**Discovery of Kinetin and cytokinins**

The discovery of Kinetin is comparatively more recent. It was discovered by Miller et al (1950), who were working on the growth of tobacco pith callus in culture and wanted it to grow indefinitely. They added various growth substances, nutrients, vitamins etc. into culture medium but failed till they noticed an old bottle of DNA kept for several years in their lab. They added the contents of that bottle to the culture medium and observed that the tobacco pith callus grow for longer periods. They obtained similar results with yeast extract.

But they did not get positive results with fresh DNA and thought the active substances to be some degradation product of DNA which had been stored for long. It could easily be precipitated by silver salts and was soluble in 90% alcohol, indicating that possibly it was a purine compound. Later on they identified it as 6-furfurylaminopurine. Because of it specific effect on cytokininesis i.e. cell division, it was called as kinetin.

Although kinetin has profound influences in inducing cell divisions, still it has not been isolated from any plant. But, certain substances which show kinetin like activity have in fact been isolated from a variety of higher plants. These substances are collectively called as cytokinins. There is now sufficient evidence to show that cytokinins do occur in plants and regulate growth and hence they are also considered as natural plant growth hormones.

Some of the very important and commonly known naturally occurring cytokinins are

1. **Zeatin**

Zeatin is the most abundant and widely distributed natural cytokinin in higher plants and in some bacteria. Although this cytokinin was known earlier but it was obtained in pure crystalline form in 1963 by Letham from immature corn grains and named Zeatin. It was synthesized by Saw and Wilson (1964).

1. **Other natural cytokinins**

Apart from zeatin, some other substituted aminopurines have been isolated from higher plants and some bacteria which are also considered as natural cytpkinins. Thses are dihydrozeatin (DZ) and N6-(∆2 isopentenyl) adenine (or ip) which differ from zeatin in nature of their side chain.

**Characteristics of cytokinins**

Cytokinins are characterized by the following features

1. Initiation of cell devision
2. Delay of senescence (Richmond-Lang effect)
3. Use in tissue culture
4. Counteract apical bud dominance
5. Induces flowering in short day plant

**Physiological roles of cytokinins**

1. **Cell division**

One of the most important biological effects of kinetin on plants is to induce cell division in the presence of sufficient amount of auxin, especially in tobacco pith callus, carrot root tissue, soybean cotyleadon, pea callus etc.

1. **Cell enlargement**

Like auxins and gibberellins, the kinetin may also induce cell enlargement. Significant cell enlargement has been observed after kinetin treatment in leaf discs cut from etiolated leaves of *Phaseolus vulgaris*, pumpkin cotyledons, tobacco pith cultures, cortical cells of tobacco roots, excised Jerusalem artchoke etc.

While cytokinins have been shown to promote cell expansion in leafy cotyledones of some plants such as mustard, sunflower, cucumber, raddish etc. but this effect is not exhibited by auxin or gibberellins i.e. they do not promote cell expansion in cotyledons.

1. **Initiation of intrafascicular cambium**

Kinetin can induce formation of intrafascicular cambium. This has in fact been shown by Sorokin et al (1962) in pea stem sections.

1. **Morphogrnrsis**

Kinetin also has ability to cause morphogenetic changes in an otherwise undifferentiated callus. For instance, the tobacco pith callus can be made to develop either buds or roots by changing the concentration of kinetin and auxin.

The effect of kinetin in the stimulating or initiating the formation of buds has also been observed in leaf cuttings of *Begonia*, *Bryophyllum* and in mosses e.g. *Tortella* etc.

A positive effect of kinetin on the regeneration offshoots from cultured root segments has also been reported in *Isatis tinctoria* and *Convolvulus arvensis*.

1. **Concentration of apical dominance**

Wickson and Thimann (1958) in one of their experiments found that the growth of the lateral buds of pea stem section in culture solutions containing IAA was inhibited. But the growth of lateral buds could continue if IAA was not included. On the other hand, the addition of kinetin along with IAA stimulated the growth of these buds. They obtained similar results with entire shoots and concluded that the apical dominance might be under the control of a balance of concentration between endogenous kinetin like substances and IAA

The cytokinins play a role in initiating the growth of lateral buds has also been proved by physiological studies made on cytokinin overproducing mutants of tobacco. The wild type tobacco plants show strong apical dominance, but in cytokinin-overproduce mutants the lateral buds grow vigourously and the plants tend to be bush like.

1. **Dormancy of seeds**

Like gibberellins, the dormancy of ceratin light sensitive seeds such as lettuce and tobacco can also be broken by kinetin treatment in dark. Furthermore, the inhibitory effect of far red light treatment on the germination of theses seeds is also overcome by kinetin treatment.

1. **Delay of senescence : the Richmond-Lang effect**

The ageing process of the leaves usually accompanies with loss of chlorophyll (i.e. yellowing) and rapid breakdown of proteins. This is called as senescence. In 1957, Richmond and Lang showed that this senescence could be postponed to several days in detached Xanthium leaves by kinetin treatment. This effect of kinetin in delaying the senescence is called Richmond-Lang effect.

Mothes (1960) and other workers have shown mobilization of nutrients and other substances including auxins to the kinetin treated areas. In intact plants, the delay of senescence at some part due to kinetin treatment may result in senescence in other part of the plant.

The observation of Osborne (1962) and other workers suggest that the high protein content in kinetin treated tissue is probably due to more synthesis of proteins than their degradation, and this in turn may be due to the regulatory action of kinetin on RNA synthesis.

One of the important factors in delay of senescence in kinetn treated leaves is their physiological age. For instance, mature leaves of *Nicotina rustica* have been found to be more responsive to kinetin treatment in delaying senescence than younger leaves.

1. **Promotion of chloroplast development**

Cytokinins are also known to greatly enhance conversion of etioplasts into chloroplasts when etiolated seedlings after treatment with cytokinins are exposed to light. In such cases chloroplasts develop extensive grana and chlorophylls and the rate of synthesis of photosynthetic enzymes is much greater in comparison to those etiolated seedlings which are illuminated without cytokinin treatment.

**Biosynthesis of cytokinins**

Cytokinins are synthesized from adenosine monophosphate (AMP) and isopentenylpyrophosphate by condensation reaction that is catalysed by the enzyme isopentenyl transferase. The product of this condensation is N6-(∆2-isopentenyl)-adenosine-5’-monophosphate [(9R-5’-P) iP] which is supposed to be precursor to all other natural cytokinins.

* The 9R-5’-P) iP is readily dephosphorylated to yield N6-(∆2-isopentenyl)-adenosine.
* Ribose sugar is now removed N6-(∆2-isopentenyl)-adenosine, so that N6-(∆2-isopentenyl)-adenine is formed.
* Isopentenyl side chain of iP is now hydroxylated to form free zeatin.

Alternatively, (9R-5’-P) iP may be hydroxylated directly to give 9-ribosyl-zeatin-5’-phosphate [(9R-5’-P) Z]. The phosphate group and then the ribose sugar are removed from [(9R-5’-P) Z] in sequence to form free zeatin.

* Reduction of the double bond in isopentenyl side chain of zeatin would give rise to dihydrozeatin (diHZ).

