**Gibberellins (Physiological Effects in detail): (contd.)**

1. **Substitute for light in Long Day plant**

Certain light sensitive seeds e.g., lettuce and tobacco show poor germination in dark. Germination starts vigorously if these seeds are exposed to light or red light. This requirement of light is overcome if the seeds are treated with gibberellic acid in dark.

1. **Breaking the dormancy of Buds:**

In temperate regions the buds formed in autumn remain dormant until next spring due to severe colds. This dormancy of buds can be broken by gibberellin treatment. In potatoes also, there is a dormant period after harvest, but the application of gibberellin sprouts the eyes vigorously.

1. **Inhibition of Root Growth:**

Gibberellins have little or no effect on root growth. At higher concentration in some plants, however, some inhibition of root growth may occur. The initiation of roots is markedly inhibited by gibberellins in isolated cuttings.

1. **Elongation of the Internodes:**

Most pronounced effect of gibberellins on the plant growth is the elongation of the internodes, so much so that in many plants such as dwarf pea, dwarf maize etc., they overcome the genetic dwarfism. For instance, the light grown dwarf pea plants have short internodes and expanded leaves. But, when treated with gibberellin the internodes elongate markedly and they look like tall plants.

1. **Bolting and Flowering:**

In many herbaceous plants the early period of growth shows rosette-habit with short stem and cauline leaves. Under short days the rosette habit is retained while under long days bolting occurs i.e., the stem elongates rapidly and is converted into floral axis bearing flower primordia. This bolting can also be induced in such plants e.g. *Rudbeckia speciosa* (It is a Long Day Plant) by the application of gibberellin even under non-inductive short days.

In *Hyoscyamus niger* (also a Long Day Plant) gibberellin treatment causes bolting and flowering under non-inductive short days. While in Long Day Plants the gibberellin treatment usually results in early flowering, its effects are quite variable in Short Day Plants. It may either have no effect, or inhibit, or may activate flowering.

1. **Parthenocarpy:**

Germination of the pollen grains is stimulated by gibberellins, likewise the growth of the fruit and the formation of parthenocarpic fruits can be induced by gibberellin treatment. In many cases e.g., pome and stone fruits where auxins have failed to induced parthenocarpy the gibberellins have proven to be successful. Seedless and fleshy tomatoes and large sized grapes are produced by gibberellin treatment on commercial scale.

1. **Induced Light Inhibited Stem Growth:**

It is common observation that the dark grown plants become etiolated and have taller, thinner and pale stems while the light grown plants have shorter, thicker and green stems, and it may be concluded that light has inhibitory effect on stem elongation. Treatment of light grown plants with gibberellin also stimulates the stem growth and they appear to be dark brown. In such cases the protein content of the stem falls while soluble nitrogen content increases prob­ably due to more breakdowns of proteins than their synthesis.

It is considered that the light in some way lowers the level of endogenous gibberellins and inhibits the stem growth.

1. **De novo Synthesis of the Enzyme-α-Amylase:**

One of the important functions of gibberellins is to cause de novo (i.e., a new) synthesis of the enzyme a- amylase in the aleurone layer surrounding the endosperm of cereal grains during germination. This enzyme brings about hydrolysis of starch to form simple sugars which are then translocated to growing embryo to provide energy source.

**Mode of Action of Gibberellins on cell division**

1. **Gibberline stimulate both Cell Division and Cell Elongation:**

Stem elongation in plants as a result of gibberellin treatment involves both cell division and cell elongation. In rosette long day plants, GA treatment causes marked increase in mitosis in the sub-apical regions of apical meristems. Internodes of tall pea plants have more cells than in dwarf ones and their cells are longer in size too. In deep-water rice, stimulation of internodes elongation is partly due to increased cell di­visions in the intercalary meristems and partly due to elongation of cells of the latter who have divided with cell elongation preceding the cell divisions.

**GA Stimulated Cell Divisions are Regulated Between G2 and M Phases of Cell Cycle:**

The mechanism of GA stimulated cell division has been extensively studied in intercalary meristems of young internodes of deep-water rice. In this case, the GA – stimulated cell divisions are believed to be regulated between G2 and M phases of cell cycle. The transitions between different phases of cell cycles are known to be regulated by cyclin- dependent protein kinases (CDKs). GA stimulates cell divisions by increasing the expression of two genes (CDC2) that encode CDKs and M cyclins which are required for entry into mitosis.

1. **Mobilization of Endosperm Food Reserves:**

As mentioned earlier, the GAs cause de novo synthesis of a -amylase in cells of aleurone layer of germinating cereal grains. (The GAs synthesized by coleoptile and scutellum of the embryo are released into the starchy endosperm from where they diffuse into the cells of aleurone layer). GAs also bring about secretion of a -amylase and other hydrolytic enzymes from the cells of aleurone layer into the starchy endosperm where complex carbohydrates are hydrolyzed into simple sugars which are then trans located to growing embryo to provide energy source. Although the mechanism of induction of a-amylase synthesis in cells of aleurone layer by GA and its secretion from cells of aleurone layer into the endosperm, have been extensively studied, but they are yet far from being completely elucidated.

**Plant Responses to Cytokinins (in detail)**

Cytokinins play a prominent role in all the phases of plant development from cell division and enlargement to the formation of flowers and fruits. They increase resistance to aging and to adverse environment.

 **(i) Cell Division:**

For continued in vitro growth and cell division of tissue accompanied by DNA synthesis, cytokinin is necessary along with auxin. While auxin and gibberellin are also able to stimulate DNA synthesis and mitosis, cytokinin alone can stimulate **cytokinesis**. Quite opposite to the pro-motive effect of auxin and gibberellin, cytokinin inhibits elongation of stem sections. Root growth is generally inhibited by cytokinins.

**(ii) Cell Enlargement:**

Cytokinins may stimulate radial growth of stem tissue by swelling rather than by longitudinal extension. Vine well-known leaf enlargement caused by cytokinin is due to an effect on cell enlargement rather than cell division. In fact, cytokinin appears to promote overall enlargement of cells and not simply elongation. Cytokinin effect on cell enlargement may be due to an influence on micro fibril orientation from longitudinal to radial direction.

**(iii) Tissue Differentiation:**

Organs in tissue culture show a spectacular response to cytokinin. With a low cytokinin supply, the tissue remains as an amorphous undifferentiated callus.

Bud formation and shoot initiation depend on higher concentrations of cytokinin by changing cytokinin auxin ratios. An interesting observation on morphogenesis in tobacco callus cultures is that a high cytokinin auxin ratio results in the production of shoots but no roots, but a low ratio leads to an opposite effect producing roots only.

In addition to their role in leaf expansion, cytokinins also play a regulatory role in chloroplast formation. When cytokinin is absent, plastids are formed but remain undifferentiated. Presence of both light and cytokinin is necessary for grana development and conversion of pro-plastids into chloroplasts.

**(iv) Retardation of Senescence:**

The retardation of senescence by cytokinin is a well-known phenomenon. Richmond and Lang first discovered that when leaf discs are kept in water, senescence appears within a few days as evident by the loss of chlorophyll and protein. But when cytokinin is added to the leaf discs, senescence is delayed through the maintenance of chlorophyll and protein.

This senescence-retarding property of cytokinin as mediated through the retention of chlorophyll is known as **Richmond-Lang** effect.

**(v) Mobilization of Nutrients:**

Mothes observed that when a particular area of leaf is treated with cytokinin, that treated area remains green showing delay of senescence, while the untreated area loses its green colour and becomes yellowish showing symptoms of senescence. Here the nutrients are drawn or mobilized from other parts of leaf so that the treated area remains green at the expense of the untreated area.

**(vi) Release of Dormancy of Seeds and Buds:**

Applications of cytokinins can stimulate germination and break dormancy. One of the remarkable characteristics of cytokinins is their ability to modify the effects of other hormones without any marked effects by themselves.

When dormancy is imposed either by high temperature (thermo dormancy) or by an accumulation of inhibitor like ABA (inhibitor dormancy), then GA alone is not capable to overcome dormancy. Addition of cytokinin opposes the action of inhibitor and permits germination.

Thus cytokinin has been documented as a permissive agent in germination by antagonizing the inhibitor action — a case of cytokinin-inhibitor antagonism. In bud growth inhibitor (preventive) and cytokinin (permissive) show opposite effects. Thus inhibitor-induced bud dormancy can be overcome by cytokinin.

**(vii) Masking of Apical Dominance:**

Cytokinin applied on lateral buds is able to mask from the effect of apical dominance whether it is due to the presence of terminal bud or due to applied auxin. This has been interpreted as an increase in IAA transport and mobilization of metabolites from the apical region to the point of application of cytokinin which is supported by the striking influence of cytokinin on phloem transport.

**(viii) Resistance to Adverse Factors:**

Cytokinins increase the resistance of plants to adverse factors such as high and low temperatures and certain disease. The nature of the action of cytokinins in bringing about these effects is still unknown.

Naturally-occurring cytokinins have been implicated in host-parasite relationship. Infection by bacterium *Corynebacterium fascines* which produces cytokinin leads to the fasciation disease symptoms in many plants. Treatment with cytokinin may induce a similar pathogenic condition.

**(ix) Stomatal Movement:**

Cytokinin has a distinct action on the mechanism of stomatal movement. Although the stomatal aperture in the isolated epidermal systems is not much influenced by cytokinins, treatment of whole leaf with cytokinin has been reported to increase the stomatal aperture and thereby transpiration.

**(x) Other Developmental Effects:**

The development of inflorescence is influenced by cytokinin treatment by increasing both the number and size. Cytokinin has been shown to cause a male-flowering plant to produce hermaphrodite flowers. Enhancement of fruit set and fruit size in grape varieties and induction of parthenocarpy in fig have also been reported.

**Mode of Action in Cytokinins:**

(i) Control of Transcription and Translation:

At present, the biochemical basis of cytokinin action is still not completely known. Still there are many evidences which make it clear that cytokinins greatly influence nucleic acid metabolism. Guttman first reported that kinetin treatment of onion roots caused a rapid increase in nuclear RNA and this observation was later confirmed by Jensen et al.

**cytokinins effect on nucleic acid synthesis**:

 Both the chemical constitution of the cytokinins and their effect on nucleic acid synthesis strongly suggest that they exert their biological activity directly in nucleic acid metabolism. Presence of cytokinin activity in specific tRNA species would suggest the influence of cytokinins on specific rather than bulk protein synthesis. Location of the cytokinins next to the anticodon in certain tRNA species with known base sequences suggests that they may function specifically in the translation step of gene- controlled protein biosynthesis.

Since various developmental pathways are influenced by cytokinins, it is pertinent to expect cytokinins to control the synthesis of many proteins either by regulating the transcription of the genes encoding these proteins or by an effect at the post-transcriptional level.

Induce the synthesis of nitrate reductase enzyme:

One well-known protein, the synthesis of which is induced by cytokinin is nitrate reductase enzyme. Nitrate reductase is a cytosolic enzyme, which reduces nitrate (NO3) to nitrite (NO2). Further reduction of nitrite to ammonia (NH+4) is catalysed by the chloroplast enzyme nitrite reductase. It is interesting to note that nitrate metabolism is induced by light, which initiates the expression of both the nitrate reductase gene encoded by nucleus as well as the chloroplastic nitrite reductase gene. Cytokinins can stimulate the synthesis of nitrate reductase in dark-grown leaves, which suggests that the light requirement for the induction of this enzyme can be partly substituted by cytokinins.

It has been further observed that along with the induction of nitrate reductase by cytokinin, there is a corresponding increase in nitrate reductase mRNA. Since cytokinin-stimulated nitrate reductase can be blocked by inhibitors of both gene transcription and protein synthesis, it may be concluded that cytokinin possibly exerts its effect both at the levels of transcription and translation.

**(ii) Respiratory Enzymes and Metabolism:**

In cultured tissue, respiratory activity can be increased by the addition of cytokinins. It has been shown that cytokinin stimulation of respiration involves a suppression of glycolytic enzymes and a shift to the hexose monophosphate shunt. In intact systems, high doses of cytokinins cause inhibition of respiration and delay of senescence can be correlated with decrease in respiration. Cytokinins have been found to influence the activity of a number of specific enzymes. Cytokinin induces the formation of tyramine methyltransferase which catalyses thiamine synthesis.

 **(iii) The Richmond-Lang Effect:**

The Richmond-Lang effect suggested that cytokinins play an active role in senescence retardation in detached leaves. The mechanism is based on the postponement of the disappearance of chlorophyll and the degradation of proteins through the activity of the corresponding hydrolases which normally accompany the senescence process.

Mothes suggested that the primary function of cytokinin in this respect is to increase the amino acid accumulation and to increase or retain the protein content.

**(vi) Cytokinin and Auxin Regulate Plant Cell Cycle:**

Both auxin and cytokinins are involved in the regulation of cell cycle by controlling the activity of cyclin-dependent kinases. The cyclins are the regulatory subunits of enzymes like cyclin-dependent protein kinases (CDKs) which regulate the cell cycle in eukaryotes by means of protein phosphorylation. Auxin has been shown to regulate the expression of the gene that encodes CDC2 (cell division cycle 2) that is the major CDK. However, CDK alone is not sufficient to stimulate cell division.

In Arabidopsis tissue cultures, two G1 type cyclin proteins, viz., δ3 cyclin and δ2 cyclin have been identified. Cytokinin has been shown to stimulate the expression of the G1 cyclin gene that encodes δ3 cyclin protein, whereas sucrose, a carbon source of cultured tissues, stimulates the expression of the other G1 cyclin gene coding for the protein δ2.

Observation suggests that the culture medium should contain a combination of auxin, cytokinins and a carbon source like sucrose that is necessary for the formation of active CDK-G1 cyclin complex. In such a culture, the cells of a dormant tissue may be induced to divide and enter the cell cycle through the action of CDK-cyclin complex which permits protein phosphorylation and cell cycle regulation.