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Analysis of Some Plant Growth Regulators in Some Medicinal Dormant Seeds of Desert Plants in Saudi Arabia II: Cytokinin Contents of *Citrullus colocynthis*

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Abstract: Dry seeds of *Citrullus colocynthis* collected in 1982 and stored at 10°C, were extracted to investigation endogenous cytokinin content. An HPLC analysis was made and profile obtained revealed the presence of two isomers of zeatin (*trans-zeatin* and *cis-zeatin*), isopentyl adenine and isopentyl adenosine. Analysis showed very high content of trans-zeatin as compared to cis-zeatin. Isopentyl adenine and isopentyl adenosine were in negligible quantity.

Key words: Desert plant, plant growth regulators, cytokinin, HPLC analysis, *Citrullus colocynthis*

Introduction

It is reported that during storage the viability of seeds may be affected and it depends Upton the storage conditions and period of storage (Dell'Aquila, 1987). Different biochemical systems might be affected by long term storage of seeds (Perlm and Kretschmer, 1988). Cytokinins play an important role in seed germination (Khan, 1971). In maize endosperm, zeatin and other purines with cytokinin like activity has been isolated (Letham, 1973). However, in mature dormant seeds low levels of cytokinin have been reported (Hocart and Letham, 1990). Present study was made to investigate the cytokinin content and their nature in *Citrullus colocynthis* seeds after a long period storage.

Materials and methods

Citrullus colocynthis seeds, collected in 1982 and stored at 10°C, were powdered in liquid nitrogen. 3 g powder was taken and cytokinin were extracted, thrice, with cold acetone in dark at 5°C. 0.5 μCi of 8 [C^{14}]-Benzyladnine was added to samples as internal standard. After centrifugation the supernant was dried *in vacua.* Residue was dissolved in leuke 38 "C warm water and acidified to pH 3.5. A double extraction of aqueous solution of cytokinin was accomplished with n-butanol saturated with acidified water (pH 3-5). Aquous phase was neutralized to pH 7. Subsequent triple extraction of this phase with alkaline butanol (butanol: amonia 9:1 v/v) transferred cytokinin into organic phase. The combined organic phase was dried *in vacuo* and final drop was dried under stream of nitrogen.

Further purification of cytokinin was achieved using "Sep Pak C_{18} cartridges. After elution of cytokin from the cartridges, the effluent was evaporated *in vacuo.* The residence was dissolved in final volume of 0.5 ml 50% ethanol and filtered on to a column of Millipore HV 0.45 μm.

Cytokinin were separated on a supersphere RP-select B analytical column. Elution was performed with 70% acetonitrile as solvent B and 2% acetic acid as solvent A.A stepped linear gradient with following profile was used on solvent B: 0 min 1% 50 min 37%, 55 min 40%, 60 min 1%. The applied flow rate was 0.8 ml min^{-1} under continuous degassing with helium and column temperature was 30°C. Different kinds of endogenous cytokinins were putatively identified by their retention times. The putative quantification of cytoking was carried out by measuring the peak areas.

Results and discussion

Cytokinin content in dry seeds of *Citrullus colocynthis,* collected in 1982 and stored at 10°C, were determined. Using 8-(C^{14}) Benzyladenine as an internal standard An HPLC profile (Fig. 1) of seed extracts was obtained. The HPLC results revealed the presence of two isomers of zatain, (*cis*-zatin and tran-zeatin , beside isopentyle, ademine and isopentyl adenosine. Tran-zeatin, content were very high (605.2 mg/g dwt) as compared to cis-zeatin (20.1 ng/g dwt), followed by isopentyl adenine and iso-pentyle adenozine which were in negligible amounts (1.5 ng/g dwt and 1-2 ng/g dwt, respectively).

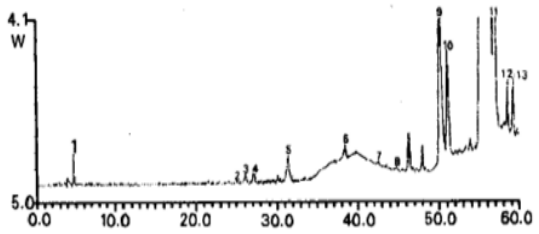

Fig. 1: An HPLC profile for cytokinins of *Citrullus colocynthis.* Peaks numbers. 2,4,7,8 show *cis-zeatin, trans-zeatin*, iso-pentyle adenine and iso-pentyle adenosine, respectively. Peaks number 1,3,5,6,9 to 13, show un-identified peaks

Some of the previous studies confirm the present results as sweet corn kernels are known to contain a complex of cytokinins and from this source zeatin and other six substituted peerine with cytokinin like activity have been isolated (Letham, 1973). Horcasitas *et al.* (1998) have shown that free zeatin were not present in any of the maize samples they studied. However, in present study, free zeatin were detected in a considerable amount which could be due to the difference of storage condition and the kind of seeds. As in the present study, Hocart *et al*. (1988) have detected free zeatin in mature maize seeds by radioimmuno analysis assay and gaschromatography-mass spectrometry. On the other hand Hocart and Letham (1990) found low levels of cytokinin in mature maize seeds and it is suggested that cytokinin biosynthesis occur during early seedling development.

Horcasitas *et al*. (1998) have found zeatin type cytokinins and their content were much higher (30-90%) for stored seed than non-stored seeds. Possible source for increment in cytokinin content might be the degradation of RNA occurring during long term storage or *de novo* synthesis of cytokinin could also contribute to the internal pool of zeatin or zeatin like compounds (Klemen and Klambt, 1974).

As less information is available about the biochemical changes that occur in seeds during long storage under different types of storage conditions, the present research will be followed by further studies to elucidate whether or not long storage of *Citrullus colocynthis* seeds in associated with viability or cytokinin content as compare to fresh collections of seeds.

Acknowledgment

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