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Cytokinin changes in the subtending axillary leaves of developing flower of *Cosmos sulphureus* Cav.

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Abstract. In the present investigation a study was conducted to record the changes in endogenous cytokinin activity of the subtending axillary leaves adjacent to flower and corelate with the flower development of *Cosmos sulphureus* Cav. using the soybean callus bioassay. Most of the nutrition moves from leaves to flower during the early development of flower and grain filling of crop plants. Cytokinin activity of flower adjacent leaves was increased from O day up to 15 days, after that the activity was decreased. In Sephadex LH-20 column chromatography of leaf extracts of different developmental stages, the cytokinin present eluted at the region of zeatin, zeatin riboside and cytokinin glucosides. The cytokinin glucoside activity appeared to be at a maximum at mature leaves.

Key words: *Cosmos*; Cytokinins; Cytokinin changes in leaves.

Introduction

Cytokinins are regraded as an integral part of senescence, regulating systems of flower, fruit and leaf development. The evidence supports the hypothesis that they are mostly produced by the roots and transported to the shoot where they are involved in the regulation of shoot process (Wareing *et al.*, 1977). It is generally accepted that root derived cytokinins are utilized and/or metabolized extensively in the leaves (Van Staden and Davey, 1979; Nagar and Saha, 1985).

In an earlier study high cytokinin activity was detected in *Cosmos* flowers just prior to full bloom (Saha *et al.*, 1985). However, cytokinin conjugates which are considered as bound or storage forms appear to be maximum at full bloom. Leaves play a vital role in maintaining high cytokinin levels in the inflorescence of certain plants.

In the present paper an attempt has been made to study the cytokinin changes during different stages of leaf development in *Cosmos* and to correlate it with their changes during flower development.

Materials and Methods

The subtending leaves of the flower buds/flowers were collected at 3 days intervals from the day of initiation till full bloom a period of 21 days. The samples were stored at -20°C prior to analysis. In all cases the material was homogenised with chilled 80% methanol in mortar and pestle and the macerated tissue was re-extracted 4 times with the same solvent each for 24 h intervals at 5°C. The extracts were evaporated to aqueous *in vacuo* and adjusted to pH 7.0 and partitioned 4 times with water saturated n-butanol. The butanol phases were separated and evaporated to dryness. The residuses were resuspended in 80% ethanol and acidified to pH 3.0 with dil. HCl and passed through a Dowex 50 cation exchange resin (H^+ form, 20-50 mesh) column at a flow rate of 50 ml/h. Compounds responsible for cell division activity were eluted from the column with 300 ml of 3 N NH_4OH. The eluate was evaporated *in vacuo* and the residue resuspended in 2 ml 80% ethanol. The concentrated ethanolic extracts were fractionated on Sephadex LH-20 column (30×2

cm) which was eluted with 35% ethanol at a flow rate of 20 ml/h. Fractions of 15 ml were collected, dried *in vacuo* at 40°C and bioassayed using the soybean callus test (Miller, 1967). All soybean callus bioassays were repeated at least once and the mean of the two bioassays are presented in the histograms.

Results

In this study after Sephadex LH-20 column chromatography the pattern of cytokinin activity was detected. From 0 day onwards two peaks of cytokinin activity were found, although present at low level in the initial stages (Fig. 1: 0 and 3 days). In the 1st and 2nd stage i. e., 0 day and 3 days the activity of zeatin riboside (ZR) is higher than zeatin (Z). But in the 3rd & 4th stages (Fig. 1: 6 and 9 days) the activity of zeatin riboside & zeatin are just similar. These cytokinin activities are frequently regarded as an active form progressively increased after 6 days and continued to increase till 15th day (Fig. 1: 12 and 15 days). After that elution volume corresponding to that of zeatin decreased appreciably (Fig. 1: 21 days). The reduction in activity of zeatin riboside (ZR) was more pronounced than zeatin (Z) from 12 days up to 18 days. But after 15 days cytokinin glucoside reached maximum activity at the last stage i. e., at 21 days. As observed in Fig. 1 (15 and 18 days) cytokinin activity in the leaves of *Cosmos* was maximum but when the leaves reached their maturity the cytokinin activity, i. e. the elution volume corresponding to zeatin riboside and zeatin was decreased and cytokinin glucoside (Fig. 1: 18 and 21 days) was reached maximum activity.

In light of these, the present investigation indicates that cytokinin level is important for young adjacent leaves to flower to the ageing process of the same leaves and also of flower. In 18th & 21st day it was found that cytokinin glucoside was maximum, before these two stages the cytokinin activity identical to zeatin riboside and zeatin reached their maximum peaks from 6 days to 15 days. From this finding it was proved that leaves have ability to convert zeatin (Z) and zeatin riboside (ZR) to their respective glucosides.

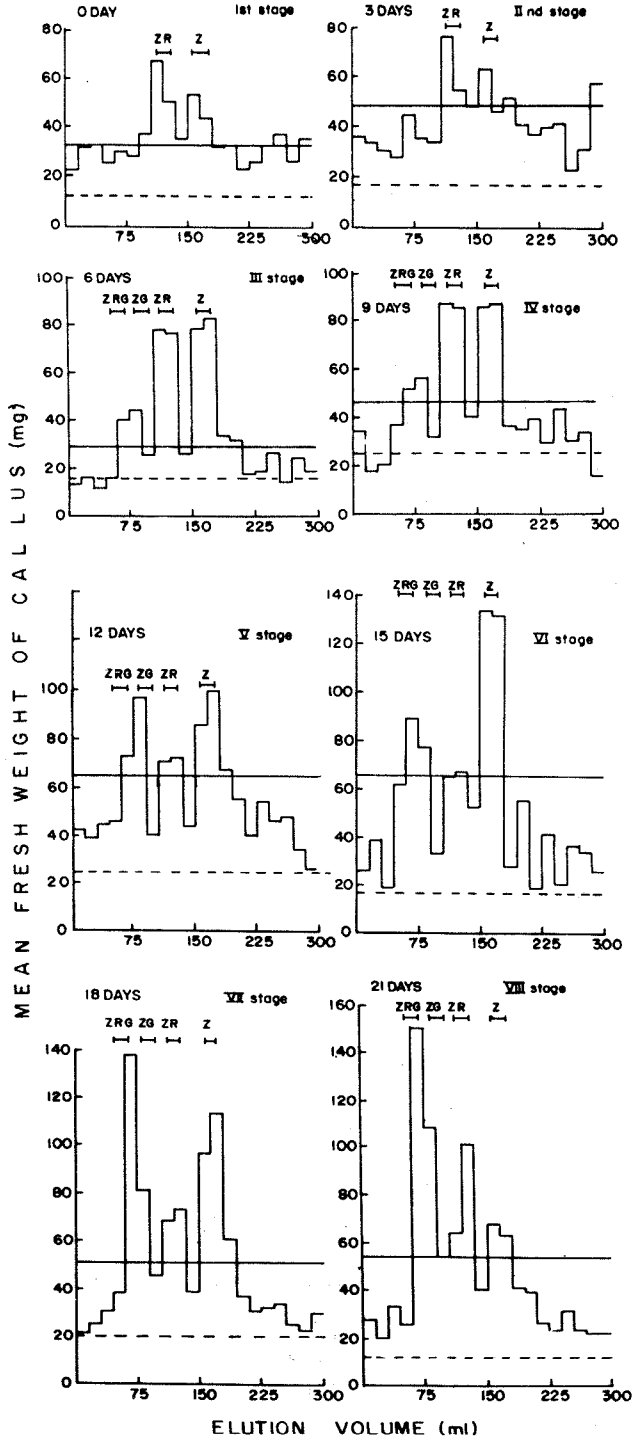

Fig. 1. Soyabean callus bioassay of cytokinin activity of 25g fresh weight of subtending axilary leaves collected from various stages of flower development. The extracts were fractionated on Sephadex LH-20 column with 35% ethanol. ZRG=Zeatin riboglucoside, ZG=Zeatin glucoside, ZR=Zeatin riboside and Z=Zeatin. Broken and solid horizontal lines represent controls and LSD at 5% level respectively.

It is generally accepted that cytokinins are glucosylated in their corresponding organ rather than their coming to the place of synthesis root or other organ(s) for glucosylation, inactivation and again moving outwards makes the whole process cumbersome and much more complicated.

Discussion

Leaves undergo both qualitative and quantitative changes in the cytokinin content during their development (Hendry *et al.*, 1981; Palmer *et al.*, 1981). The most significant aspect related to a changing pattern of cytokinin metabolism in developing leaves (Henson, 1978; Van Staden, 1977) is that with leaf maturity these substances are stabilized by reduction and glucosylation (Hewett and Wareing, 1973; Palmer *et al.*, 1981). Cytokinin glucosides have been found to fluctuate depending on age and were found to increase when leaves reached maturity (Hewett and Wareing, 1973). These cytokinin are considered as a storage form and possibly can be reutilized (after conversion) to provide other cytokinin dependent plant organs. During rice seed maturity cytokinins are inactivated by glucosylation and again they are reutilized during germination (Saha *et al.*, 1984). Due to gene unfoldment and synthesis of hydrolysing enzymes these cytokinins are properly utilized. Although it is not know whether these are exported from leaves but transport is suggested in certain cases (Van Staden and Brown, 1977; Van Staden, 1976).

With increase in leaf maturity cytokinins activity also increased in the leaf tissues. This coincides with flower development and while the total cytokinin activity tended to decrease after 15 days an increase in cytokinin glucoside activity was evident with flower maturity. The accumulation of cytokinin glucosides appear to be a characteristic feature of mature and ageing leaves of woody as well as herbaceous annuals (Van Staden, 1976). In this study towards leaf maturity the cytokinins identical to zeatin (Z) and zeatin riboside (ZR) decreased while zeatin glucoside increased. It seems that leaves may convert Z and ZR to their respective glucosides. This may be a means of inactivating and storing excess cytokinins supplied by the transpiration stream (Henson and Wareing, 1976; Van Staden and Papaphilipou, 1977).

Work on endogenous cytokinins and with ^{14}C zeatin has established that zeatin like derivatives are transported to the leaves via the transpiration stream where they are metabolised to glucosylated forms (Davey and Van Staden, 1978). The exact function of the cytokinin glucoside in plants is still obscure. They are considered as inactive form. As they are present in yellow senescing leaves in relatively high concentrations they apparently do not retard senescene. It is quite likely that the attachment of glucose moiety to zeatin (Z) and zeatin riboside (ZR) inactivates these compounds (bound form) which enables the plant to transport them through the phloem.

In cosmos flower the zeatin (Z) and zeatin riboside (ZR) predominated prior to full bloom (Saha *et al.*, 1985). The total cytokinin activity tended to decrease after 15 days and an increase in cytokinin glucoside activity was evident with flower maturity i. e., at full floom stage. In case of *Cosmos* leaves the endogenous cytokinin activity was similar to that of flower development. In mature leaves the level of cytokinin glucosides reached maximum peak. So from this study it is concluded that within mature leaves the cytokinins derived from the roots are converted to inactive or storage forms by means of glucosylation. The glucosylation of zeatin like derivatives may represent an attempt to inactivate excess cytokinins or alternatively prepare these compounds for export from the leaves to other parts.

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黃波斯菊發育中花朵的腋生苞葉內細胞分裂素之變化

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本文在探討花朵相鄰之腋生苞葉內細胞分裂素活性的變化及其與開花之關係，以大豆之癒傷組織做爲測定細胞分裂素活性之材料。農作物在花和穀粒發育早期，大部份養分會由葉移至花。和葉相鄰花朵內的細胞分裂素活性自 0 至 15 天會增加，以後開始下降。以 Sephadex LH-20 管柱層析法分析來自不同發育時期之葉部的萃取物，發現細胞分裂素活性出現於玉米素、玉米核酸核苷和細胞分裂素糖苷等流洗物處。成熟葉的細胞分裂素糖苷活性最高。