ROLE OF HORMONES ON SEED GERMINATION- A REVIEW

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ABSTRACT

Hormone is a Greek word derived from ‘hormao’ which means to urge or to stimulate. Thimann suggested to use the term ‘phytohormone’ for hormones of plant origin. According to him phytohormones are organic substances which are naturally produced in plants that regulate the growth or other physiological functions at a site remote from its place of production and active in extremely minute quantities. In plants growth is regulated through interactions of several growth substances. This can be well explained taking the example of vegetative growth in plants which depends upon cell division, enlargement and differentiation. All these are influenced by auxins, gibberellins, cytokinins, abscisic acid, ethylene etc. These have variable effects on the processes e.g. auxins increase cell division in cambium but inhibit the growth of lateral buds. Gibberellins also influence cambial growth and promote cell division in subapical (internodal) regions of shoot. Cytokinins promote cell division in callus tissues and promote growth of lateral buds. Abscisic acid inhibits cell division but its effect can be overcome by exogenous auxins, gibberellins, cytokinins etc. Ethylene has little effect on cell division but it has strong effect on cell enlargement.

Role of auxin in seed germination: Cholodny (1930) was first to suggest the use of hormone in agriculture who reported fifty five per cent increased yield of wheat by hormonisation of grains. Later it was reported by several scientists. In India auxins have been used widely to break the dormancy of seeds. Now-a days IAA, IBA, NPA, 2,4-D are most widely used in soaking of seeds for germination. Thus, auxins at a very low concentration promote germination but these effects are subjected to variation depending upon form and species of plant. Higher concentrations and prolonged treatment retard and reduce germination. The methylester of NAA is used to prevent the sprouting of potatoes.

Eiji Hirasawa suggests that the development of alpha – amylase in the detached cotyledons is regulated by endogenous phytohormone, probably by auxin. Then how is the alpha-amylase activity in the attached cotyledons regulated?. In intact seedlings an auxin from the embryonic axis may induce alpha-amylase activity in the cotyledons during germination. Treating the seeds of onion with IAA (100, 200 and 300 ppm) increased the seed germination followed by plant height, number of leaves, bulb diameter over control (Mathur, 1965).

Cell elongation:

- Loosening of cell wall
- Dissolution of cell wall material
- Breaking chemical bonds
- Synthesis of new cell wall

Loosening of cell wall

Decreased WP ZTP

Increased water uptake

Increased size of vacuole - cell stretches
Gibberelline:

1. Discovery:

   1. In 1920, it was discovered that the fungus Gibberella fujikuroi causes a disease in rice plants called "Foolish seedling" disease. Extracts from the fungus were applied to healthy plants and caused similar symptoms as those observed in diseased plants.

   2. In 1958, GA was isolated from immature seeds of Phaseolus coccineus. GA is a complex, 5-rings structure, with over 70 naturally occurring gibberellins identified. Out of these, some are active and some are not active.

Role of GA in seed germination:

   1. Imbibition of water stimulates the embryo to release gibberellins.

   2. Gibberellins stimulate the transcription of genes for hydrolytic enzymes in the aleurone layer of seeds. GA stimulates the transcription of amylase mRNA.

   3. Hydrolytic enzymes are secreted by dictyosomes into the endosperm, resulting in starch hydrolysis.

GA action in wheat seed germination:

At the cellular level, hormones attach to a protein receptor which sends a signal down a transduction pathway to switch on particular genes. Through transcription and translation, these lead to the production of an enzyme protein which actually causes the change in plant growth. A good example from the early stages of plant development is the role of GA in cereal seed germination. As the seed imbibes, the embryo produces GA. This induces synthesis of amylase in the aleurone layer which secretes the enzyme to the endosperm. Amylase breaks down starch to glucose which diffuses to the embryo and is used for the early stages of plant growth.

The increase in germination due to gibberellic acid might be due to the promoter role in releasing the dormancy mechanism and enhancement of alpha-amylase synthesis.

Abscisic Acid:

1. Discovery:

   In 1963, Frederic Addicot discovered a compound that stimulated abscission of cotton fruit and named it Abscisic acid. Abscisic acid is defined as a plant hormone that mainly acts to inhibit growth, promotes dormancy and helps to tolerate stressful conditions. Mosolov and Mosolova (1959) reported increased assimilation of nutrients by onion plants when GA3 at 50-100 ppm was treated, leading to higher germination and higher seed yield over control.

Seed maturation and dormancy:

Seeds are not only important agents of reproduction and dispersal, but they are also essential to the survival of annual and biennial plants. These angiosperms die after flowering and seed formation is complete. ABA plays a role in seed maturation, at least in some species, and also enforces a period of seed dormancy. As in buds, it is important the seeds not germinate prematurely during unseasonably mild conditions prior to the onset of winter or a dry season. ABA in the seed enforces this dormancy. Until

Table 2. Effect of dormancy breaking treatments on germination and seedling growth of seeds in rice variety ADT38, (Selvaraju, 2001)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Germination (%)</th>
<th>Root Length (cm)</th>
<th>Shoot Length (cm)</th>
<th>Dry Matter production (mg 10 seedlings(^{-1}))</th>
<th>Vigour Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>67</td>
<td>21.8</td>
<td>11.7</td>
<td>132</td>
<td>2244</td>
</tr>
<tr>
<td>GA 100 ppm</td>
<td>80</td>
<td>18.7</td>
<td>18.8</td>
<td>138</td>
<td>3000</td>
</tr>
<tr>
<td>GA 200 ppm</td>
<td>81</td>
<td>18.7</td>
<td>19.4</td>
<td>138</td>
<td>3086</td>
</tr>
<tr>
<td>GA 500 ppm</td>
<td>84</td>
<td>21.0</td>
<td>19.7</td>
<td>139</td>
<td>3419</td>
</tr>
<tr>
<td>GA 1000 ppm</td>
<td>91</td>
<td>23.5</td>
<td>19.9</td>
<td>143</td>
<td>3949</td>
</tr>
</tbody>
</table>
the seed has been exposed to a prolonged cold spell and/or sufficient water to support germination dormancy is not lifted.

**Abscission**: ABA also promotes abscission of leaves and fruits (in contrast to auxin, which inhibits abscission). It is, in fact, this action that gave rise to the name abscisic acid.

**Cytokinins**: Cytokinins play a key role in the life of higher plants. Skoog (1955) has demonstrated that when pith tissues of *Nicotiana tabacum* were separated from vascular and cortical elements, they grew similar to auxin containing medium and showed enormous enlargement without cell division. If vascular tissues were placed in contact to them, pith tissue resumed cell division. This observation proved instrumental in the discovery of cytokinins.

**Role of Cytokinins**: (i) Breaking the dormancy of seeds (*Lactuca sativa*) by mollifying the effect of ABA (ii) Root initiation

Treatment of ber seeds with kinetin at 100 ppm for 12 hours significantly improved seed germination and vigour index over control.

**Ethylene**:

I. **Discovery**: Neljubow (1901) discovered ethylene gas.

II. **Chemistry**: It is gas, colorless and smells like ether: 

\[ \text{H}_2\text{C} = \text{CH}_2 \]

III. **Functions of ethylene**:

- Abscission of leaves, fruits and flowers petals
- Stimulates fruit ripening
- Release of dormancy which is important for germination
- Drooping of leaves
- Flower formation in some species

**Role of Ethylene in seed germination**: Many seeds produce ethylene during germination, but the detailed role of this phytohormone remains unclear. To provide insight into the action of ethylene, experiments were performed in which the germination medium was enriched with ethylene (ethephon), Amino Cyclopropane 1-Carboxylic acid (ACC) substances that alter the biosynthetic pathway or compounds that inhibit the action mechanism (Baashi, 1991). In other studies, the experimental protocols included an examination of the effect that ethylene exerts on seed responses to an exogenous phytohormone whose action mechanism on some physiological process is relatively well understood (i.e., ABA, GA, auxins or kinetin).

Thus, it was demonstrated that exogenous ethylene accelerates germination in cocklebur, *Amatanthus retroflexus* (Bglye, 1980; Schonbeck and Egley, 1980), aged *Striga lutea* (Bglye and Dale, 1970) and aged *Brassica napus* seeds. From these data, it was suggested that ageing deteriorated the ethylene production system (Takayanagi and Harrington, 1971). Baashi et al. (1975), submitting cocklebur germination, demonstrated that exogenous ethylene boosted the germination percentage even more than anaerobiosis.

Ethylene reversed ABA inhibition of the germination of *A.hypogea* (Ketring and Morgan, 1972), *C. album* (Karssen, 1976) and also reversed the polyethylene glycol (PEG) inhibition

**Table 3. Effect of kinetin on ber seed germination and seedling growth at 90 days after growth.**

(Hiwale and Raturi, 1996)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Germination Days</th>
<th>Germination Percentage</th>
<th>Height Cm</th>
<th>Diameter Cm</th>
<th>Vigour Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kinetin 50 ppm</td>
<td>7</td>
<td>70.00</td>
<td>30.27</td>
<td>4.12</td>
<td>1816.66</td>
</tr>
<tr>
<td>Kinetin 100 ppm</td>
<td>7</td>
<td>83.33</td>
<td>30.82</td>
<td>4.27</td>
<td>3466.66</td>
</tr>
<tr>
<td>Kinetin 200 ppm</td>
<td>7</td>
<td>73.33</td>
<td>30.00</td>
<td>4.03</td>
<td>2280.00</td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>46.66</td>
<td>29.75</td>
<td>3.77</td>
<td>1090.00</td>
</tr>
</tbody>
</table>
of A. retroflexus seeds. Schnobeck and Egley (1980) hypothesized a timing sequence for redroot pigweed seeds, taking into account light, ethylene sensitivity, temperature, water stress and CO₂.

Germination of recalcitrant Quercus robur seeds exposed to light was inhibited by ethylene, ACC and ABA (Finch-Savage and Clay, 1994). In some seeds the promotion of germination by ethylene depends on an interrelationship with CO₂ (i.e. cocklebur, lettuce, Spargula arvensis or redroot pigweed), and the sequence of the CO₂ and ethylene sensitive phases could be changed by seed conditioning (Esashi et al., 1986, 1988). Secondary dormancy in cocklebur seed germination, was capable of counteracting CO₂ action in some cases, but was incapable of reversing the action of ethylene, suggests that Nitro-2-1,3-Benzoxa Diazol-4-yl (green fluorescent dye used to track synthetic lipids (NBD) acts with some side-effects besides being a competitive inhibitor of ethylene action (Ishizawa et al., 1988). Exogenous ethylene was capable of alleviating the chilling injury in peas, while Amino ethoxy Vinyl Glycine (AVG) and NBD tended to increase the chilling injury (Petruzzelli and Harren, 1997). By contrast, Echinacea angustifolia seeds need a continuous light treatment pre-chilling and ethephon in order to reach 100% germination (Feghahati and Reese, 1994).

In addition, short chain saturated fatty acids are known to inhibit the germination of chick pea seeds, and was reversed by ACC or ethylene (Gallardo et al., 1994). The mechanism of short-chain saturated fatty acid action is unknown, but in Cardaminus seeds these fatty acids negatively affected all steps in the transformation from AdoMet to ethylene (Gallardo et al., 1994). Furthermore, the sensitivity of various plant tissues (e.g. peanut and cyclopia seeds) to ethylene, increased as a result of short-chain saturated fatty acid incorporation into cell membranes (Whitehead and Nelson, 1992).

Finally, strigol, isolated from root exudates of cotton (Cook et al., 1972), stimulates germination in witchweed by promoting ethylene biosynthesis in the seed (Babiker et al., 1993a). Ethylene is a good germination promoter in witchweed (Egley and Date, 1970) and the soil atmosphere can contain ethylene (Smith, 1976) which positively or negatively affects germination in various seeds (Taylerson, 1979). Applying ethylene to soils can encourage germination of this devastating plant, after the plant becomes photosynthetically competent, it can be killed with herbicides (Egley, 1999).

Seed germination involves a series of hormonally regulated metabolic processes. Consequently, as germination involves the revival of the growth of the organ that breaks the seed coat, this part of the seed may contain the true target cells for certain phytohormones (e.g. ethylene). Egley (1999) rightly considers germination and dormancy in this light: “Dormancy (a reduced ability to germinate) results when some pre-germination events do not follow an essential sequential pattern and perhaps an orderly sequence of metabolic events is necessary to ‘set the state’ for germination” (Basu et al., 1974).

Various studies have demonstrated that ethylene production in certain seeds increased before radicle protrusion and this protrusion in certain seeds increased before reduced on trapping the ethylene produced. However, in wild oat an early temporary rise in ethylene production was reported in both dormant and non-dormant seeds (Adkins and Ross, 1981) and in seeds of peanut and bean, the application of Amino ethoxy Vinyl Glycine AVG effectively inhibited ethylene production without reducing germination (Hoffman et al., 1983). The use of inhibitors of ethylene synthesis and action
indicate the dependence of seed germination in some species on endogenous ethylene. The possibility that other compounds could replace the need for GAs in the GA-deficient mutants of Arabidopsis was studied by Karrsen et al. (1998). Thus, ethylene and light together induced full germination in the gal mutant in the absence of applied GA, the effect being much weaker in darkness (Groot and Karssen, 1987); fusicoccin was only compound tested that partly replaced the need for applied GA.

Little is known about the action and mechanism of ethylene in the germination of ethylene-dependent seeds. Logan and Stewart (1991, 1995) proposed that cytokinins elicited germination of *S. hermonthica* by stimulating ACC-oxidase activity. However, Babiker et al. (1993b) proposed that cytokinins affected ethylene biosynthesis and germination of *S. asiatica* by increasing ACC-oxidase activity rather than ACC-synthase. This germination can be inhibited by AVG and NBD. It is possible that stimulation of germination by ethylene and ACC in *S. hermonthica* led to a higher rate of cell division prior to radicle protrusion and that cell division required a higher rate of aerobic respiration than elongation (Logan and Stewart, 1991, 1995). Levy et al., (1972) reported enhanced flowering and fruit set in onion with the application of ethephon up to 960 ppm.

Baashi et al. (1987) later concluded that ethylene action could not be explained only in terms of regulation of the respiratory system. In a number of recent publications, the activity of Beta - cyanoalanine synthase (CAS), the enzyme likely to be involved in cyanide metabolism (Maruyama et al., 1998), is related to regulation of the cocklebur germination. Ethylene is capable of activating electron transport through both cyanide-sensitive and resistant pathways in cocklebur seeds (Baashi et al. 1982). The stimulation of aerobic respiration by ethylene in cocklebur was associated with increased mitochondrial development during inhibition (Baashi et al., 1975). Ethylene as well as cysteine and/or HCN (both substrates of CAS) increased the amino acid content in dormant and non-dormant cocklebur seed simultaneously with increased CAS activity (Maruyama et al., 1997). This ethylene induced, amino acid accumulation also occurred under anoxic conditions (Yoshiyama et al., 1996a,b). It appears that mitochondrial CAS activated by ethylene provided asparagines and aspartate and increased the amino acid pool during the pre-germination period (Maruyama et al., 1997). Ethylene action may also be related to amino acid accumulation in primed seeds (Yoshiyama et al., 1996a,b).

Chick-pea germination may require activation of mRNA transcription for ACC-oxidase, which can be inhibited by ABA and osmotic stress and stimulated by IAA and polyamines. Exogenous polyamines (i.e. putrescine or spermine) or the presence of inhibitors of their synthesis (i.e. cyclohexylamine or methylglyoxal - bis-guanylhydrazone), activated the transformation of AdoMet to ethylene, resulting in a strong stimulation of radicle protrusion under optimal (25°C) as well as non-optimal (30-35°C) germination conditions (Gallardo et al., 1992, 1994c, 1996). Cyclohexylamine (25°C) stimulated the mitotic index in the sub-apical and apical zones of radicle apex and plumule, respectively (Gallardo et al., 1994c). However, ethylene did not seem to have a significant effect on the mitotic activity of the radicle meristem, since the mitotic index was not altered by the addition of ethephon.

Finally, it is essential to mention osmopriming (pre-soaking of seeds in osmotic solutions that allow the seeds to imbibe water and initiate germination, but which do not permit radicle protrusion through the seed coat) and its relationship to ethylene biosynthesis. Osmopriming improves germination in certain seeds at suboptimal temperatures, and it was associated with increased respiration and gene
expression as well as ethylene production and ACC-synthase activity (Fu et al., 1988). Osmopriming of sunflower with PEG-6000 enhanced the conversion of ACC to ethylene (a good indicator of seed vigour, as indicated above) and probably increased ACC-oxidase activity (Chojnowski et al., 1997). The low ACC dependent ethylene production in aged sunflower seeds could be related to a reduced in vivo ACC-oxidase activity as in pea and cocklebur (Gidrol et al., 1991).

### Ethylene and thermoinhibition in seeds:

Most studies on the alleviation of thermoinhibition by ethylene have used lettuce seeds (Abeles, 1986). Lettuce germination (i.e. radicle protrusion) is sensitive to many internal and external factors (plant growth regulators, light, temperature and water availability) and depends on cell expansion initiated within the embryo (i.e. hypocotyl region). As the temperature of germination or inhibition is raised from optimal (25°C) to supraoptimal (30-35°C), germination is inhibited. This effect is called thermoinhibition. Such thermoinhibition can be overcome by treating seeds with ethylene. Therefore, this system is highly useful in studying the action of ethylene in the germination process.

Effect and interactions among GA, kinetin and ethylene with CO₂ on the relief of thermoinhibition have been reported (Negm et al., 1972; Keys et al., 1975; Rao et al., 1975; Khan, 1980/81) however, these reports were based on germination tests conducted in sealed systems where metabolic activities of the seeds could dramatically change the concentrations of gases such as ethylene and CO₂ (Negm et al., 1972; Keys et al., 1975), possibly resulting in modified effects of other treatments (Keys et al., 1975; Saini et al., 1986). Ethylene synthesis was essential for the relief of thermoinhibition in the dark, the action of exogenous ethylene required the presence of at least another hormone, CO₂ or light, or a combination of these factors (Saini et al., 1986). A similar requirement for ethylene under nonthermoinhibitory conditions (25°C) in the light was reported by Abeles (1986). Endogenous ethylene was also essential for the relief of thermoinhibitory (Saini et al., 1989).

Over the course of dicot embryogenesis, Amino Cyclopropane 1-Carboxylic acid oxidase mRNA can be expressed in cotyledons and embryonic axis. However, as maturation proceeds, it disappears. In some seeds that develop primary dormancy, ethylene synthesis can be among the prerequisites for breaking dormancy. The use of inhibitors of ethylene biosynthesis or its action has provided data implicating as ethylene requirement for seed dormancy or germination in some species. Recent studies with Xanthium pensylvanicum seeds suggested that α-cyanalanine synthase is involved in ethylene dependent germination. Regulation of partitioning of 3-Adomet between ethylene vs polyamine synthetic pathways may be a way of controlling germination in some seeds. These results suggest that ethylene plays an essential role in lettuce seed germination, regardless of the conditions for germination or the means used to induce it.

### Abscisic acid:

Abscisic acid (ABA) inhibits germination. ABA has been shown to suppress GA-responsive genes essential for seed germination and seedling growth, including the GA-responsive amylase gene. Transcription factor proteins are also involved in ABA-mediated gene expression. In barley, ABA is found in the aleurone layer (Rylott and Hewitson). Studies of gibberellin (GA)-deficient, abscisic acid (ABA)-deficient, and signaling mutants in Arabidopsis and tomato have identified the crucial role of ABA in seed dormancy, as well as the requirement for GA for germination. The observation that inhibitors of ABA biosynthesis, such as norflurazon, promote germination indicates that the maintenance of dormancy in imbibed seeds is
an active process involving de novo ABA synthesis. It was recently found that the ethylene insensitive2 (ein2) and ethylene response (etr) mutants of Arabidopsis are also hypersensitive to ABA. These findings, in combination with the non-dormant phenotype of the ein2 abi3-4 double mutant, indicate that ethylene may suppress seed dormancy by inhibiting ABA action. Germination of lettuce (Lactuca sativa L. cv. ‘Grand Rapids’) seeds was inhibited at high temperatures (thermoinhibition). Thermoinhibition at 28 °C was prevented by the application of fluridone, an inhibitor of abscisic acid (ABA) biosynthesis. At 33 °C, the sensitivity of the seeds to ABA increased, and fluridone on its own had no longer effective. However, a combined application of fluridone and gibberellic acid (GA) was able to restore the germination. Exogenous GA decreased endogenous ABA content in the seeds, enhancing catabolism of ABA and export of the catabolites from the intact seeds. The fluridone application also decreased the ABA content. Consequently, the combined application of fluridone and GA decreased the ABA content to a sufficiently low level to allow germination at 33 °C. There was no significant temperature-dependent change in endogenous GA contents. It is concluded that ABA is an important factor in the regulation of thermoinhibition of seed germination, and that GA affects the temperature responsiveness of the seeds through ABA metabolism (Gonai et al., 2003).

Morphactins: Morphactin-butyl ester (a flavone-9-carboxylic acid derivative) inhibited seed germination of two strains of lettuce. Morphactin induced inhibition of germination could be partially or wholly reversed by simultaneous addition of gibberellic acid. However, gibberellic acid played very little part in reversing the inhibitory effect of morphactin on seedling growth. It is concluded that gibberellin can reverse all the growth effects induced by morphactin (Sankhla and Sankhla, 1968).

CONCLUSION

By exploring the various literatures on role of plant hormones on seed germination, it could be concluded that GA and ethylene have strong influence on seed germination of different plant species. It is possible by GA to remove starch granules by production of alpha-amylase which might be either a first step towards the breaking of dormancy or an accompanying phenomenon of germination. The presence of a large number of starch granules could inhibit the germination of the embryo since the soluble carbohydrates and their breakdown products. Ethylene may play a role in the control of secondary dormancy. The inability of secondary dormant seeds to germinate might be related to an insufficient level of endogenous ACC in order to produce the required concentration of ethylene. ABA induces the seed dormancy in vivipary seeds.
REFERENCES


Rylott and Hewitson, in website www-saps.plantsci.cam.ac.uk.


