, in respect to cellular differentiation (de Castro *et al.*, 2000)

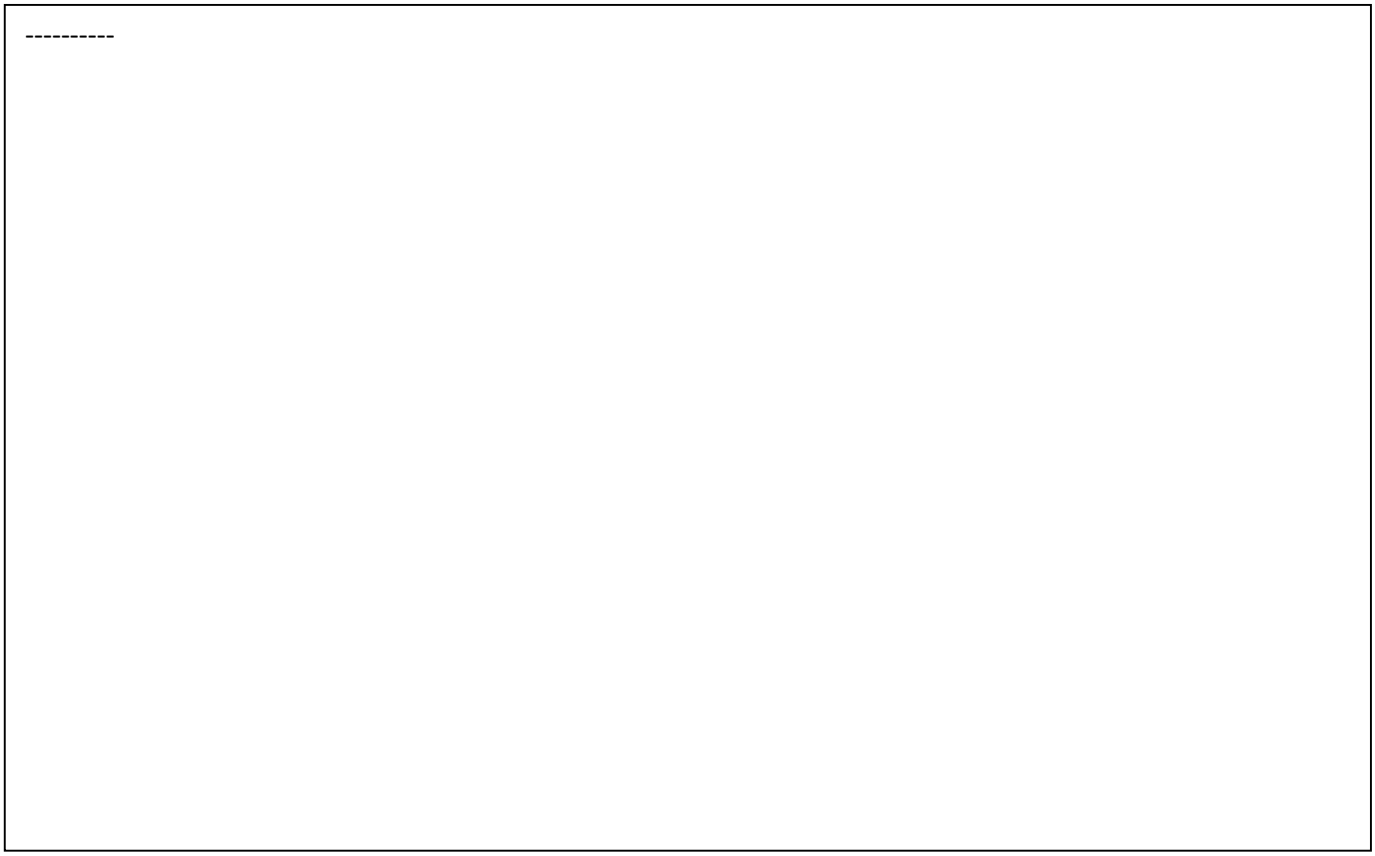

Figure 2.1 Embryo developmental stages (Zygotic embryogenesis) in *Arabidopsis*. The stages are shown here from octant to heart stages. The upper and lower tiers of the pro-embryo are established at the octant stage, which has been shown here **with light and deep green colors** respectively. Gradually, when the embryo enters to globular stage, the topmost suspensor (**here shown in grey color**) cell is described as the hypophysis **(in orange color)**. Later, the hypophysis divides asymmetrically, gives rise to an apical lens-shaped cell (**in yellow color**), which act as the precursor of the QC (quiescent center) and a basal cell (in orange color), which act as the progenitor of the columella stem cells. Abbreviations: SAM, Shoot Apical Meristem; RAM, Root Apical Meristem. [Image adapted from (Radoeva and Weijers, 2014)].

2.3.1.2 Seed maturation phase

The maturation phase is started once the embryo and endosperm have completed the morphogenesis and patterning stages (Wobus and Weber, 1999). This phase is characterized by a growth arrest, followed by the synthesis and accumulation of reserves, whose degradation upon germination will provide nutrients to the growing seedling before the photosynthetic capacity is fully acquired (Baud et al., 2002). Early and mid phases of maturation are dominated by the action of ABA, initially synthesized in the maternal tissues and later on, although to a lower extent, in the embryo and endosperm (Nambara and Marion-Poll, 2003). Transcription of major seed storage protein genes occur mainly during this

period. Subsequently, ABA levels decline and late maturation follows characterized by the synthesis of LEA (Late Embryogenesis Abundant) proteins, associated to the dehydration process and acquisition of desiccation tolerance. During this stage, accumulation of storage metabolites prevails in the form of carbohydrates (endosperm) or lipids (embryo), a quiescent state is accomplished and dormancy, the inability to grow under otherwise favourable conditions, can be established (Holdsworth *et al.*)

Loss-of function studies is a useful tool to unravel the significant contributors of seed development. Either a mutant shows lethality if it suffer deformities during morphogenesis, or its seedling shows the phenotype (Mayer *et al.*, 1991). In a loss-of-function mutant of seed germination, the characteristics of dormancy as well as germination changes, which sometimes causes multiple genetic effects associated with maturation, there after desiccation intolerance (Bentsink and Koornneef, 2008; Goldberg *et al.*, 1994).

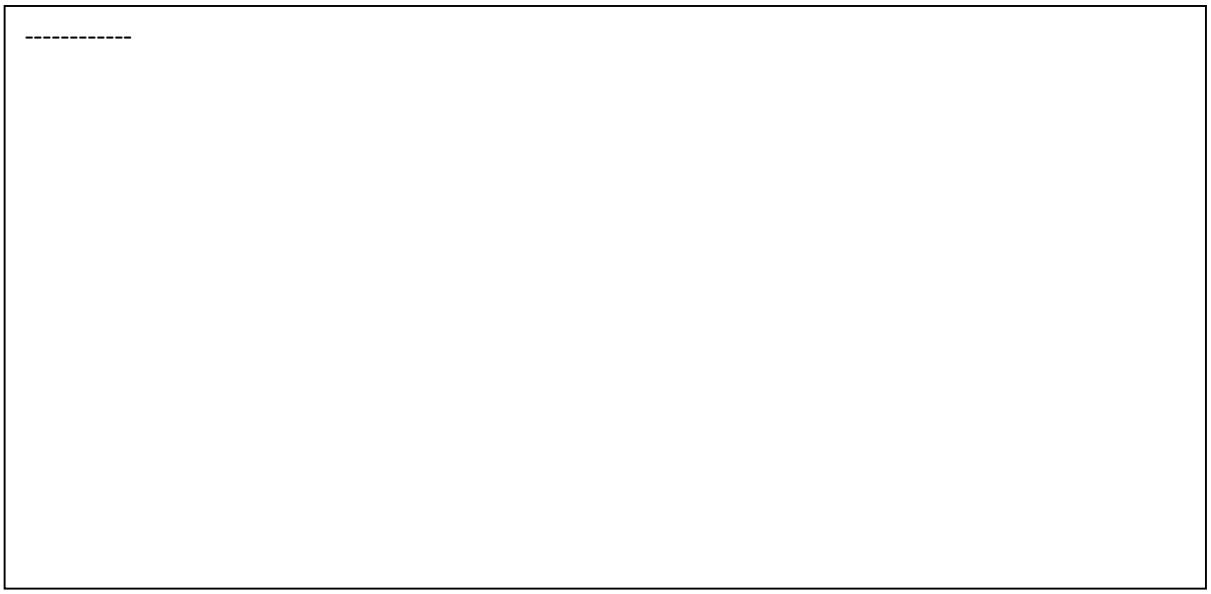

Figure 2.2 Over view of seed development stages[Adapted and modified from (Martin *et al.*, 2012)].

2.3.2 Genes involved in seed development process

The genes which are the key regulatory factors of embryo development, was found out by molecular genetics approach.The genes that are involved in the different stages of Arabidopsis embryogenesis are *ATML1*(*Arabidopsis thaliana* meristem layer1) (Sessions *et al.*, 1999), *WOX2,WOX8 (WUSCHEL RELATED HOMEOBOX)(*Breuninger *et al.,* 2008*) ,PIN1,3,4 and 7 (*Friml *et al.,* 2002*;* Reinhardt *et al.,* 2003*) MAP Kinase* (Pearson *et al.*, 2001)*, LEC1*(**Fig 2.3**), *LEC2 and FUS3* (**Fig 2.3**) (Curaba *et al.*, 2004), *ASIL1, ASIL2,*

HDA6/SIL1 (Willmann *et al.*, 2011), *PNH* (Pinhead), *SIN1*(Short Integuments1), *SUS1*(Suspensor1), *Zll* (Zwille) (Martin *et al.*, 2012). Small RNAs are also significant regulators of many features of plant growth and development (described later). The development of seed is also significantly regulated by small RNAs and their target molecules. Different small RNA biogenesis pathway mutants significantly exhibit defects in seed development. In brief, the key components in regulation of small RNAs that play a crucial role in seed development are *DCL1* (Chen, 2009; Martin *et al.*, 2012)*, HYL1, HEN1(Bartel, 2004; Chen, 2009), AGO (*Chen*,* 2009*;* Jones-Rhoades *et al.,* 2006*;* Martin *et al.,* 2012*),* SE(Serrate) (Martin *et al.*, 2012) etc.

2.3.2.1 *ARABIDOPSIS THALIANA* MERISTEM LAYER1 (*ATML1)*

The *ARABIDOPSIS THALIANA* MERISTEM LAYER1(*ATML1)* is expressed in the epidermis of developing embryo and shoot meristems (Sessions *et al.*, 1999). Expression begins in the apical cell of the one cell embryo, become restricted to the protoderm at the 16-cell stage of embryogenesis, and is articulated later in the L1 of shoot and floral but not root meristems (Sessions *et al.*, 1999).

2.3.2.2 *WUSCHEL RELATED HOMEOBOX2* and 8 (*WOX2, WOX8*)

WUSCHEL RELATED HOMEOBOX2 (*WOX2*) and *WOX8* are initially co expressed in the egg cell and the zygote, but become restricted to the apical (*WOX2*) and basal (*WOX8*) lineages after the zygotic division (Breuninger *et al.*, 2008). Distinct expression domains of *WUSCHEL-RELATED HOMEOBOX* (*WOX*) gene family members are involved in patterning and morphogenesis of the early embryo in *Arabidopsis* (Mayer *et al.*, 1991). Recently, it has been found that *WOX2* is important for protoderm and suspensor development in the gymnosperm Norway spruce (Zhu *et al.*, 2016). *WOX8* expression pattern requires the plant specific zinc-finger transcription factor WRKY2 (Ueda *et al.*, 2011). WRKY2 directly activates *WOX8* transcription in the zygote to regulate polar organelle localization and asymmetric division of the zygote, thus linking zygote polarity to regulators of asymmetric embryo development (Ueda *et al.*, 2011).

2.3.2.3 *PIN-FORMED1*, 3, 4, 7 (*PIN1, 3, 4 and 7)*

The PIN-FORMED (PIN) proteins are known as secondary transporters acting in the efflux of the plant signal molecule auxin from cells. They are localized within cells in asymmetrical

way and their polarity itself determines the directionality of intercellular auxin flow (Friml *et al.*, 2003). PIN1/ PIN7 are involved in maintaining basipetal polar fluxes in the developing embryo and along the apical–basal embryonic axis throughout the developmental process (Friml *et al.*, 2003; Jönsson *et al.*, 2006). PIN1 and PIN2 mediate polar auxin fluxes in organogenesis (Reinhardt *et al.*, 2003). Eight pin proteins in *Arabidopsis* are known so far (Friml *et al.*, 2003).

2.3.2.4 *MITOGEN-ACTIVATED PROTEIN KINASE (MAP Kinase)*

Mitogen-activated protein kinases (MAPKs) are evolutionary conserved and act as a signal transduction module in eukaryotes (Zhang *et al.*, 2006). A MAPK mainly consists of three kinases: a MPK, a MPKK, and a MPKKK, which regulates its downstream targets through phosphorylation and MPKs could regulate the different gene expressions by phosphorylating different transcription factors and several substrates (Andreasson and Ellis, 2010). MAPKs are well known for regulating hormone signaling, abiotic and biotic stress resposes and different development related factors (Colcombet and Hirt, 2008; Rodriguez *et al.*, 2010a). There are 20 different MPKs so far was identified in *Arabidopsis*. The research group (López-Bucio *et al.*, 2014)was first to find out the involvement of MAPK in embryo development. They found that the double mutants *mpk3* and *mpk6* become lethal at the embryo stage where as they are viable but developmentally arrested at the cotyledon stage (López-Bucio *et al.*, 2014; Wang *et al.*, 2007).

2.3.2.5 *DICER-LIKE1* (*DCL1*)

Dicer-like 1(DCL1) is a RNase III endonuclease, required in the processing of miRNA as well as small RNA biogenesis steps (Chen, 2009). It is known that *Arabidopsis* has only four DCL proteins with several connected functions in biogenesis of small-RNA. *DCL1* produces the most of the miRNAs, however very small young miRNAs from evolutionary context are developed by *DCL4* (Park *et al.*, 2002; Rajagopalan *et al.*, 2006; Reinhart *et al.*, 2002). The *dcl1* mutant in *Arabidopsis* embryo, shows early maturation than its wild type counterpart (Willmann *et al.*, 2011).

2.3.2.6 *HYPONASTIC LEAVES1* (*HYL1*)

HYL1 (Hyponastic leaves1) is a double stranded RNA binding protein, which is essential for processing of pri- and pre-miRNA (Chen, 2009; Laubinger *et al.*, 2008b).Recent localization studies have shown the existence of HYL1, SE and DCL1 inside the nucleus (Hiraguri *et al.*,

2005; Lobbes *et al.*, 2006); also it was revealed that DCL1 and HYL1 may act together (Fujioka *et al.*, 2007; Hiraguri *et al.*, 2005; Kurihara *et al.*, 2006). Loss of function mutants of HYL1 in plants produces increased level of mature miRNAs and lower level of pri-miRNAs. It has also the phenotype of abnormal development of embryo (Yang *et al.*, 2006).

2.3.2.7 *SERRATE* (*SE*)

Serrate (SE) is a zinc-finger protein, which is also essential for the small RNA biogenesis (Laubinger *et al.*, 2008a). The mutants of *SE* and *HYL1* show deformity in embryo development (Yang *et al.*, 2006). Among the mutants some are hypersensitive to ABA during seed germination (Bezerra *et al.*, 2004; Lu and Fedoroff, 2000). During the heart stage of embryo development the strong *SE* mutants like *se-4* shows improper cell division, abnormal development and also unable to develop proper cotyledon primordium which is basically embryo lethal (Lobbes *et al.*, 2006) like *se-3* mutants.

2.3.2.8 *HUA-ENHANCER 1(HEN1)*

Hua-Enhancer1 (HEN1) is a RNA methyl transferase protein that plays a role in the maturation of small RNAs in plants and also prevents the degradation of small RNAs through uridylation activity (Yu *et al.*, 2005). For the proper activity of a mature miRNA, its stability is very important and this is provided by *HEN1*, which incorporate a methyl group to the 3'end of a miRNA duplex (Park *et al.*, 2002). Although *hen1* was primarily isolated as a floral mutant, but also other various developmental defects was observed in the stronger mutants of *hen1* which is similar to *sin1/caf-1* mutants (Chen *et al.*, 2002; Park *et al.*, 2002). In recent studies it was found that for the stability of miRNAs the methylation of the miRNA miRNA* duplex is very essential, probably because it prevents the 3' uridylation and its subsequent destruction. The evidence of this event is the reduction or absence of some miRNAs in *hen1* mutants compare to its wild type counterparts (Li *et al.*, 2005b; Park *et al.*, 2002; Yu *et al.*, 2005).

2.3.2.9 *ARGONAUTE 1(AGO1)*

The **ARGONAUTE** protein family plays a crucial role in gene regulatory mechanisms like RNA silencing processes, as essential components of the RNA-induced silencing complex (RISC). RISC is responsible for the gene silencing phenomenon known as RNA interference (RNAi).

Current studies have unravel that ARGONAUTE protein plays a crucial role in maintaining RNA stability, translational regulation, genome integrity along with the proper production of specific mature small RNAs (Hutvagner and Simard, 2008). The ARGONAUTE proteins are categorised into three paralogous groups: (i) ARGONAUTE-like proteins, similar to *Arabidopsis thaliana* AGO1; (ii) Piwi-like proteins, closely related to *D. melanogaster*; and (iii) *Caenorhabditis elegans*- specific group 3 ARGONAUTES7 (Hutvagner and Simard, 2008).

The presence of ARGONAUTE-like proteins were observed in both archaea, bacteria and eukaryotes, which indicates its ancient origin.The abundance of ARGONAUTE proteins may vary from specie to species. They are 10 in numbers in *Arabidopsis thaliana*. It has four different domains, namely the N-terminal, PAZ, PIWI and Mid domains which are very critical for the proper gene regulation and functioning of an ARGONAUTE protein (Hutvagner and Simard, 2008). Interestingly, miR168 targets AGO1 (Rhoades *et al.*, 2002), which require for the proper embryo development, as earlier it was shown that *ago1* and *ago10* double mutant is embryo lethal (Lynn *et al.*, 1999; Mallory and Vaucheret, 2006) and the *ago1* mutant shows the phenotypic similarity with *dcl1*, *hen1*, and *hyl1* mutants (Vaucheret *et al.*, 2004).

2.3.2.10 *LEAFY COTYLEDON (LEC*) and *FUSCA3 (FUS3*)

LEAFY COTYLEDON1 (LEC1), *LEAFY COTYLEDON2 (LEC2)* and *FUSCA3 (FUS3*) are the most important genes principally expressed in the embryo, and required to maintain cell fate. They are also known as embryonic regulators. They are involved in activation of seed storage protein genes, repression of premature germination and in other seed developmental stages (Curaba *et al.*, 2004). Current studies have shown that inside the endosperm, the expression of these genes could be detectable at the early stages of seed maturation. Thus their functions could act as a landmark for the overall seed development starting from embryo development to seed maturation phase. Mutation of any of these genes could cause serious affect on seed phenotypes (Bäumlein *et al.*, 1994; Gazzarrini *et al.*, 2004; Locascio *et al.*, 2014; Lotan *et al.*, 1998; Meinke *et al.*, 1994).

2.3.2.10.1 *LEC1*

The *Arabidopsis LEAFY COTYLEDON1* (*LEC1*) gene is required for the specification of cotyledon identity and the completion of embryo maturation. The *LEC1* gene encodes a

transcription factor homolog, the CCAAT box–binding factor HAP3 subunit. *LEC1* RNA accumulates only during seed development in embryo cell types and in endosperm tissue. Ectopic postembryonic expression of the *LEC1* gene in vegetative cells induces the expression of embryo specific genes and initiates formation of embryo-like structures (Lotan *et al.*, 1998; Meinke *et al.*, 1994).

2.3.2.10.2 *LEC2*

The *LEAFY COTYLEDON2 (LEC2)* transcription factor with a plant-specific B3 domain plays a central role in zygotic and somatic embryogenesis (SE). LEC2 over expression induced in plant leads to spontaneous somatic embryo formation, but impairs the embryonic response of explants cultured in vitro under auxin treatment. The auxin-related functions of LEC2 appear during SE induction (Wojcikowska *et al.*, 2013). LEC2 is expressed in zygotic embryos between 4 and 14 days after pollination in a developmentally regulated pattern (Kroj *et al.*, 2003; Stone *et al.*, 2008)

2.3.2.10.3 *FUS3*

FUSCA3 (FUS3) is considered as a member of *LEC* group of genes and it is a B3 domain transcription factor (Wang and Perry, 2013). In the above it has discussed that *FUS3, LEC1* and *LEC2* are crucial for plant embryo development. The loss-of-function mutants of these three genes in *Arabidopsis* directly enter into the seedling developmental stage excluding embryogenesis prematurely (Wang and Perry, 2013). Direct targets of *FUS3* reveal that it acts exclusively as a transcriptional activator. *FUS3* could indirectly repress its target gene through miRNA mediated regulation, *FUS3* could also positively regulate *VP1/ABI3-LIKE1 (VAL1)* directly by feedback mechanism. In the transition from embryo to seedling development *VAL1* along with *VAL2* and *VAL3* play a significant role. Many genes are regulated by both FUS3 and *VAL1/VAL2* , but their way of regulation is complement to each other (Wang and Perry, 2013).

2.3.2.11 *ASIL1, ASIL2, HDA6/SIL1*

The three genes which are concerned in causing interruption in seed maturation at some point in early seed developmental stages and down regulation of the post germination embryonic activity are trihelix transcription factors *Arabidopsis 6B-Interacting Protein1-Like1 (ASIL1), ASIL2*, and *Histone Deacetylase6 (HDA6/ SIL1*). These three genes are repressed during the

torpedo stage embryos of *dcl1-15* mutants. Recent studies of single and double mutants like *asil1-1*, *asil2-1*, and *sil1-1* have shown that these genes act downstream of the miRNAs and repress the seed maturation programs at the very early stages of embryogenesis (Willmann *et al.*, 2011).

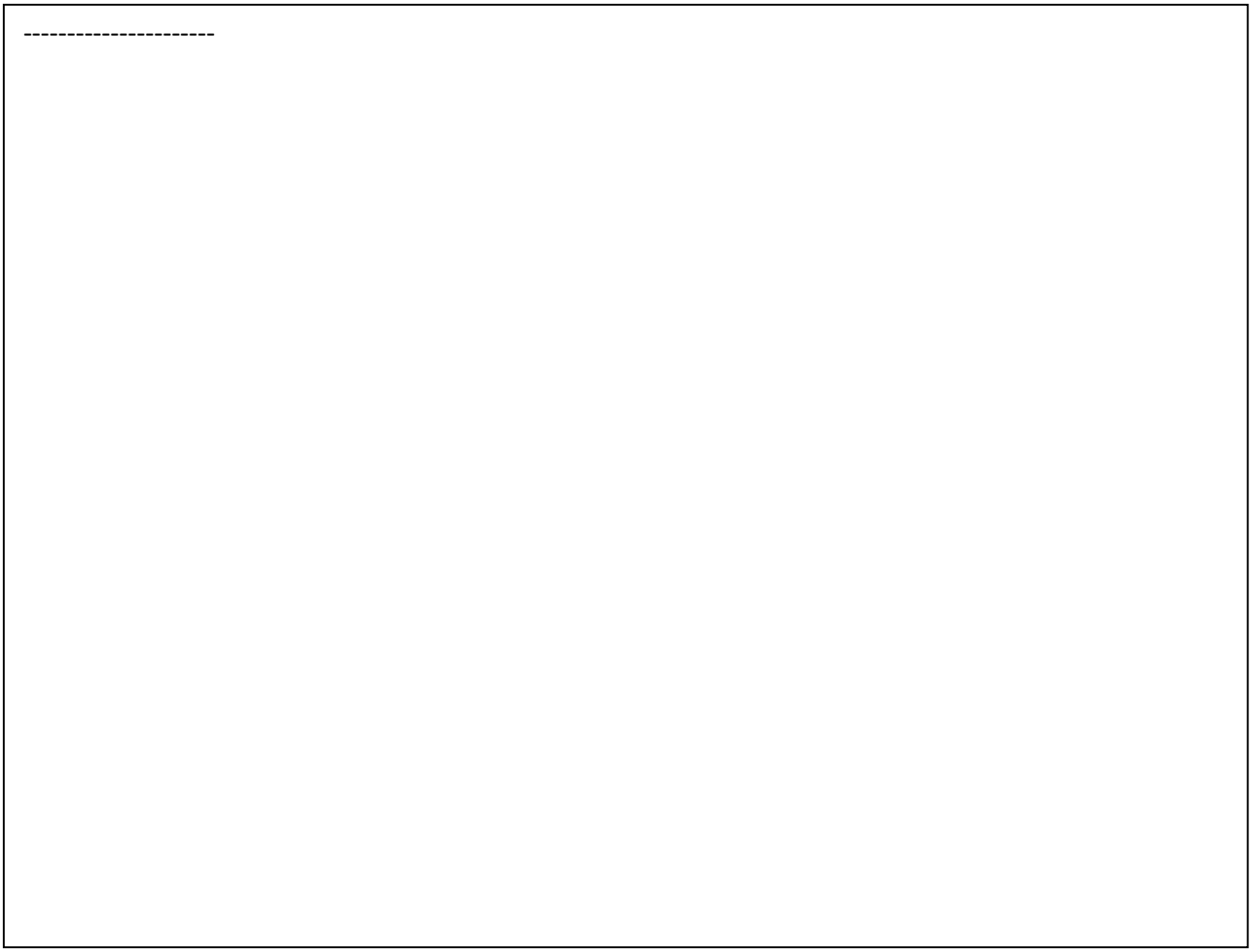

Figure 2.3 A summarised model of regulatory network depicting the genetic and molecular interactions during embryogenesis in radish. Embryo development can be categorised into two phases, morphogenesis and maturation. Arrows in blue and black colours indicate gene regulation and affiliation, respectively, whereas red arrows between genes indicating the transcriptional repression of the genes. The circle indicated with solid and dotted line representing those genes identified or not in radish and the corresponding miRNAs are shown in red color. Genes indicated in yellow boxes were connected with miRNAs by purple arrows which is indicating the miRNA-mRNA interactions [Image adapted from (Zhai *et al.*, 2016)].

2.3.3 What is seed germination?

Germination begins with the absorption of water by the inactive dry seed and conclude with the emergence and further growth of the embryonic axis (Derek Bewley and Black, 1985). It is assumed that germination is complete when the radicle emerges out from the surrounding structure of an embryo. On the perspective of germination there are two kinds of seed, one is dormant and another is termed as non-dormant. It was observed that both non-dormant and imbibed dormant seeds undergo through all of the metabolic and cellular programs before the

completion of germination, though they are different in manner but some unknown reasons dormant seeds are unable to protrude the radicle outside (Bewley and Black, 1994).

2.3.3.1 Water uptake (imbibitions) and the Metabolism Resumption

Earlier studies had shown that the water uptake procedure by a mature dry seed could be divided into three different phases (triphase) (**Fig 2.4B**).A rapid primary uptake of water occur during the first phase, followed by a plateau phase in phase-II. It is assumed that during phase-II, germination become complete. During phase-III the embryonic axis begins to elongate after further uptake of water. Therefore it is obvious that a imbibed non-dormant seed cannot enter into phase-III of germination. When the external water come inside the cell, especially into the cell membrane, then suddenly it faces some pressure changes which could cause a temporary structural interference, which is the immediate result for solutes leakage including the metabolites (of low molecular weight) within the adjacent imbibition solution. This occurring is the indication of a switch of the phospholipid membrane apparatus from its gel phase, which was established during maturation drying to the hydrated, normal, liquid-crystalline condition (Crowe and Crowe, 1992).Very shortly after rehydration, the membranes appear back to their more steady pattern, in the mean time the leakage of solute is gradually slowed down. Till date it is unknown to us that how the repair mechanism works after desiccation tolerance and the damage inside the membrane and organelles induced by rehydration. However, some phosphor lipid armed with membrane stabilizing property was observed in cotton seeds during imbibitions, like N-acetylphosphatidylethanolamine. It could therefore be assumed that this kind of molecules might help to maintain the cell membrane stability and integrity during germination (Sandoval *et al.*, 1995). Shortly following imbibitions, the dry seed starts its metabolic activities. It is assumed that the bio-molecules like enzymes and other necessary components might be in proper working condition or be partially intact condition within the dry seed for its survival after undergoing through desiccation tolerance. During imbibitions, water uptake occur by the dry seed is sufficient for the resumption of its metabolic activities. After imbibitions, another metabolic event starts is respiration which could be detectable within few minutes. Initially a sharp peak of oxygen consumption was found which gradually decreases with the progression of the completion of germination. Another event of respiratory metabolism happen when the radicle starts to elongate (Bewley and Black, 1994; Botha *et al.*, 1992). During phase I, The glycolytic and oxidative pentose phosphate pathways both restart their activities and during this time the Kreb's cycle enzymes start to activate (Nicol *et al.*, 1979; Salon *et al.*, 1988). At the phases of

germination a deficiency of oxygen level often occur within seeds which could be due to the restrictions of gaseous diffusion because of the surrounding dense structures of most of the seeds.This deficiency in oxygen level probably gives rise to excess amount of pyruvate to carry out the Krebs cycle and electron transport chain. As a consequence of that in many species the seeds frequently generate ethanol (Morohashi and Shimokoriyama, 1972) during germination. As a result of maturation drying, mitochondria, which present in the dry seed tissues, were poorly differentiated, so contain sufficient terminal oxidases and Krebs cycle enzyme to supply enough ATP to support the metabolism for a couple of hours even after imbibitions in seed (Attucci *et al.*, 1991; Ehrenshaft and Brambl, 1990). Depending upon the nature of stored reserves two types of mitochondrial development occur during seed germination, those could visible in cotyledons. Activation along with repair of pre-existing organelles prevails in starch accumulating seeds, whereas fresh mitochondria are produced in the oil storing seeds (Morohashi, 1986; Morohashi and Bewley, 1980).Except that, an initiation of coordination in the regulation of mitochondrial and nuclear genome also occur through the early hours of germination. As for example, in germinating maize embryos the mitochondrial biogenesis (that mainly store up oil in the scutellum, while starch is considered as the foremost endosperm reserve) entails the production of the enzyme cytochrome c oxidase that determined by the organellar genome, that act in accordance with quickly by the creation of nuclear-encoded subunits (Ehrenshaft and Brambl, 1990).

2.3.3.2 Translation during Germination

For protein synthesis within mature dry seeds following after imbibitions, every required component is present there except polysomes. Shortly after imbibitions every single ribosome are getting involved in polysomal protein synthesizing complexes, so a significant number of reduction in total single ribosomes was observed after imbibitions. Primarily the proteins are synthesized with the extant ribosomes, latter on the newly synthesized ribosomes are utilized for this purpose (Dommes and Van de Walle, 1990). The existence of some residual mRNAs also observed within the dry matured seeds. These are involved in the earlier developmental programs (Comai and Harada, 1990; Lane, 1991) and are believed to be utilized through early germination stages. The late embryogenesis abundant (LEA)proteins is called the message encoding proteins are critical throughout seed maturation and desiccation. This protein gradually degraded following after imbibitions (Han *et al.*, 1997; Jiang and R. Kermode, 1994). On the contrary, those proteins that are required during the early hours in germination (e.g., the messages of ribosomal protein) are substituted with indistinguishable new messages

afterward, as protein synthesis becoming more reliant upon the latest transcripts with time (Bewley and Black, 1994).The properties of the already reserved messages within the mature seeds aren't studied in details until now. But it could be pre assumed that these messages are encoded with proteins known as mRNP complexes (Ajtkhozhin *et al.*, 1976). The fresh mRNAs are synthesized as germination continued (Hammett and Katterman, 1975). Majority of these mRNAs encode proteins that are involved in normal cellular metabolism as well as the reactions for growth continuance but they are not specific for germination. Till date, no particular protein marker has been found which is exclusively for germination. Therefore in many literatures where there is a report for the germination specific mRNAs, care should be taken there. Those mRNAs are related to post germination growths of young seedlings, but unrelated to germination (Bewley and Marcus, 1990). However, few alterations in embryo mRNA collections and freshly synthesized proteins do occur in germination of several species of conifers (e.g., loblolly pine); (Mullen *et al.*, 1996); monocots (e.g., maize) and dicots (e.g., peas); (Lalonde and Bewley, 1986); (Mullen *et al.*, 1996). The significance of these freshly synthesized proteins needs to study more. Among those one interesting candidate for a "germination-specific" gene could be the one which encodes the protein germin (Lane, 1991), which is an oxalate oxidase (Bewley, 1997).

2.3.3.3 Extension of Radicle is the landmark for completion of Germination

The radicle growth across the structures neighboring the embryo is regarded as the incident that starts with the termination of germination and establishes the starting of the growth of seedling. After imbibitions inside the radicle two separate stages of DNA replication occur. Among these the first one happens shortly following imbibitions. It was reported that this synthesis of DNA also include the repair of damaged DNA during desiccation tolerance of mature seeds and also during rehydration after imbibitions. The second one is the synthesis of mitochondrial DNA. The growth of the radicle is a turgor-directed development which needs the extension of cell walls of the embryonic root axis that consists amid the embryonic root cap and hypocotyls base.

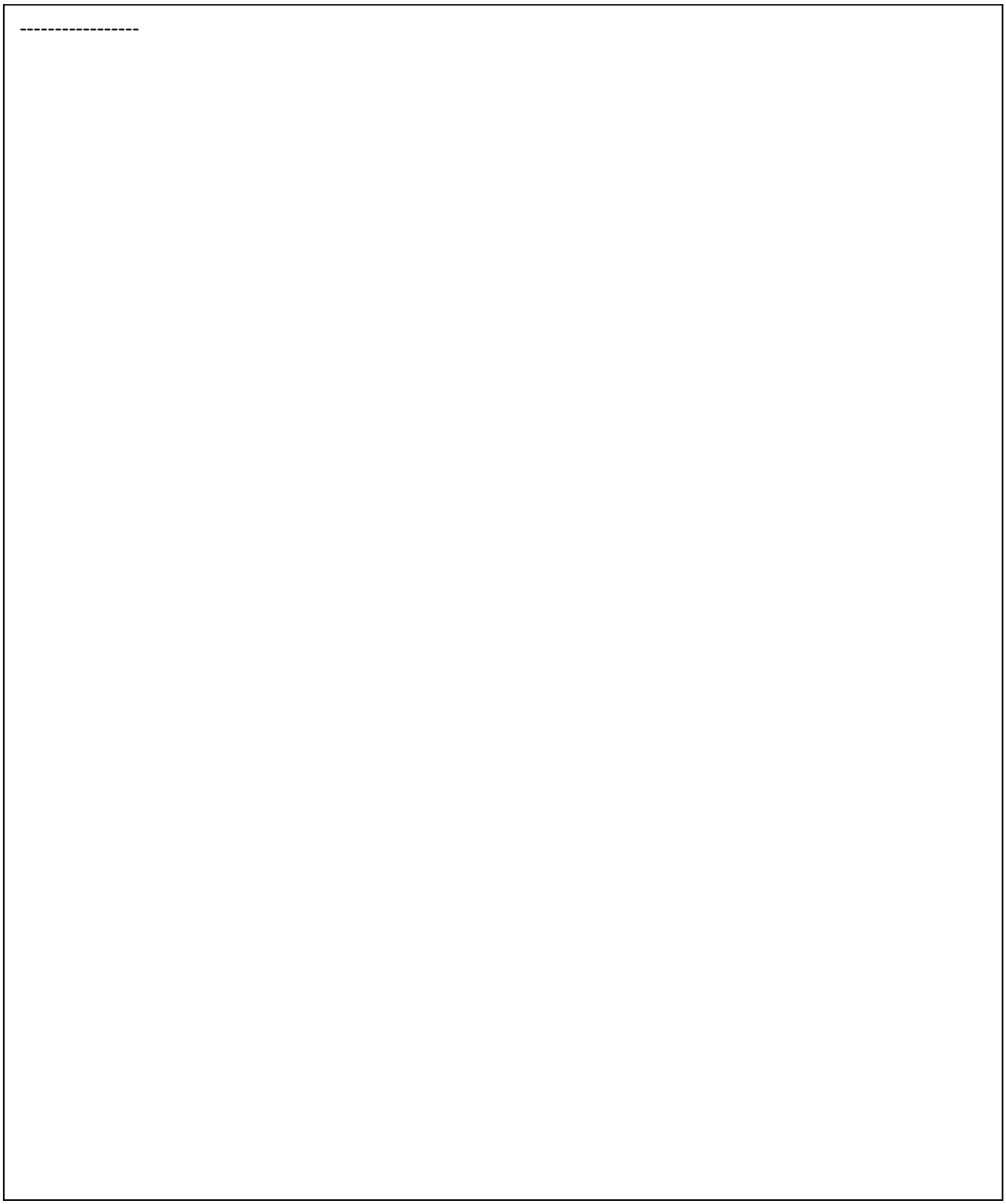

Figure 2. 4A Schematic representation of different parts of Seeds and Seed Germination stages. Seeds and germination stages of dicotyledonus **(Chickpea)** and monotyledonus **(Maize)** plants have been shown in upper and lower panels, respectively. **B. Major Events associated with Seed Germination and Post-Germinative growth phases. Germination stages are represented by phase 1 and phase 2; post-Germinaton events includes phase 3.** The time **(X-Axis)** for events varies from couple of hours to many weeks, based on different plant species and germination conditions. Uptake of water and related increase in biomass is indicated in (**Y-Axis)** and shown in line graph during three phases. Some specific events **(such as DNA repairing,**

transcription, translationand mitochondira production etc.) are spread over more than one phases and indicated with shaded color; where dark colors indicate more activity and light colors indicate less activity.

It is assumed that behind the initiation of radicle growth there might act three reasons (Bewley, 1997). The first one could be that after germination, because of the accumulation of solutes, the osmotic potential inside the cells of radicle turns more negative, the consequence might be the effect of hydrolysis of some polymeric reserves located within the radicle cells themselves (Bewley, 1997). This significant decrease in osmotic potential may trigger the enhancement of water absorption and ultimately the increment of turgor may act as the driving force for radicle cell extension. The second reason would be the extension property of the radicle cell walls allow it to elongate for growth. Till date, it is not clear the reason behind the more extensibility of radicle compare to other tissues.

One explanation would be that the splitting and re-association of xyloglucan molecules give rise to the loosening of cell walls. These xyloglucan molecules tie up adjacent cellulose microfibrils, which would allow the growth by microfibril separation. The amount of the enzyme xyloglucan endotrans glycosylase (XET) enhances within the root apical region of the maize seedlings throughout their extension after germination. This enzyme could cleave the xyloglucan molecules reversibly. Another alternative protein that are involved in cell wall loosening is expansins (Bewley, 1997). It can break the hydrogen bonds in between the cell wall polymers (e.g., cellulose microfibrils and matrix polysaccharides). Previously, it was reported that this expansins are critically involved in the hypocotyls elongation of cucumber (McQueen-Mason and Cosgrove, 1995).

2.3.4 Dormancy and its biological roles

Under favorable conditions when an intact viable seed is not able to germinate then this phenomenon is termed as seed dormancy. When the surrounding structure of an embryo restrains the seed from germination then this kind of dormancy is called as coat enhanced dormancy. In some species it has been observed that when the embryos were isolated from their seeds then they are no more dormant. Another category of dormancy is called embryo dormancy when the embryos are dormant itself. Till date we have no sufficient information regarding the concept of dormancy. Also we don’t have proper definition of seed germination. Therefore excluding this basic information it is bit difficult to explore the reasons that working as the driving force of a dormant seed to be dormant (Bewley, 1997). However, study of seed germination is itself challenging, since all the seed population donot

germinate synchronously. To predict dormancy is even more difficult since the effective stimulating energy requires for boosting germination after breaking the dormancy varies widely and non-specifically within individual seeds. Recently, a "biotime" concept has emerged which include a mathematical model that could predict the seed germination behavior in relation to dormancy and other influencing factors (Bradford, 1996). Though several interesting observations have been made so far with the isolated embryos from the seeds with coat enhanced dormancy, yet it is not very satisfactory to reach any final conclusion on dormancy with the isolated embryos since there is the difference in tissue types, also the cellular information for the imposition and then breaking of seed dormancy of isolated embryo and its counterpart (seeds having coat enhanced dormancy) may differ. The major questions that normally come in our mind that does dormancy is the consequence of the deficiency of some critical cellular events of germination; or is there exist any dormancy-imposed program that are non-functional before germination? But the most concerned issue is the liberation from dormancy and then germination is moderated through a common signal transduction pathway that are linked with different cellular responses which could also vary within seeds of different species and dormancy types. Earlier studies had revealed that within the embryonic cells (plasma membrane) there are common receptors for dormancy-breaking molecules. When these receptors are excited then they begin to start a signal transduction cascade and possibly involves in the synthesis of germination promoting hormone gibberellins (GAs), which could lead to the completion of germination (Hilhorst and Karssen, 1992; Vleeshouwers *et al.*, 1995). Another event i.e. the changes in the phosphorylating activity of Ca^{2+}-dependent, membrane associated protein kinases also lead to dormancy and then germination as well (Trewavas, 1987). However, these all suggestions are without any solid evidences, therefore these suggestions could be considered as an interesting reference for further research.

2.3.4.1 Metabolism of Dormancy Maintenance and Termination

Some seeds miss their dormancy, when the rate of their metabolism is extremely low during dry state. Although in dormant state, but when they are in imbibed condition, then this dormant, imbibed seeds become very energetic and they could percept the environmental signals like light, varying temperatures, chemicals as well as hormonal treatment to stimulate germination. The primary event to release from dormancy (**Fig 2.5**) is the reception of environmental stimulus followed by a well organized signal transduction cascade which leads to the further secondary events associated with several hormonal and metabolic changes. At

the end, the embryonic axis oozes out from seed coat which acts as a signal for the completion of germination. In these over all primary events several phyto hormones play a significant role.

In the earlier reports the associated secondary actions in the way of release from dormancy were described critically (Bewley, 1997). The key events could be concluded like the following ways: (i).The environmental and chemical stimuli those help in breaking dormancy may work at the phase of transcription, whether they act in translation or not is not known to us till date. (ii).From the perspective of metabolism no significant differences were noticed in between non dormant and imbibed dormant seeds. (iii)No strong evidences were observed in favor of the involvement of respiratory enzymes or pathways during the maintenance of dormancy. And lastly (iv).The cell membranes could help in the maintenance of dormancy but how is not known to us.

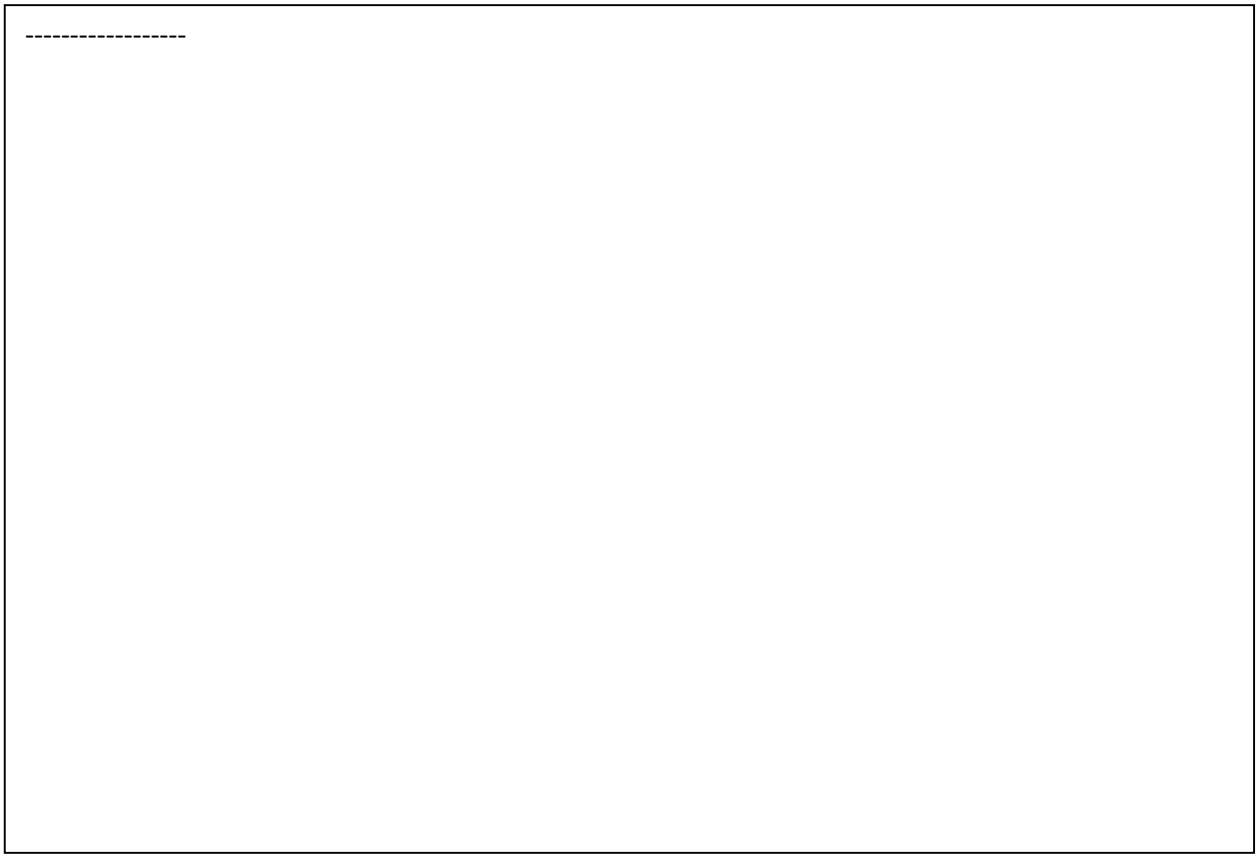

Figure 2.5 Major Events which are the integral part of breaking Seed Dormancy (Image adapted from Bewley 1997 after modification).

2.4 During seed germination and seed development, the miRNA regulation on different phyto hormones is essential

Several atmospheric conditions such as oxygen, light, soil, temperature, humidity, moisture stress etc and some physiological factors such as dormancy period, viability of seeds, thickness of seed coat, etc also play significant roles in seed germination stages (Martin *et al.*, 2010; Weitbrecht *et al.*, 2011). Several recent studies have implicated that the interactions between different phyto hormones like auxin, abscisic acid (ABA), gibberellins (GAs), cytokinins (CKs), ethylene and brassinosteroids (BRs) participate in a vital role in conducting the organized molecular actions which control discharging from dormancy and activation stages of seed germination (Finkelstein *et al.*, 2008; Liu *et al.*, 2007). As some phyto hormones exert crucial but contrasting influence on the process, therefore the activity of plant hormones needs to be precisely regulated. It is reported that ABA acts as a negative regulator of seed germination, but it acts as an upregulator of dormancy and its maintenance (Finkelstein *et al.*, 2008). The insensitivity or absence of ABA during later stages of seed development gives rise to viviparous or precociously germinating seeds; such as *Arabidopsis* ABA-deficient (*aba*), ABA-insensitive (*abi*), maize viviparous (*vp*) and tomato sitiens (*sit*) mutant seeds etc (Finkelstein et al., 2008). GA helps to release from dormancy and its role is quite similar to ethylene and BR in promoting germination by counteracting ABA effects. The crosstalk between ABA and auxin has also been come into light recently (Finkelstein *et al.*, 2008). The study of microRNA (miRNA), a class of small non-coding RNAs (of 19–24 nucleotides length) has added a new dimension to the understanding of the regulation of cellular environment (Axtell *et al.*, 2007; Bartel, 2004). It has been shown that in both plants and animals they play multiple significant roles in case of growth, development, morphogenesis and various stress responses (Chen, 2012). From double stranded RNA precursors with the help of RNA-dependent RNA Polymerase (RDR), DICER-like (DCL), and ARGONAUTE (AGO) proteins, the mature and functional miRNAs are produced (Allen *et al.*, 2005; Axtell, 2013; Mallory *et al.*, 2008; Rajagopalan *et al.*, 2006). miRNAs bind to the complementary sequences of their target genes and thus negatively regulate them. At the mRNA level, the small RNAs may be involved in chromatin remodelling (Huettel *et al.*, 2007; Pontier *et al.*, 2012; Xie and Yu, 2015) while at the post-transcriptional level, they can bring about the cleavage of the target mRNA (Rajagopalan *et al.*, 2006; Vaucheret, 2006) or can block their translation depending upon the nature of homology (Bartel, 2009; Poethig *et al.*, 2006; Vaucheret, 2006). The molecular functions and biosynthesis pathway of many of

these small RNA genes are also regulated by various phyto hormones and environmental stresses (Khraiwesh *et al.*, 2012; Mallory *et al.*, 2005; Martin *et al.*, 2010; Reyes and Chua, 2007; Sanan-Mishra *et al.*, 2013; Shukla *et al.*, 2008; Sunkar *et al.*, 2007).

2.5 Biogenesis of miRNA

The majority of *MIR* genes are located in the regions among protein coding genes (Rajagopalan *et al.*, 2006; Reinhart *et al.*, 2002). miRNA biogenesis consists of several process including transcription, processing, modification, and association with RISC loading complex (**Fig 2.6**). With the help of Pol II a *MIR* gene is transcribed (RNA polymerase II) into a poly adenylated and

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Figure 2.6.Diagramatic representation of Plant miRNA biogenesis. [Image adapted with slight modification from (Chen, 2009)].

caped pri-miRNA, that further turned into the stem-loop precursor, ~70–100 nt long pre-miRNA, by a DCL (Dicer-like) protein, generally in *Arabidopsis* DCL1 (Park *et al.*, 2002; Reinhart *et al.*, 2002). In *Arabidopsis* there are four DCL proteins are known so far (Schauer *et al.*, 2002). DCL1 is known for miRNA production (Park *et al.*, 2002), DCL2 is responsible for virus induced siRNA production, where as DCL3 is involved in RDR2 dependent siRNA production (Xie *et al.*, 2004) and DCL4 is involved in the production of mature ta-siRNA (Gasciolli *et al.*, 2005). After further processing by DCL1, the precursor miRNA then formed into a miRNA and miRNA* duplex (**Fig 2.6**). With the help of two partner proteins HYL1 (Hyponastic leaves1) and SE (Serrate) DCL1 performs its activities (Han *et al.*, 2004; Lobbes *et al.*, 2006; Vazquez *et al.*, 2004; Yang *et al.*, 2006). In vivo within nuclear dicing bodies these three proteins reside together (Fang and Spector, 2007; Fujioka *et al.*, 2007; Song *et al.*, 2007). Most of the miRNAs are accumulated with the help of nuclear cap-binding complex (CBP) which is hetero dimeric in nature (Gregory *et al.*, 2008; Laubinger *et al.*, 2008a). With HEN1 the duplex miRNA-miRNA* is methylated at the 2'OH position of the 3' end of nucleotides (Yu *et al.*, 2005). Among the two strands only a single strand out of the duplex is loaded within a ribonucleo protein complex that contains AGO1, called RNA-induced silencing complex (RISC) (Baumberger and Baulcombe, 2005; Qi *et al.*, 2005) (**Fig 2.6**). Probably with the help of the protein HASTY and along with other export factors the miRNA is exported to the cytoplasm from the nucleus (Park *et al.*, 2005). In order to limit the steady state levels of single stranded miRNAs, the SDN1 (small RNA degrading nuclease1) family of exonucleases degrade them. The strand selection widely depends on the relative stability of the two ends of the duplex (Axtell, 2013). It is observed that generally the strand whose 5′ end is comparatively loose, gets incorporated into the RISC (Allen *et al.*, 2005; Axtell, 2013). The RISC complex containing the miRNA identifies its target transcripts based on perfect or nearly perfect sequence complementarity. In plants the stringency of target recognition is very high and the target transcripts are normally cleaved, however, the central mismatches in the miRNA:mRNA pair direct the inhibition of translation (Allen *et al.*, 2005; Axtell, 2013).

2.6 Biogenesis of ta-siRNA

ta-siRNAs, another class of small non coding RNAs are generated from *TAS* (Trans-Acting SiRNA locus) gene derived non-coding transcripts through specific miRNA guided cleavage. The cleaved precursors of ta-siRNAs are bounded and stabilized by *SUPPRESSOR of GENE*

SILENCING3 (*SGS3*) and further synthesized into double stranded RNAs by *RDR6 (Allen and Howell, 2010; Axtell, 2013; Chen, 2009)*. The double stranded RNAs are cleaved several times with the help of *DCL4* from the miRNA cleavage sites, so that 21nt long phased ta-siRNAs are produced. Similar to miRNAs, the ta-siRNAs are incorporated into RISCs (**Fig 2.7**), where

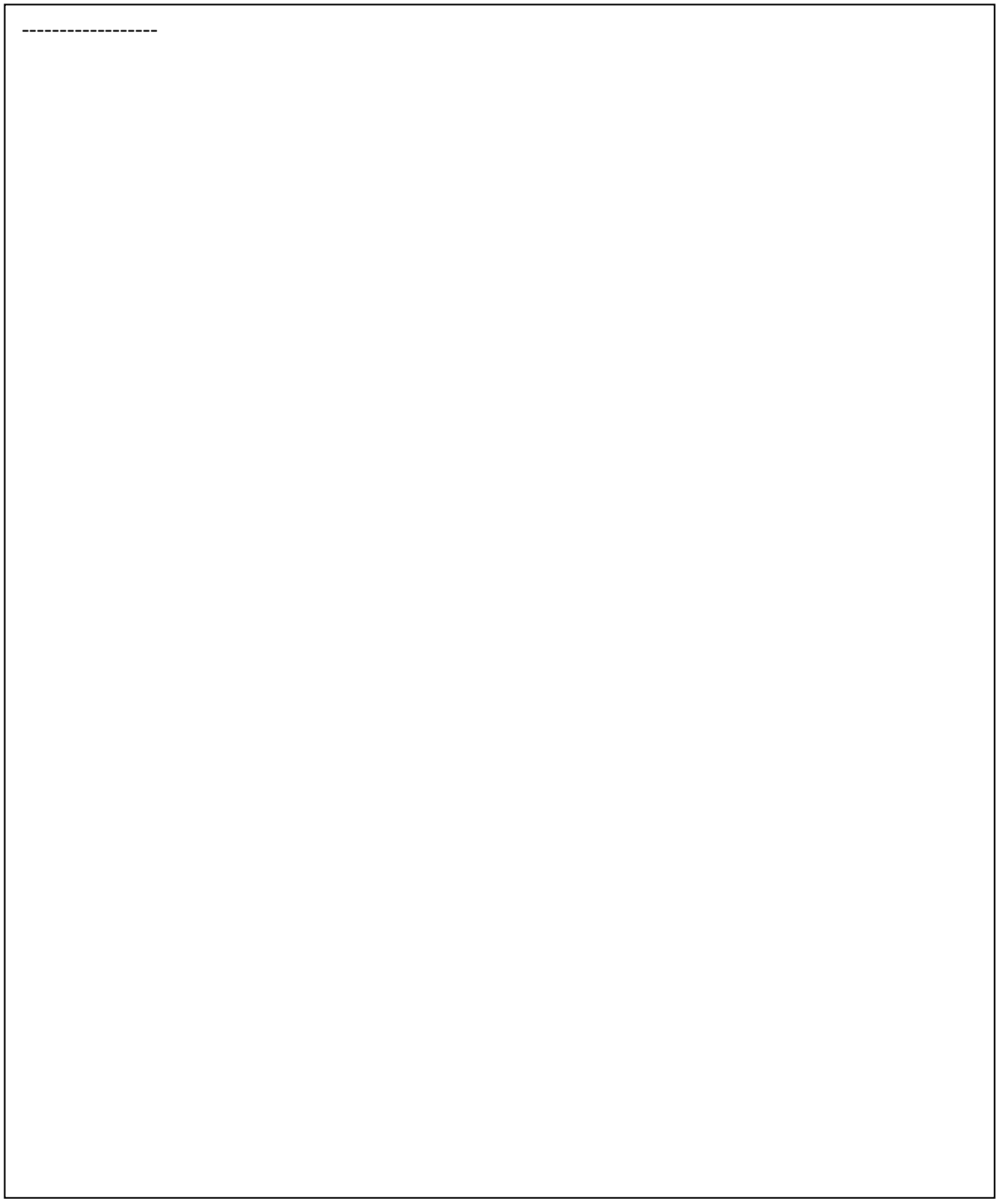

Figure 2.7 Diagramatic representation of the biogenesis of Plant ta-siRNAs. (A) Long non coding precursor transcripts at the ***TAS1*** locus, are cleaved by **miR173/AGO1**, and the 3' cleavage products are bound by ***SGS3*** and copied into dsRNAs by ***RDR6***. The dsRNAs again processed into siRNAs by ***DCL4*** in a step-by-step

manner from the end specified by the initial cutting/cleavage. **(B)** Long non coding precursor transcripts at the ***TAS3*** locus, are familiar at two sites by **miR390/*AGO7***, which cleaves the transcripts simply at the 3' site. The 5'cleavage products are ultimately channelized into mature ta-siRNA production by ***SGS3***, ***RDR6***, and ***DCL4*** [Image adapted from (Chen, 2009)].

they cleave the target mRNAs or repress translation (Allen and Howell, 2010; Allen *et al.*, 2005). There are four families of *TAS* gene in Arabidopsis, namely *TAS1*, *TAS2*, *TAS3*, *TAS4 (Allen and Howell, 2010; Rajagopalan et al., 2006)*. For the initial processing *TAS1* and *2* require miR173 whereas *TAS3* and *TAS4* require miR390 and miR828 respectively, for initial processing (Allen and Howell, 2010; Axtell, 2013; Chen, 2009). These four TAS families can be separated by two classes: those that require one miRNA binding site (*TAS1*, *TAS2*, *TAS4*), and those that require two (*TAS3*) miRNA binding sites (Allen and Howell, 2010). The first characterized ta-siRNA families were *TAS1* and *TAS2* from *Arabidopsis*. The *TAS1* family consists of three loci, *TAS1a*, *TAS1b* and *TAS1c*. *TAS2* is in close proximity to *TAS1c*.

miR173 is required by both *TAS1* (**Fig 2.7**)and *TAS2* families, incorporated with *AGO1* to guide the cleavage of the transcript for the production of mature ta-siRNAs. The precursor *TAS3* transcripts and the stimulator miR390 have been found in mosses and in many higher plants which indicates a very high conserved mechanism for the production of mature and functional ta-siRNAs (Axtell *et al.*, 2006). *TAS3* transcripts require only two miR390 binding sites unlike *TAS1/2* and *TAS4* families. Till date in *Arabidopsis* three *TAS3* loci have been identified. Those are: *TAS3a*, *TAS3b*, and *TAS3c* (Howell *et al.*, 2007). In these transcripts, miR390 guides cleavage at the 3' site to produce functional ta-siRNAs (tasi*ARF*s), which indicates the significance of miR390 mediated cleavage. *TAS3* transcript also requires *AGO7* (**Fig 2.7**) instead of *AGO1* for cleaving 3' miR390 binding site (Allen and Howell, 2010).

2.7 The Role of small RNA in plant growth and developments

The miRNAs are one of the major classes of small RNAs which play important and diverse roles in various aspects of plant growth and development. A huge portion of both conserved and non-conserved miRNAs significantly regulate their target genes which have direct or indirect roles in developmental patterning. Here are some classical examples of the developmental functions of the small RNAs.

2.7.1 Hormone Biosynthesis and Signalling

MYB33, *MYB65* and *MYB101* are the targets of miR159 in *Arabidopsis*. These target genes are linked to *Myb* gene *GAMYB*, that triggers gibberellin-responsive (*GA*) genes in the

aleurone layer (Millar and Gubler, 2005; Reyes and Chua, 2007).The aforesaid *MYB* genes are required in developmental processes which involves GA, like flowering in short day condition, male fertility etc, although these genes don't mediate GA signaling process. Over expression of miR159 causes reduction of *MYB65* and *MYB33* transcripts and delayed flowering under short days due to reduction in male sterility (Achard *et al.*, 2004; Millar and Gubler, 2005).

In seed germination stages, miR159 is responsible for the reduction of expression of *MYB101* and *MYB33* whereas over expression of miR159 /or, synonymously loss-of-function mutations in *MYB101* and *MYB33* cause hyposensitivity towards abscisic acid response (Reyes and Chua, 2007). The *ARF* genes are targeted by miR160, miR167, and tasiR*ARF*s. The regulation of miR160 in many developmental concerns of root and shoot by *ARF10*, *ARF16* and *ARF17* is regarded as the most significant. The phenotype of miR160-resistant *ARF10, ARF16* or *ARF17* exerts developmental (pleiotropic) deficiency in maximum aerial organs including roots (Liu *et al.*, 2007; Mallory *et al.*, 2005; Wang *et al.*, 2005). *ARF6* and *ARF8* are the target genes of miR167. Studies had shown that miR167- resistant *ARF6* expression guide to detained anthers and ovule development. Therefore it could be inferred that both ARF6 and ARF8 play significant roles in ovule and anther development. From the precursor *TAS3* locus, the functional ta-siRNAs target *ARF3* and *ARF4,* which sponsor the adult vegetative traits and lateral (abaxial) organ polarity in leaves. *NAC1* is the target gene of miR164, which mediates auxin signaling during the lateral root growth and development (Guo *et al.*, 2005). miR393 targets *TIR1*, which encodes an auxin receptor and associated genes (Jones-Rhoades and Bartel, 2004). miR319 negatively regulates the biosynthetic genes of jasmonic acid (Schommer *et al.*, 2008). The gene, *LIPOXYGENASE2* (*LOX2*), plays a critical role in the biosynthesis of jasmonic acid, is possibly the target of *TCP4*, which is also the target of miR319 (Chen, 2009).

2.7.2 Boundary formation/organ separation

miR164 targets *CUP SHAPED COTYLEDON* (*CUC*)*1*, *2*, and *3* and *NAC* gene family members. These two gene families share some common functions in the initiation of shoot apical meristem (SAM) and in organ boundary formation processes. These three target genes are mainly expressed in the terminal regions of cotyledons within embryo and further express in the terminal regions of floral organs. The loss-of-function mutations in these two genes together exert an enhanced rate of cotyledon fusion on equal directions of the cotyledons and

this double mutant plant consisting *cuc2* and another *cuc* alleles don't contain the Shoot Apical Meristem (SAM) (Aida *et al.*, 1997). Also a fusion of cotyledons (embryonic organ), sepals and stamens (floral organ) was observed in the phenotype of this double mutant. These results indicate that those genes might regulate a common mechanism that is responsible for the separation of organs in both embryo and flower development (Aida *et al.*, 1997). Though obvious but also in vivo it had observed that over expression of miR164 in wild type background with 35S promoter causes both floral organ and cotyledon fusions (Chen, 2009). Experimentally it has been observed that miR164-resistant*CUC2* can rescue sepal separation in miR164 over expressing lines. Whereas the miR164-resistant *CUC2* in wild type back ground has reduced sepal number, higher number of petals and enlarged leaves (Mallory *et al.*, 2005) and increment in the boundary width area between the sepals (Laufs *et al.*, 2004). This last property of enhancement of sepal terminal width of domain was also marked in mutants like *hen1, hyl1*and *dcl1* (Laufs *et al.,* 2004). The *CUC2* and *CUC1* transcript amounts were higher in mutant *miR164c* which is not applicable in case of other four targets of miR164 (Baker *et al.*, 2005). Additionally, the phenotype of *cuc2 cuc1 mir164* mutant flowers are identical with *cuc2 cuc1* flowers, indicating that the reason behind the improved number of petal in *mir164c* is the over expression of *CUC2* or *CUC1* (Baker *et al.*, 2005).

2.7.3 Organ polarity

The portion of an organ that is in front of the meristem in the primordium is termed as adaxial side. On the contrary, the portion of the organ that appears distant from the meristem is known as abaxial side. These two sides differ from each other in lateral organs like floral and leafy organs. They are also termed as polar organs. The distinctions of these two sides are clearly visible through their epidermis structure and also through the mesophyll tissues on both sides.

In lateral organs and stem the xylem is established on the adaxial region, where as the phloem is set up on the abaxial region. This differentiation in vasculature is very significant for necessary leaf performances as the proper positioning of vasculature on leaves is responsible for maximum photosynthesis along with water and gas exchanges. Apparently, this adaxial/ abaxial polarity specification is considered as the first developmental event where the roles of small RNAs were implicated (Juarez *et al.*, 2004; Li *et al.*, 2005a).The opposed interactions among two most important groups of genes, the class III homeo domain

leucine zipper (*HD-ZIP*) family of genes **(Fig 2.8)**, *PHAVOLUTA* (*PHV*), *REVOLUTA* (*REV*) and *PHABULOSA* (*PHB*) and the *KANADI* family (*KAN1*, *2*, and *3*) of genes (Emery *et al.*, 2003) Kerstetter et al, 2001) is considered as the main reason behind the polarity of the lateral organs. In lateral organs the HD-ZIP genes are expressed in the meristem and adaxial regions whereas the KAN genes are expressed in the abaxial region of the same. The phenotype of the gain-of-function mutants of *PHV*, *PHB* and *REV* genes show in floral and leafy organs that are adaxialized, which is alike to the phenotypes of *kan* mutants. The phenotype of triple mutant *phv phb rev* shows cotyledons that are abaxialized and also losses the SAM, which is identical with the transgenic lines over expressing *KAN1*. Therefore it could be inferred that these genes also regulate the vasculature polarity in a similar manner like polarity of leaf (Chen, 2009).

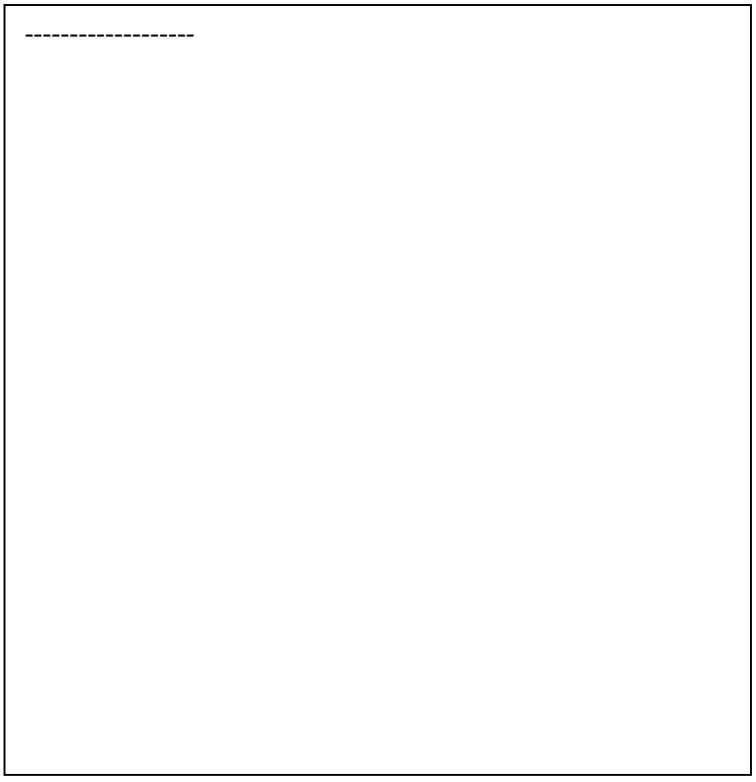

Figure 2.8 Interactions between miRNAs and their respective targets in determination of adaxial-abaxial leaf polarity. Black arrow indicates the positive regulation and the **T-lines** indicate the negative regulation**. Dashed lines** denote speculative behaviours and interactions [Image adapted from (Rubio-Somoza and Weigel, 2011)].

Earlier it has been reported that at the adaxial region the restriction of *PHB* expression is controlled by miRNA-mediated regulation. However, the mechanism behind this is not clear till date. miR165/166 mediates the transcript level cleavage of *PHB*, *PHV*, and *REV*, therefore it could be inferred that HD-ZIP transcripts get restrictions via miR165/166 in the abaxial domain that clears the mRNAs from this region (**Fig 2.8**). miR165/166 is also

responsible for DNA methylation of the two genes of *HD-ZIP* gene family, *PHB* and *PHV*. Therefore, it seems that there is a substitute mechanism through which the mRNAs of transcription factors in the abaxial region got resistance by the miRNAs. The regulation of *HD-ZIP* genes mediated by miR165/166 is evolutionarily conserved, and the sequences of these miRNAs are highly conserved among gymnosperms, angiosperms, ferns, mosses etc (Chen, 2009). Simultaneously, miR390–*TAS3* node is the determinant of abaxialization (Rubio-Somoza and Weigel, 2011). The crosstalk between miR165/166 and miR390–*TAS3* node depends upon the activity of *ARF3* and *ARF4* which have a positive regulation on miR165/ miR166 (**Fig 2.8)** (Li *et al.*, 2005a; Nogueira *et al.*, 2009).

2.7.4 Developmental transitions

During the vegetative phase and reproductive phase of development in plant, the leaves and flowers respectively are produced from the shoot apical meristem (SAM). During the vegetative phase, the juvenile leaves (come out earlier) are normally distinct from the adult leaves (that made later). Current findings suggest that various developmental transitions of SAM are regulated by small RNAs (Chen, 2009; Rubio-Somoza and Weigel, 2011). Apart from *AP2*, miR172 governs a number of *AP2*-like genes (**Fig 2.9**), *TOE1*, *TOE2*, *TOE3*, *SNZ* and *SMZ*.

The mutant *toe1-1* causes a little early flowering whereas the *toe2-1* mutant has no effect on flowering time. On the other hand the double mutant *toe1-1 toe2-1* flowers much prior compare to wild type, indicating that *TOE2* and *TOE1* are not essential repressors of the vegetative-to-reproductive phase transition (Aukerman and Sakai, 2003). These studies suggest that miR172-*TOE* node significantly controls the vegetative-to-reproductive transition (Aukerman and Sakai, 2003). As the over expression of miR172 does not cause to reduction in *TOE1* transcript level, therefore might be another mode of regulation called translational inhibition is the possible mode of regulation of miR172-mediated *TOE1* node.

Interestingly, over expression of miR172 leads to a reduction in *TOE2* transcript amount, which indicates miRNA172 regulates several targets of it with a number of mechanisms (Aukerman and Sakai, 2003; Chen, 2009). Developmental transitions in plants

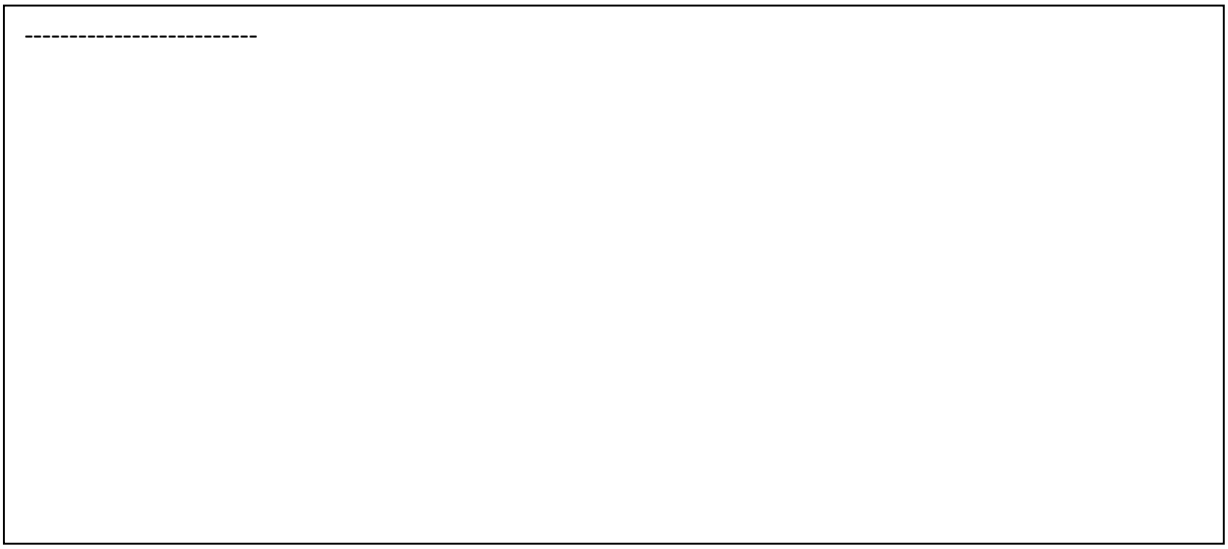

Figure 2.9 The interactions between miRNAs and their targets during developmental phase transition.Solid black arrows indicating positive regulation and **solid T-lines** indicating the negative regulation. Whereas the **dashed Lines** specify the speculative behaviours and interactions **[Image adapted from (Rubio-Somoza and Weigel, 2011) after modification].**

are maintained by the antagonistic activities of two miRNAs, miR156 and miR172 (Aukerman and Sakai, 2003; Wu and Poethig, 2006) (**Fig 2.9**). While miR156 expression level reduces with leaf age, there miR172 level of expression increases. The transcription factors *Apetala 2 (AP2)* and *Squamosa Promoter Binding Protein-Like (SPL)* are the respective targets of miR172 and miR156 and their expression patterns are complementary (**Fig 2.9**). Lower level activity of miR172 or higher activity of miR156 gives rise to delay of flowering, extended juvenile characteristics and pin down developmental transitions. The *SPL* can be divided into two categories: First one contains the small *SPL*s like *SPL5, SPL4* and *SPL3* and another one contains large *SPLs* like *SPL15, SPL10* and *SPL9.* From the recent studies it appears that larger *SPLs* are critical for leaf patterning, where as in trichome production and for flowering both of the groups are involved (Wu *et al.*, 2009; Yamaguchi *et al.*, 2009). During the transition from juvenile to reproductive phase, the larger SPLs specifically *SPL9* play a significant role by directly binding to the promoter region of *miR172b.*

A third set of sRNA nodes involved in regulating the vegetative phase transition includes trans-acting siRNAs (ta-siRNAs) from the *TAS3* locus. Mutant plants defective in ta-siRNA biogenesis pathway, which lack *RNA-dependent RNA POLYMERASE6* (*rdr6*), *DICER-LIKE4* (*dcl4*) and *ARGONAUTE7* (*ago7*), have enhanced level of *ARF3* and *ARF4.*

Consequently, they come into the adult phase prematurely, which can be evidenced by precocious commencement of abaxial trichomes and defective leaf pattern (Adenot *et al.*, 2006; Yant *et al.*, 2010). Interestingly, apart from targeting *ARF3* and *ARF4*, *TAS3*-tasiRNAs target members of the AP2-like family of transcription factors (Rubio-Somoza and Weigel, 2011) (**Fig 2.9**).

2.7.5 Floral organ and reproductive development

The origin of floral organs is floral meristem. The combined actions of three main classes of floral homeotic genes, called as the A, B, and C genes specify the floral organ primordia identities (Jack, 2004). The genes A and C perform antagonistically in order to confine each other within the floral meristem (Chen, 2004). One example of this phenomenon is the class C gene *AGAMOUS* (*AG*) mutations, which gives rise perianth organs in replacement of reproductive organs whereas class A gene *APETALA2* (*AP2*) mutations gives rise to the reverse effect. Basically, the transcript of *AG* was observed in floral meristem (inner two whorls) only, that will later convert into reproductive organs, where as the transcript of *APETALA1 (AP1)*, a class A gene, was only observed in the outer two whorls of the meristem which will soon be the perianth organs and the *AP2* mRNAs are present throughout the floral meristem. Though the transcripts of *AP2* are cleaved by miR172, but this couldn't completely explain the way of ruling of *AP2* by miR172. The over expression of miR172 produces the lower levels of AP2 protein but this transcript level only couldn't suggest that miR172 has a translational inhibition mode of action (Aukerman and Sakai, 2003; Schwab *et al.*, 2005). miR172 may cause to decreased amount of AP2 production in the inner two whorls even though the uniform production of *AP2* RNA was observed in all over the floral meristem.

In the reproductive development, miR159 also plays considerable role by the regulation of two *MYB* genes, *MYB65* and *MYB33*. The function of miR159 is very critical in restricting the *MYB65* and *MYB33* expressions to anthers. Transgenic plants consist of the miR159-resistant version of *MYB33* blocked or inhibited from growth at different stages, clearly indicates that the limitation of *MYB33* expression by miR159 is significant for plant growth and development (Millar and Gubler, 2005).

2.7.6 Leaf growth

Leaves appear from undifferentiated association of cells in the shoot apical meristem (SAM), while local auxin maxima help to decide where leaf primordia are formed. The boundary region between the undifferentiated cells is established by *CUP-SHAPED COTYLEDON1*

(CUC1) and *CUC2* which are targets of miR164. Plants compromised in *CUC2* and *CUC1* action have fused cotyledons, nearly similar mutants affected in miR319–*TCP4* node of regulation (Aida *et al.*, 1997; Palatnik *et al.*, 2003). The phenotypic association to miR319–T*CP* and miR164–*CUC* nodes was again confirmed by the finding that *TCP*s can straight bind to miR164a regulatory sequences, which governs its expression level (**Fig 2.10**). The regulation of cell proliferation in *Arabidopsis* by miR319–*TCP* nodes is moderately intervened by the conserved miR396–*GRF* (Growth Regulator Factor) node (Rodriguez *et al.*, 2010b) (**Fig 2.10**). Any kind of dispute in the regulation of miR319–*TCP4* node gives rise to decrease in GRF expression and also lower the mitotic activity (Rodriguez *et al.*, 2010b) (**Fig 2.10**). In both *Lotus japonicas* and *Arabidopsis*, the miR390–*TAS3–ARF* node is regarded as to regulate the leaf margin growth (Fahlgren *et al.*, 2006; Yan *et al.*, 2010).

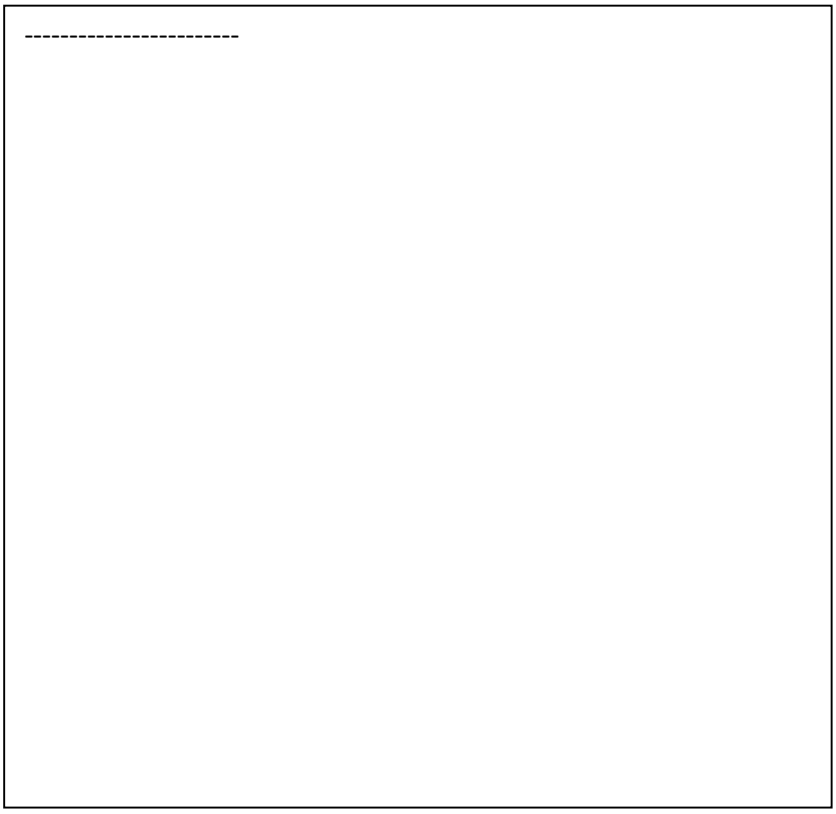

Fig 2.10. miRNAs and their target interactions regulating cell proliferation in leaves (Image adapted from (Rubio-Somoza and Weigel, 2011) after modification.

2.8 Plant hormones act as crucial player for breaking seed dormancy and initiation of germination:

Although required in very low concentrations, but also the phytohormones like abscisic acid (ABA), gibberellins (GA), ethylene, brassinosteroids (BR), auxins, cytokinins and other

signalling molecules have noteworthy effects on plant growth and development. These bio molecules act like chemical messengers in order to maintain communications in between cells, tissues and several organs in higher plants. For the successful maintenance and regulation of seed dormancy and germination also these plant hormones are awfully essential (Koornneef *et al.*, 2002); Till date we have very limited information regarding the molecular mechanism controlling seed dormancy and the release from seed dormancy in response to environmental and hormonal cues.

2.8.1 Abscisic acid (ABA):

ABA helps to keep seed dormancy and restricts seed germination. Therefore we can interpret that ABA acts as an up or positive regulator of seed dormancy and down or negative regulator of seed germination. It is assumed that following maturation and dessication tolerance, seed development become completed through successful completion of primary dormancy, when water content decreases, ABA and storage compounds are accumulated. For receiving the sensitivity of ABA no receptors are known so far (Finkelstein *et al.*, 2002). Only few probable candidate genes are recognized till date, like *RPK1* (Osakabe *et al.*, 2005; Razem *et al.*, 2004). During early ABA signalling in seed and seedlings, *RPK1* may act as a key membrane-bound regulator, which is a leucine-rich repeat receptor-like protein kinase. The knock out mutant of *RPK1* seeds showed reduced ABA sensitivity during germination. It was reported that for the induction and mainly for the maintenance of the dormant state in several plant species, endogenous ABA is required (Bewley, 1997; Hilhorst, 2008; Koornneef *et al.*, 2002; Leubner-Metzger, 2007). Similarly, the gain of function mutant of ABA biosynthetic genes showed higher rate of accumulation of ABA content which in turn enhance seed dormancy as well as reduces germination potential (Frey *et al.*, 1999; Lindgren *et al.*, 2003; Nambara and Marion-Poll, 2003; Thompson *et al.*, 2000). Due to obstruction of catabolism of seed ABA, enhanced seed dormancy along with increased ABA content was observed in *Arabidopsis cyp707a2* mutants (Kushiro *et al.*, 2004). In *Arabidopsi*s, the protein ABSCISIC ALDEHYDE OXIDASE 3 (AAO3) is known as the final product in ABA biosynthesis. This protein itself acts as an objective of a loop (self regulatory) governing ABA biosynthesis (Xiong *et al.*, 2001). The *aao3* mutant seeds exhibit lesser biosynthesis of ABA and as a consequence of that show reduced seed dormancy level (Gonzalez-Guzman *et al.*, 2004). *abi1* to *abi5* and *abi8* are the ABA-insensitive (abi) response mutants in *Arabidopsis.* These mutant seeds were identified from their control counterpart wild type

seeds through selecting well germinating and good seedling growth after treating with the presence of exogenous ABA concentrations, which inhibit seed germination in wild type seeds (Koornneef, 1994). The three well characterised transcription factors like *ABI3, ABI4, ABI5* belong to several transcription factor families: *B3-, APETALA2-* and *bZIP* domain and govern the overlapping subsets of specifically seed-related and/or ABA-associated genes (Finkelstein, 1994; Finkelstein and Lynch, 2000; Finkelstein *et al.*, 1998). The characterisation of maize ABA-insensitive *viviparous1 (vp1)* mutant was done by extreme seed responses, like decreased seed germination sensitivity in response to exogenous ABA and vivipary (Li and Foley, 1997; McCarty, 1995; Schwechheimer and Bevan). For proper ABA activity, the essential transcription factors belonging to B3 domain class are encoded by the two orthologous genes, the *Arabidopsis ABI3* and the maize *VP1.* In *FUS3* and *LEC2* of *Arabidopsis* (Rohde *et al.*, 2000) and *AfVP1* in wild oat *(Jones et al., 2000)* are the other members of this B3 domain class that influence germination.

MARD1 (Mediator of ABA-regulated dormancy1) is another component of ABA signalling pathway. *Mard1* mutant seeds are super capable in germination, they are less dormant compare to their wild-type counterpart, exogenous ABA couldn't affect them and interestingly they are capable to get release from dormancy even in absolute dark circumstances (He and Gan, 2004). A number of mutants which are ABA-hypersensitive in *Arabidopsis* like *enhanced response to ABA1 (era1)* and another *supersensitive to ABA and drought (sad1)* show increased dormancy (Brady and McCourt, 2003; Cutler *et al.*, 1996; Ghassemian *et al.*, 2000; Xiong *et al.*, 2001). It had been observed that with the application of a very tiny concentration of ABA, the germination of these seeds are inhibited, where as this couldn't affect the control wild type seeds. It is regarded that protein farnesylation act as downregulator of ABA sensitivity, and the β-subunit of a farnesyltransferase is encoded by *ERA1.* In *Arabidopsis* the protein arabinogalactan (APG30) plays a significant role in seed germination since it controls the schedule of germination by tuning the perception of ABA and seemingly by changing the force created by the protruding radicle (Van Hengel and Roberts, 2003). The induction of *Class I β-1,3-glucanase* (*βGlu I*) in Nicotiana seeds does happen post testa rupture and just at the beginning of endosperm rupture (Leubner-Metzger, 2007). That induction is entirely confined to the micropylar endosperm from where the radical cell will come out. Also this induction of *βGlu I* during seed germination in tobacco is inhibited by ABA which distinctively delays endosperm rupture.

2.8.1.1 ABA biosynthesis and signalling:

The various physiological events like seed dormancy, maturation and various adaptations in response to different stresses are regulated by the phytohormone abscisic acid (ABA). In accordance with the developmental progression and changing environments the level of endogenous ABA varies, which acts as a signal to the regulation of that physiological processes. The appropriate stability between biosynthesis and catabolism is the major controlling factor of the endogenous ABA level. Not only in plants, but also in several other organisms ABA is synthesized.

The fifteen carbon atom containing structure C15 sesquiterpene is regarded as Abscisic acid (ABA).This originates from the precursor isoprene, also called isopentenyl pyrophosphate (IPP). Different genes act significant roles in ABA biosynthesis, like *9-CIS-EPOXYCAROTENOID DIOXYGENASE (NCED)*, *ZEAXANTHIN EPOXIDASE PROTEIN (ZEP), ALDEHYDE OXIDASE(AAO)* and *SHORT-CHAIN ALCOHOL DEHYDROGENASE/REDUCTASE (SDR)* **(Fig 2.11).** ABA biosynthesis involves two individual pathways (Nambara and Marion-Poll, 2005; Schwartz and Zeevaart, 2010). First one is the direct pathway, occurs in phyto pathogenic fungi; second one is an indirect pathway, occurs in only plants. The synthesis of IPP in direct pathway include mevalonate (MVA) pathway which happen in most of the eukaryotes and in some prokaryotes (Newman and Chappell, 1999).Whereas, as a source of IPP the indirect pathway includes the methylerythritol phosphate (MEP) pathway. The second pathway (MEP) generally practiced by all photosynthetic eukaryotes and cyanobacteria (Lichtenthaler, 1999).

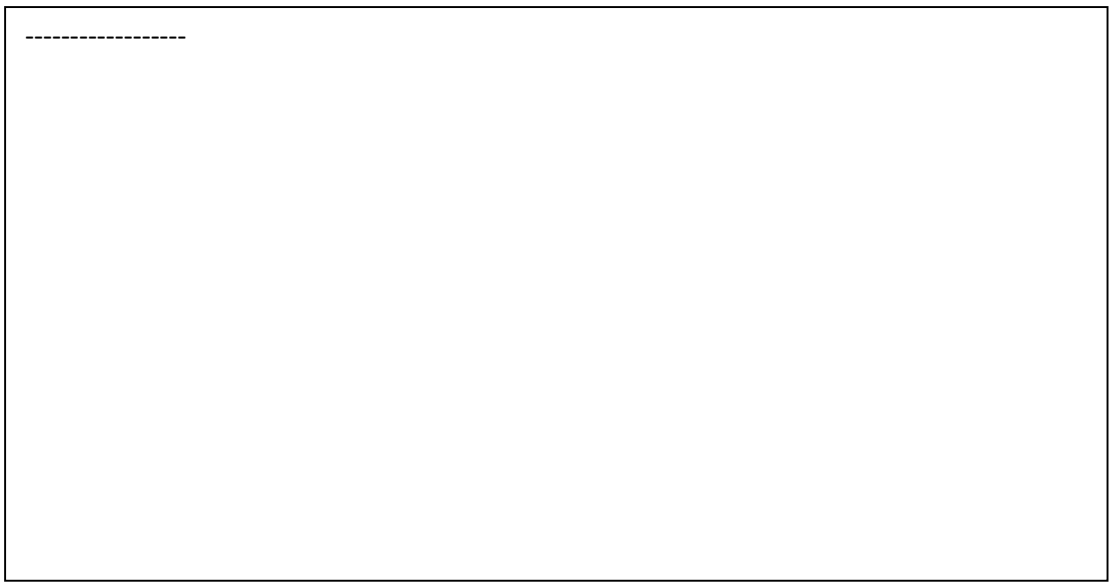

Fig 2.11 Biosynthetic pathway of ABA in higher plants. In plastids through an oxidative cleavage reaction from precursors **C_{40} epoxycarotenoid** ABA is derived. **In cytosol,** the **Xanthoxin** (C_{15} intermediate)is converted to ABA after two-step reaction via ABA-aldehyde. **Different abiotic stresses such as drought and salt activate the biosynthetic genes,** most probably through a Ca^{2+} dependent cascade as shown on the left. ABA feedback excites the expression of the biosynthetic genes, which is also presumably through a Ca^{2+} dependent phosphoprotein series of interaction. Among several biosynthetic genes, ***NCED*** is strongly upregulated by abiotic stress whereas ***SDR*** is regulated by sugar. ABA biosynthetic genes are denoted with small ovals. The ***NCED*** step probably limits ABA biosynthesis in leaves (denoted with a dashed arrow). ***NCED*: *9-cis-epoxycarotenoid dioxygenase*; *ZEP*: *zeaxanthin epoxidase ; MCSU*: *MoCo sulfurase; AAO: ABA-aldehyde oxidase*** [Image adapted from (Xiong and Zhu, 2003)] .

2.8.2 Gibberellins (GAs):

ABA and GA perform mutually during the entire life cycle of a seed. Earlier reports suggest that ABA enhances dormancy during seed maturation, where as GAs release dormancy, endorse germination and work against ABA effects. Therefore collectively we can say that GA plays a significant role in the release from seed dormancy and in the execution of seed germination process. In many species the GA biosynthesis within developing seeds gives rise to storage and accumulation of either bioactive GAs or bio inactive GA precursors (Yamaguchi *et al.*, 2001). GA biosynthesis itself indicates other characteristics of seed development like fertilization, growth of embryo and fruit and avoidance of seed abortion (Hays *et al.*, 2002; Koornneef *et al.*, 2002; Singh *et al.*, 2002). In maize mutants, upon analysis on ABA insensitive mutants, ABA deficient mutants and with the aid of GA biosynthesis inhibitor it has come out that GA acts as an enhancer of vivipary, although it is the ratio of GA/ABA, not the total hormone quantity that regulate vivipary (White *et al.*, 2000). During *Arabidopsis* seed germination the different expression pattern of GA biosynthesis genes have been observed (Ogawa *et al.*, 2003; Yamaguchi *et al.*, 2001; Yamauchi *et al.*, 2004). At two distinct locations within the embryo the bioactive GAs become concentrate just before the advancement of radicle protrusion. One is the early biosynthetic pathway in the provascular tissue, which also includes the geranyl geranyl diphosphate cyclization reaction, that again catalysed by ent-copalyl diphosphate synthetase (CPS); and another is the late biosynthetic pathway in the cortex and endodermis of the root , that includes the development of bioactive GA by GA 3- oxidase. In this reaction GA3ox2 and GA3ox1 mRNAs mount up, and the localisation of the promoter activity of GA3ox2 gene become established. This indicates that for the production of bioactive GAs an intermediary (possibly ent-kaurene) is essential which acts as intercellular transport. Generally two specific functions of GA are considered very significant during seed germination (Bewley, 1997; Hilhorst, 2008; Koornneef *et al.*, 2002; Leubner-Metzger, 2007).

(1) GA enhances the growth potential of the embryo. (2) GA is essential to prevail over the mechanical restraint vested by the seed-coating layers, through debilitating the tissues that surround the radicle (Bewley, 1997).

Several environmental signals like temperature, light etc. can act as the regulator in GA biosynthesis localization which is tissue specific (Yamauchi *et al.*, 2004). The expression of GA biosynthetic genes and its responses are equally significant for the GA regulated response of seeds. The GA-insensitive (*gai)* mutant is considered as one of the significant among GA-response mutants of *Arabidopsis,* which is characterised by increased GA content, a dwarf phenotype and complex seed characteristics accompanied with critically lower GA sensitivity during liberation from dormancy and seed germination (Derkx and Karssen, 1993; Koornneef, 1994; Richards *et al.*, 2001). For *gai* mutant, no significant seed germination was observed in dark, but interestingly, after-ripening a blend of light with either dry or chilling effect gives rise to release from dormancy and establishes germination. The *GAI* gene in *Arabidopsis* and its orthologues in other species encode some nucleus-localized proteins which function as a transcription factor that repress the GA-signal transduction pathway. In *Arabidopsis repressor-of-ga1-3(RGA), RGA-like1 (RGL1), RGL2 and RGL3* act as a repressor of GA responses. These regulators belong to DELLA subfamily of GRAS regulatory proteins, which also include GAI protein (Peng and Harberd, 2002; Richards *et al.*, 2001). In *gai* mutant there is the deletion of DELLA domain region, which is responsible for fine tuning the GA response. This in turn acts as a gain-of-function mutation in the *gai* mutant, which is categorized by prevailing GA-insensitive repression of GA responses. The absence of four DELLA genes (*GAI, RGL1, RGL2 and RGA*) causes gibberellins and light independent seed germination in *Arabidopsis* (Cao *et al.*, 2005). It is assumed that SLY1 and CTS proteins are the master regulators of GA signalling within seeds. The two mutants in *Arabidopsis, comatose (cts)* and the *sleepy1 (sly1*) mutants exert significantly lower seed germination potential which even couldn't be compensated with GA (Steber *et al.*, 1998; Strader *et al.*, 2004). The CTS encode an ATP-binding cassette (ABC) transporter class of protein, which is also a proximal protein. This protein acts as the critical control unit for the promotion from dormancy to germination (Footitt *et al.*, 2002). Seed stratification (cold treatment of seeds at 4˚C), generally regarded as to synchronise and promote seed germination, also cause to increase in bioactive GA content and responsible for GA3ox1 transcripts into the entire radicle and in the alurone layer of seeds (Yamauchi *et al.*, 2004). Therefore both reduced temperature and light could alter the pattern of expression of GA

biosynthetic genes. The seed germination of N. tabacum is also controlled by phytochrome, and GA substitute which are required as a trigger for red light, essential for photo dormancy release, that include the induction of dark germination (Leubner-Metzger, 2007). Therefore in summary we can say that GA helps to release from embryo and coat enhanced dormancy, directly or indirectly check the inhibitory effects of ABA and of course promotes seed germination.

2.8.2.1 GA biosynthesis and signalling:

The GAs (gibberellins) comprise a large group of diterpenoid carboxylic acids that are ubiquitous in higher plants, in which certain members function as endogenous growth regulators, promoting organ expansion and developmental changes such as stem elongation, germination, flowering, and fruit ripening (Hedden and Thomas, 2012). GAs are usually produced from the methylerythritol phosphate (MEP) pathway in higher plants (Hedden and Thomas, 2012). In this pathway, bioactive GA is produced from trans-geranylgeranyl diphosphate (GGDP) (Hedden and Thomas, 2012). In the MEP pathway, three classes of enzymes are used to yield GA from GGDP: 1) terpene synthases (TPSs), 2) cytochrome P450 monooxygenases (P450s), and 3) 2-oxoglutarate–dependent dioxygenases (2ODDs) (Yamaguchi, 2008). There are several steps, included in the MEP pathway which is shown in (**Fig 2.12**).

There are two classes of gibberellins, based on the presence of either 19 or 20 carbons. The 19-carbon gibberellins, such as gibberellic acid, have lost carbon 20 and, in place, possess a five-member lactone bridge that links carbons 4 and 10. The 19-carbon forms are, in general, the biologically active forms of gibberellins. Hydroxylation also has a great effect on the biological activity of the gibberellin. In general, the most biologically active compounds are dihydroxylated gibberellins, which possess hydroxyl groups on both carbon 3 and carbon13 positions. Gibberellic acid is a dihydroxylated gibberellins. The The bioactive GAs are GA1, GA3, GA4, and GA7 (Yamaguchi, 2008). Biologically active GA binds to GID1 receptor, then they are together transduced to the nucleus, if the binding occurred in the cytoplasm. In the nucleus, the GA–GID1 complex binds to a DELLA protein, and they together form a triplicate complex where the conformation of the molecule is changed (for instance, by phosphorylation; however, it is not compulsory). Then, F-box of the DELLA-protein binds to the GID2 and SLY1 proteins and the E3-ubiquitinligase–Skp–Cullin–FBox complex. Such a large complex is recognized by 26S proteasome and destroyed.

Fig 2.12 The biosynthetic pathway of Gibberellins (GA) [indicated with red arrow] and GA signalling by inhibition of DELLA proteins (indicated as T-line). [Image adapted from (Ferguson *et al.*, 2011)].

In the absence of GA (the left side of Fig. 2.13), the DELLA protein negatively regulates the GA response: SCF^{SLY1}E3-ubiquitin-ligase is unable to interact with the DELLA proteins

(Chebotar and Chebotar, 2011). Thus, the DELLA proteins are stored in the cells and inhibit GA responses, such as seed germination, stem elongation, flowering, etc.

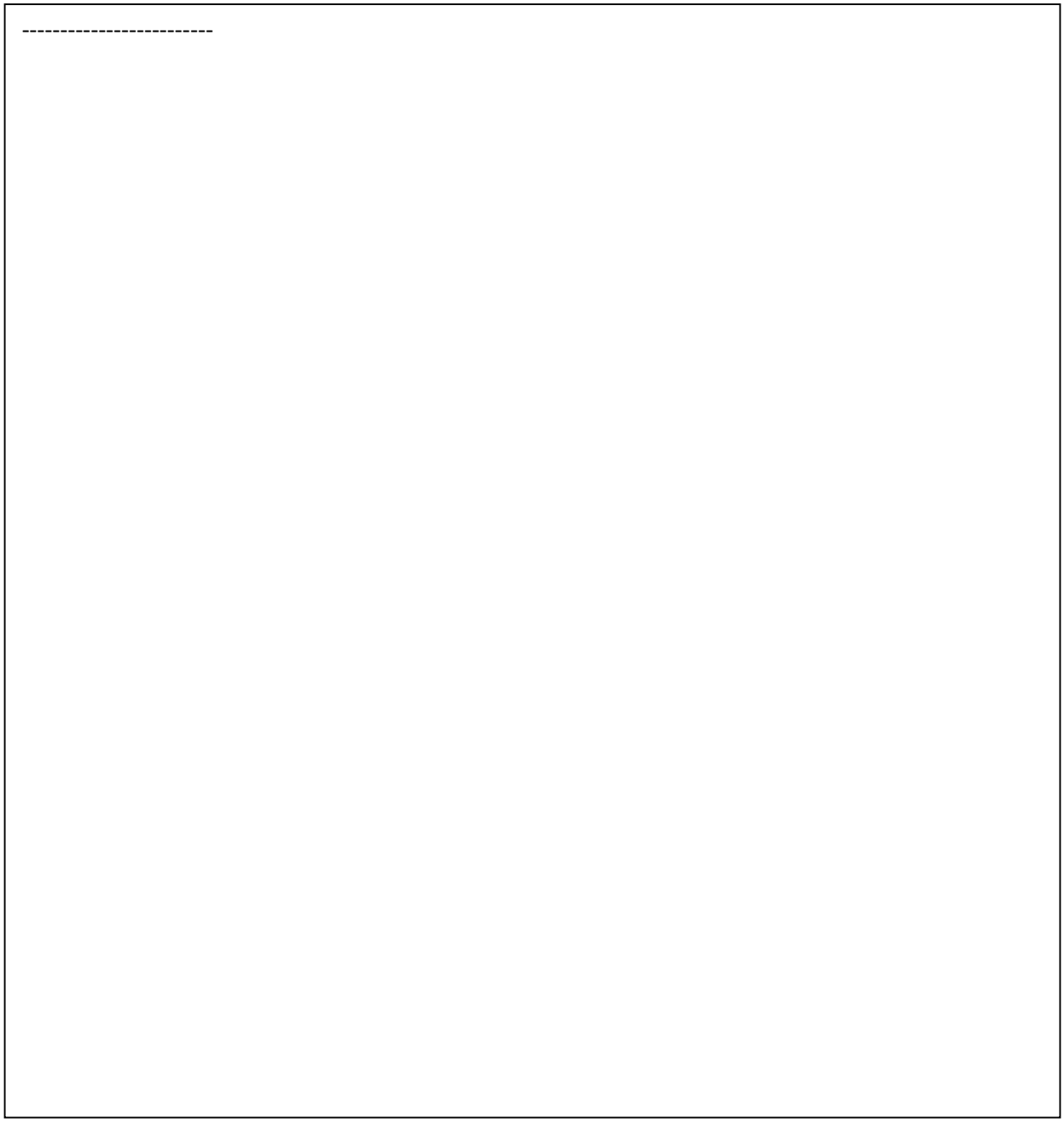

Fig 2.12 Diagrammatic presentation of the interactions between the Abscisic acid (ABA), Gibberellin (GA) and Ethylene signalling pathways in the regulatory process of Seed Dormancy and Germination. This hypothetical representation is mostly based on hormone mutational analyses in *Arabidopsis.*The interactions based on extranal enhancer or suppressor are represented by thin grey lines. Interactions are indicated by bold arrows and blocks, respectively. Small black arrows specify the upregulation (upward arrow) or downregulation (downward arrow) of seed dormancy. Whereas small blue arrows specify increase (upward arrow) or decrease (downward arrow) of seed ABA sensitivity of the mutant of the corresponding proteins.The hormonal mutant of *Arabidopsis thaliana* mentioned here are : *rga = repressor-of-ga1-3; rgl 1, rgl2 = rga-like1, 2; aba1, aba2 = ABA-deficient1,2*; *abi1 to abi5 = ABA-insensitive1* to *ABAinsensitive5; era3 = enhanced response to ABA3*; *ein2, ein3 = ethylene insensitive2, 3*; *ctr1 = constitutive triple response1; gai = GA insensitive; sly1 =sleepy1; spy = spindly;* **Other abbreviations: *GA3ox*** *= GA 3-*

oxidase; ***Man*** = *mannanase;* ***vp1*** = *viviparous1 (*maize mutant*);* **EREBP** = ethylene responsive element binding protein; **ERF** = ethylene responsive factor; **ACS** = ACC synthase; **ACO** = ACC oxidase [**Image adapted from (Kucera *et al.*, 2007)].**

2.8.3 Ethylene (ET):

Counteraction of ABA effects and promoting seed germination are one of the significant characteristic of ethylene. The amount of ethylene produces in non dormant seeds is higher compared to dormant seeds (Matilla, 2007). There are several hypotheses to elucidate the device(s) of how ethylene works in germinating seeds (Matilla, 2007). It is assumed that in the embryonic hypocotyls the advancement of radial cell expansion, improved seed respiration or enhanced water potential is the primary ethylene action. In the micropylar endosperm of tobacco, elevated level of stimulation of ABA sensitive *class I β-1, 3-glucanase (βGlu I)* gene, require endogenous ethylene for the promotion of endosperm rupture (Leubner-Metzger, 2007). It is regarded that a family of receptors linked to ETR1 of *Arabidopsis* are responsible for the perception of ethylene. During seed germination it had been found that the mRNA quantity of three ethylene receptor genes [βGlu I, cysteine proteinase and 1-aminocyclopropane-1-carboxylic acid (ACC, the ethylene precursor) oxidase (ACO, the ethylene-forming enzyme)] become increased (Cervantes *et al.*, 1994; Lashbrook *et al.*, 1998; Leubner-Metzger, 2007; Puga-Hermida *et al.*, 2003). Both biosynthesis and sensitivity of ethylene are considered as vital for tobacco (Leubner-Metzger, 2007) and *Arabidopsis* (Beaudoin *et al.*, 2000; Gallardo *et al.*, 2002; Ghassemian *et al.*, 2000) seed germination. Earlier report suggests that seed germination of *ctr1(constitutive triple response1)* mutant is not enough sensitive to ABA response, which reflects through the germination of freshly harvested *ctr1* seeds, as a little faster germination rate of it was observed compared to its wild type counterpart (Beaudoin *et al.*, 2000). When ethylene is absent, *CTR1* is activated by *ETR1,* and *CTR1* is known as the repressor of downstream signalling components. Also *CTR1* remain quiet (inactive) if ethylene is present.

In the absence of ethylene, *EIN2,* which itself is a downstream signalling component, is negatively regulated by *CTR1.* In *Arabidopsis,* like *etr1*, the *ethylene insensitive2 (ein2)* mutants show hypersensitivity to ABA response and higher rate of seed dormancy. Interestingly, in seed germination ABA-insensitive *abi1-1* mutant, the *ctr1* and *ein2 mutants* act like an enhancer and suppressor mutants (Beaudoin *et al.*, 2000). Another mutant *Arabidopsis enhanced response to ABA3 (era3)* is characterised by enhanced sensitivity to ABA of seeds and excess build up of ABA (Ghassemian *et al.*, 2000). The alleles of *era3* are known to be the new alleles of the *EIN2* locus. The allele *ein2-45* enhances seed dormancy, however this characteristic is absolutely checked by strictly ABA-insensitive mutations, such as *abi3-4* (Beaudoin *et al.*, 2000). As a result of that, the *ein2-45 abi3-4* double mutant

becomes non dormant like *abi3-4* single mutant. This phenomenon suggests that ethylene acts like a suppressor of seed dormancy by impeding ABA action. Therefore, *EIN2* is considered as a probable down regulator of ABA response. Thus, ethylene appears to work against the inhibitory effects of ABA during seed germination by making obstruction to ABA signalling.

2.8.3.1 Ethylene biosynthesis and signalling:

The pathway of ethylene synthesis is well established in higher plants (Bleecker and Kende, 2000). Ethylene is formed from methionine via S-adenosyl-Lmethionine (AdoMet) and the cyclic non-protein amino acid 1-aminocyclopropane-1-carboxylic acid (ACC). ACC is formed from AdoMet by the action of ACC synthase (ACS) and the conversion of ACC to ethylene is carried out by ACC oxidase (ACO) (Alexander and Grierson, 2002). In addition to ACC, ACS produces 5'-methyl thioadenosine, which is utilized for the synthesis of new methionine via a modified methionine cycle. This salvage pathway preserves the methylthio group through every revolution of the cycle at the cost of one molecule of ATP. Thus high rates of ethylene biosynthesis can be maintained even when the pool of free methionine is small.

2.8.4 Brassinosteroids(BRs):

Brassinosteroids interact with GA, together with light, and are regarded as the up regulator of seed germination (Altmann, 1999). The existence of endogenous BR have been observed in seeds of different species (Schmidt *et al.*, 1997). In *Arabidopsis* brassinosteroids are recognised by the leucine rich repeat receptor kinase, *BRI1* (BR insensitive 1) which is regarded as plasma membrane-localized, and different homologues have been reported, making use of mutants of other species that are BR-insensitive (Kinoshita *et al.*, 2005; Szekeres, 2003). The exogenous application of BRs increase germination of specific parasitic angiosperms (Takeuchi *et al.*, 1995), mutants of *Arabidopsis* (Steber and McCourt, 2001), cereals (Yamaguchi et al., 1987) and tobacco (Leubner-Metzger, 2001), but it couldn't affect the germination of non-endospermic, non-photo-dormant cress seeds that imbibed in the absence of light (Jones-Held *et al.*, 1996). In presence of light, in pre-chilled (non-dormant) BR-insensitive response mutant *bri1-1* and BR-deficient biosynthesis mutant *det2-1* of *Arabidopsis,* BR promotes the germination (Steber and McCourt, 2001). The seed germination of *bri1-1* and *det2-1* mutants is robustly inhibited by ABA compared to

germination in control wild type, which implies that BR is capable to partly prevail over the inhibition of germination due to ABA. Exogenous BR treatment helps to rescue the phenotype of seed germination of the strong GA-deficient biosynthesis mutant *ga1-3* which needs usually GA treatment from the release of dormancy and germination. BR treatment can also save the germination phenotype of the strong GA-insensitive mutant *sly1* to some extent, which also can't be done by GA treatment. After screening for BR-dependent germination a new allele for *sly1* has been found out, which indicates connections between GA and BR signalling in seeds (Steber *et al.*, 1998; Steber and McCourt, 2001). The above results indicate that BR might play important role to promote germination of *Arabidopsis* seeds. These findings and other several reports collectively suggest that BR and GA work parallel while counteracting ABA effects to establish cell elongation and germination.

2.8.4.1 BRs biosynthesis and signalling:

Brassinosteroid is a plant steroidal hormone. Comparison studies between BR-deficient mutants and wild type plants were widely used to determine the catalytic functions of certain enzymes in the BR biosynthetic pathway (Kwon and Choe, 2005). These efforts reveal that the BR-specific biosynthetic precursor campesterol (CR) is first converted to campestanol (CN) and then to BL via two that the BR-specific biosynthetic precursor campesterol (CR) parallel pathways, named the early and the late C-6 oxidation pathways (Zhao and Li, 2012). For the early C-6 oxidation pathway, CN is chiefly converted to 6-oxocampestanol (6-oxoCN) and then to cathasterone (CT), teasterone (TE), 3 dehydroteaserone (3DT), typhasterol (TY), and castasterone (CS), respectively. In the second parallel pathway, CN is initially hydroxylated at C-22 to form 6-deoxocathasterone (6-deoxoCT) and is then converted to corresponding intermediates similar to those in the early C-6 oxidation pathway but in a C-6 deoxy forms. The two pathways converge at CS, which ultimately leads to the biosynthesis of BL. Brassinolide (BL), the final product of BR biosynthetic pathway and which is the most active BR (Zhao and Li, 2012). Some BR deficient mutants are *det* (de-etiolated) and *cop* (constitutive photo morphogenesis), *cpd* (constitutive photo morphogenesis and dwarfism) etc (Akira and Shozo, 1997).

2.8.5 Auxin(IAA):

Several previous studies indicate that auxin (IAA) signalling is essential for viability as well as pattern formation in early embryos during embryogenesis, although we have very limited

information till date about the role of auxin at the molecular level during seed germination. During imbibitions of Sorghum grains the amount of free indole acetic acid (IAA) reduces (Dewar *et al.*, 2008) and in maize kernels, auxin also governs the expression of catalase in the scutellum during germination (Guan and Scandalios, 2002). A correlation between dormancy, IAA and sprouting of wheat grains (pre-harvested) has been reported earlier (Ramaih *et al.*, 2003). During germination elevated level of IAA synthesis occurs in bean seeds (Bialek *et al.*, 1992). During the advent of radicle the content of free IAA decreases, and the synthesis of new IAA is recognized in the growing seedling. In bean seeds an IAA-modulated protein called lAP1 is considered to be responsible for fast development at some point in seed maturity (Walz *et al.*, 2002). Also, during germination this protein experiences fast degradation. The role of auxin in seed germination came into limelight recently, that auxin is known to affect seed germination in the presence of ABA (Brady and McCourt, 2003; Liu *et al.*, 2007).

2.8.5.1 Auxin biosynthesis and signalling:

Auxin is considered as an essential hormone for almost every aspect of plant growth and development (Zhao, 2010). Indole-3-acetic acid (IAA), the most important natural auxin in plants, is mainly synthesized from the amino acid tryptophan (Trp). Recent genetic and biochemical studies in *Arabidopsis* have established the first complete Trp- dependent auxin biosynthesis pathway. The first chemical step of auxin biosynthesis is the removal of the amino group from Trp by the TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS (TAA) family of transaminases to generate indole-3-pyruvate (IPA). IPA then undergoes oxidative decarboxylation catalyzed by the YUCCA (YUC) family of flavin mono oxygenases to produce IAA. This two-step auxin biosynthesis pathway is highly conserved throughout the plant kingdom and is essential for almost all of the major developmental processes (Zhao and Li, 2012).

The auxin signalling pathway involves the protein families TIR1 (transport inhibitor response1), ARF (auxin response factor), Aux/IAA transcriptional repressors, and the ubiquitin ligase complex that is a part of the ubiquitin-proteasome protein degradation pathway (Vanneste and Friml, 2012; Wang *et al.*, 2013). ARF proteins have DNA binding domains and can bind promoter regions of genes and activate or repress gene expression. Aux/IAA proteins can bind ARF proteins sitting on gene promoters and prevent them from doing their job. TIR1 proteins are F-box proteins that have three different domains giving

them the ability to bind to three different ligands: an SCF^{TIR1} ubiquitin ligase complex (using the F-box domain), auxin (so TIR1 proteins are auxin receptors), and Aux/IAA proteins (Dharmasiri *et al.*, 2005). Upon binding of auxin, a TIR1 protein's specific domain has increased affinity for Aux/IAA repressor proteins, which when bound to TIR1 and its SCF complex undergo ubiquitination and subsequent degradation by a proteasome. The degradation of Aux/IAA proteins frees ARF proteins to activate or repress genes at whose promoters they are bound (Delker *et al.*, 2008). The process, polar auxin transport, is directional, very strictly regulated, and based in uneven distribution of auxin efflux carriers on the plasma membrane, which send auxins in the proper direction. While PIN-FORMED (PIN) proteins are vital in transporting auxin in a polar manner (Benková *et al.*), the family of AUXIN1/LIKE-AUX1 (AUX/LAX) genes encodes for non-polar auxin influx carriers (Swarup and Péret, 2012).

2.8.6 Cytokinin:

The cytokinins were observed in developing seeds and mostly build up in the liquid endosperm (Emery *et al.*, 2000; Mok and Mok, 2001). It has been assumed that cytokinins are accumulated within endosperm, which is essential for the progression of cell division in the embryo. It was also suggested that they might play critical role in pattern formation within embryo during embryogenesis and in grain filling of cereals at very early stage. Interestingly, the content of cytokinin increases in the embryo, become low within endosperm, and started to decrease during water uptake or imbibitions (Dewar *et al.*, 2008). A cytokinin peak is correlated with the build up of α-amylase after radical extension. Cytokinins are involved in cell division and expansion of radicle. Within the embryonic regions it could also be reorganised in the regions where it can efficiently accumulates and control root growth. During germination high amount of ABA and a very small amount of bioactive GA was observed within embryo and it was reported that cytokinin/ABA interface plays a critical role in regulation of germination in Sorgham (Dewar *et al.*, 2008). There are evidences in some species where just cytokinins can break the seed dormancy (Cohn and Butera, 2017). During the release of lettuce thermo inhibition and conditioning of parasitic Striga species and Orobanche, by increasing ethylene biosynthesis cytokinins helps to release from dormancy following germination (Babiker *et al.*, 2000; Saini *et al.*, 1989). The correlation between cytokinins and ethylene was also reinforced by the invention that *Arabidopsis cytokinin-resistant1 (ckr1)* mutant is allelic to the *ethylene-insensitive mutant*

ein2 and also insensitive to ethylene (Fischer-Iglesias and Neuhaus, 2001). The mutants of *N. Plumbaginifolia* which is cytokinin-resistant have been isolated, show decrease in seed dormancy and also its seed phenotypes (pleiotropic) specify the ABA-cytokinin interactions (Rousselin *et al.*, 1992).

2.8.6.1 Cytokinin biosynthesis and signalling

Adenosine phosphate-isopentenyl transferase (IPT) catalyzes the first reaction in the biosynthesis of isoprene cytokinins. It may use ATP, ADP or AMP as substrates and may use dimethylallyl pyrophosphate (DMAPP) or hydroxymethyl butenyl pyrophosphate (HMBPP) as prenyl donors (Ildoo and Hitoshi, 2006). This reaction is the rate-limiting step in cytokinin biosynthesis. DMADP and HMBDP used in cytokinin biosynthesis are produced by the methyl erythritol phosphate pathway (MEP) (Ildoo and Hitoshi, 2006). Auxin is known to regulate the biosynthesis of cytokinin (Nordstrom *et al.*, 2004).

2.9 Molecular crosstalk of several miRNAs, hormones and abiotic stresses during Seed Germination and Dormancy

In the complete life cycle of an angiosperm plant, it is considered that from the developmental point of view there are two main phase transitional periods. Firstly it is germination, extended from seed to seedling stage (Huang *et al.*, 2013); second one is emergence of flowering, extended from vegetative to reproductive stage (Wu *et al.*, 2009). Recent studies indicate that those genes that are involved to flowering transition are also take part in dormancy to germination or embryo to seedling growth stages or vice versa (Huang *et al.*, 2013). Additionally different plant hormones and environmental factors also influence the seed germination programme (Finkelstein *et al.*, 2008; Liu *et al.*, 2007). Detailed characterization and analysis of multiple small RNA biogenesis pathway genes like *DCL1, HEN1, AGO1* and *HYL1* had shown that they exert severe developmental defects in embryo during embryogenesis and overall seed development (Willmann *et al.*, 2011). One fine example is *dcl1* mutant which produces early seed maturation phenotype than its control normal wild type seeds. The concept of the implication of small RNAs during seed germination and dormancy also has been focused following these studies. Leafy cotyledon (*LEC*) genes like *LEC2* and *FUS3* are the positive regulator of *DCL1.* Whereas *ASIL1, ASIL2* and *HDA6/SIL1* are considered as the repressor or negative regulator for early embryo

maturation *(Willmann et al., 2011)*. Multiple miRNAs like miR156, miR160, miR158, miR159, miR165/166, miR167, miR164, miR172, miR395, miR402, miR417 (Table 2.1) etc. simultaneously both act as activators and repressors during seed germination and dormancy phases (Huang *et al.*, 2013; Jung and Kang, 2007; Kim *et al.*, 2010a; Kim *et al.*, 2010b; Liu *et al.*, 2007; Martin *et al.*, 2010; Reyes and Chua, 2007). Upregulation of miR156 and downregulation of *SQUAMOSA-PROMOTER BINDING PROTEIN-LIKE* (*SPL*) and miR172 (Table 2.1) in mature embryo could also down regulate the developmental transition and also keep dormancy in intact condition (Huang *et al.*, 2013; Martin *et al.*, 2010).The imbibition process itself has been implicated to differentially down-regulate twelve miRNA families, miR156, miR159, miR164, miR166, miR167, miR168, miR169, miR172, miR319, miR393, miR394, and miR397; while four families, miR398, miR408, miR528 and miR529 were up-regulated during the seed germination (Li *et al.*, 2013) condition. Interestingly, two closely related miRNAs namely miR156 and miR157 have also been implicated in vegetative to reproductive phase change (Wu *et al.*, 2009), which clearly indicates their functional diversification. Through the findings of two ABA supersensitive mutants for germination likely *absg1* and *absg2* as the alleles of *dcl1* and *hen1*, the complex regulatory cross-talk between the hormones and the small RNAs was come into light. These two mutants showed positive regulation of the expression of ABA responsive genes (Zhang *et al.*, 2008). miR159 is one of the efficient player in the regulation of seed germination process by modulating GA and ABA hormone signalling (Table 2.1). Another way we can say that the expression of miR159 is maintained by both GA and ABA (Martin *et al.*, 2010). Earlier reports suggested that *GAMYB* acts as an activator and *DELLA* acts as a repressor for the GA signalling cascade (Finkelstein *et al.*, 2008; Peng and Harberd, 2002; Weitbrecht *et al.*, 2011). The *GAMYB* transcripts are governed by miR159 during the development of flower, fertility and seed germination (Reyes and Chua, 2007). Recent studies had revealed that alurone vacuolation, a GA-mediated programmed cell death (PCD) process in alurone layer of seed is essential for seed germination (Alonso-Peral *et al.*, 2010; Finkelstein *et al.*, 2008; Peng and Harberd, 2002). miR159 targets *MYB33* and *MYB101* which act as a positive regulator of seed germination and dormancy (Martin *et al.*, 2010; Reyes and Chua, 2007). The upregulation of miR159 was observed in case of *rdr2* and *dcl2 dcl3 dcl4* triple mutants. Interestingly *DCL2, 3, 4 and RDR2* are the critical factors for small RNA biogenesis, especially for heterochromatic siRNA biogenesis pathway (Allen and Howell, 2010; Axtell, 2013). These findings suggest that besides miRNAs, different kind of other small RNAs also could

essentially play significant role in seed germination and dormancy. The role of the plant hormone auxin in seed germination was come into light when (Liu *et al.*, 2007) showed that ARF10 is repressed by miR160 which plays actually very significant role in seed germination (Table 2.1) (Liu *et al.*, 2007). Auxin response factors (ARFs) play very important role in auxin signalling pathway during many plant growth and development. The miR160 is likely to act as the converging point of auxin and ABA regulated cross-talk during seed germination, as mutation in *ARF10* gives rise to developmental defects and over expression of ABA responsive genes (Liu *et al.*, 2007). In the same way it was found that over expression of miR160 caused hyposensitivity to ABA during germination (Liu *et al.*, 2007). Previous reports suggest that auxin homeostasis is essential for proper embryo development which is mediated by multiple miRNA actions like miR158, miR160, miR164, miR165/166 and miR167 (Martin *et al.*, 2010). This observation implies that miRNAs play a significant role during embryo and seed development by mediating suitable auxin signalling (Table 2.1). Therefore it could be inferred that several miRNAs not only act as an essential factor for maintaining dormancy but also actively helps in breaking from dormancy to promote embryo into seedling stage through seed germination (Huang *et al.*, 2013; Martin *et al.*, 2010; Zhang *et al.*, 2013). Ethylene, as a gaseous hormone promotes seed germination through interaction with ABA signalling (Finkelstein *et al.*, 2008). The two mutants namely *ethylene resistant1* (*etr1*) and *ethylene insensitive2* (*ein2*) or*, enhanced response to aba3* (*era3)* show up regulation of ABA responsive genes and delay in seed germination (Finkelstein *et al.*, 2008).Whereas wild type seeds treated with ethylene precursor ACC (1-aminocyclopropane -1-carboxylic acid) show down regulation of ABA response factors (Finkelstein *et al.*, 2008). Again, *etr1-2* mutant shows over accumulation of GA content, which could be a compensation to over accumulation of ABA (Finkelstein *et al.*, 2008). As miR159 and miR160 both have regulatory effects on ABA and GA, and ethylene has a cross talk with ABA and GA, therefore, it could be pre assumed that these miRNAs may have direct or indirect control over ethylene mediated regulation during seed germination and dormancy. Plant steroid hormone brassinosteroid(BRs) that mainly effect stem elongation and leaf unfurling also effect seed germination. Mutational analysis has revealed that BR biosynthetic and signalling pathway are sensitive to ABA leading to decrement in the germination potential (Finkelstein *et al.*, 2008). For the activation of miR160, the possibility of a cross talk between BR and ABA signalling cannot be ruled out in seed germination (Liu *et al.*, 2007). Again, BRs induce the expression of distinct EXPANSIN (EXP) family

members, which are cell wall loosening proteins that can indirectly influence seed germination (Bewley, 1997). Parallel studies indicate that those small RNA biogenesis pathway mutants which exposes higher expression of ABA, are strongly sensitive towards osmotic and salt stresses (Zhang *et al.*, 2008), thereby indicating the overlap with the environmental cues. miR395 (Table 2.1) acts both as an activator and repressor in case of seed germination under abiotic stress conditions (Kim *et al.*, 2010b). In *Arabidopsis* genome miR395 has six family members and they target *ATP Sulfurylases1, 3, 4(APS1, APS3, APS4)* and *Sulfate transporter (SULTR)* which are involved in sulfate assimilation and transport. Studies had shown that in spite of single nucleotide difference between miR395e and miR395c, miR395e cannot target *APS1* and *APS4* (Kim *et al.*, 2010b). In *Arabidopsis,* these miRNAs have also diverse effects on seed germination under dehydration and high salinity stress conditions. Over expression of miR395c reduces the germination potential under high salt or dehydration stress condition; whereas over expression of miR395e enhances the germination potential under the same stress condition in *Arabidopsis thaliana (Kim et al., 2010b)*.

Likewise over expression of miR402 (Table 2.1) increases the seed germination potential in *Arabidopsis* under dehydration, salinity and cold stress conditions (Kim *et al.*, 2010a). Another conserved miRNA, miR402 down regulates its target gene *DML3* (DEMETER-LIKE protein3), which is involved in DNA demethylation which is an epigenetic regulatory process of plants under various stress conditions (Kim *et al.*, 2010a). miR417 (Table 2.1) also exhibits a negative regulation over seed germination under salinity stress condition (Jung and Kang, 2007). However, the molecular mechanism of its action is not clear till date.

Table 2.1. List of miRNAs that are involved in seed germination & dormancy.

miRNAs	Targets of miRNA	Upregulation (↑)	Downregulation (↓)	Seed germination related function	References
miR395	ATP Sulfurylases (*APS1, APS3, APS4*); Sulfate transporter (*SULTR2:1*)	↑ (*APS1, APS4, SULTR2:1*by miR395c)	↓ (*APS1, APS4, SULTR2:1*by miR395c and *APS3 by miR395e*)	Regulatory effects on seed germination under salt &dehydration stress conditions	Kim et al, (2010b).

miR402	*DML3* (*DEMETER-LIKE protein 3*)		↓	Regulatory effect on seed germination & seedling growth under salt, dehydration & cold stress conditions	Kim et al, (2010a)
miR417	Unknown	Unknown	Unknown	Plays a role as a negative regulator of seed germination in *Arabidopsis thaliana*under salt stress condition.	Jung and kang, 2007
miR160	*ARF10, ARF16, ARF17*		↓ (*ARF10*)	*ARF10* mutant show up regulation of ABA responsive genes during germination	Liu et al, 2007
miR159	*MYB33, MYB65, MYB101*	↑ (*MYB33, MYB101)*		*MYB33*& *MYB101* are the positive regulators of ABA signalling during seed dormancy & germination.	Reyes & Chua, 2007
miR165/ 166	*PHB, PHV, REV* etc.	Unknown	Unknown	Maintain the auxin signal during seed development & maturation .So could have role in seed germination &dormancy also.	Huang et al, 2013
miR164	*NAC1, CUC1/CUC2*	Unknown	Unknown	Maintain the auxin signal during seed development & maturation.	Huang et al,2013
miR167	*ARF6, ARF8*	Unknown	Unknown	Maintain the auxin signal during seed development & maturation.	Huang et al,2013;
miR156	*SPL* 3, 4, 5		↓	Seed development & maturation.	Huang et al, 2013;

					Li et al, 2013
miR172	*AP2*	↑		Seed development & maturation.	Huang et al,2013; Li et al, 2013
miR158	Unknown	Unknown	Unknown	Seed development & maturation.	Huang et al,2013

(The first five miRNAs in the grey shaded region of the table are also involved in mediating the stress response signals during germination)

2.10 Future Perspective

Agriculture exclusively depends on growing crops; sothe success of cultivation as well as productivity largely depends on seed viability, seed germination and efficiency of seed development. miRNAs play critical roles in regulation of gene expression in developing and germinating seeds. miRNA regulated nodes, play crucial roles in regulating seed germination in response to different phyto-hormones and abiotic stresses. But the mechanism of action and the interconnection of the various signalling cascades with their regulatory networks remain largely unknown till date.Thus, functional analysis of miRNAs expressed in seeds or during germination process will provide useful information for seed biology. Future studies are required to unravel the molecular details of small RNAs As well as miRNA regulated pathways.