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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 4 (1): 50-52, 2001

Analysis of Some Plant Growth Regulators in Some Medicinal Dormant Seeds of Desert Plants in Saudi Arabia I: Cytokinin Contents of *Rhazya stricta* Seeds

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Abstract: Three populations of *Rhazya stricta* seeds, non-stored (1999 collection), short-term stored (1998 collection) and long-term stored (1982 collection) were used to study their endogenous level of cytokinins. Using appropriate internal standards, an HPLC profile revealed the absence of free zeatin in quiescent seeds of all of the *R. stricta* seed populations used. Zeatin ribosides predominated other cytokinins in all three types of seeds. Trans-zeatin and dihydrozeatin contents were high in the 1998 collection followed by 1982 and 1999 collections. Isopentyladenine was also detected, but in very small amounts, in the samples of seeds tested.

Key words: Plant growth regulators, desert plant, cytokinin, *Rhazya stricta*, dormant seeds

Introduction

Seeds exhibit a unique form of life. In the mature dry seed metabolic activity is almost negligible in the embryo tissue until the seeds absorb water (Barnes *et al.*, 1980). During storage, viability and vigour of seeds may be affected and that depends upon the time span and storage conditions (Del' Aquila, 1987). There are many reports concerning the accelerated loss of seed viability (Abdul Baki *et al.*, 1972; Perl, 1988). Biochemical parameters are investigated as indicators of seed viability and vigour (Perl and Kretschmer, 1988). However, different biochemical systems might be affected by long term seed storage. Cytokinins play an important role in seed germination and counter act the effects of inhibitors (Khan, 1971). Many reports have been cited in literature about the cytokinin levels in seeds. In maize, the endosperm of both immature (Letham, 1973) and mature (Smith and Bray, 1982) kernels, have been shown to contain a complex of cytokinins. From this source zeatin and other purines with cytokinin activity have been isolated (Lethman, 1973). Low levels of endogenous cytokinins are present in mature seeds (Davey and VanStaden, 1978; Hocart *et al.*, 1988). It would seem likely that cytokinin biosynthesis occurs during germination and early seedling development (Hocart *et al.*, 1990).

The present study was undertaken to elucidate whether changes, in cytokinins levels and their nature occur during storage with age of *Rhazya stricta* seeds, collected on different dates.

Materials and Methods

Rhazya stricta seeds were collected from Riyadh during 1982, 1998 and 1999. These seeds were used to compare the cytokinin content in dry seeds stored for a long period (1982 collection), a short period (1998 collection) and non-stored (1999, collection).

Rhazya stricta seeds (3 g dried weight) were powdered by pestle and mortar in liquid nitrogen and cytokinins extracted with cold acetone (three times within 24 hours in the dark at 4 °C). To estimate the recovery, 0.5 μCi of 8-[^{14}C]-Benzyladenine (specific activity 54 mCi mmol^{-1}. Amersham Pharmacia Biotech, Buckinghamshire, UK) was added to samples as an internal standard.After centrifugation (8,000 g, 10 min, 4 °C), the supernatant was reduced to 2 ml (at 35 °C), 5 ml distilled water added, frozen in liquid nitrogen, thawed and centrifuged at 5,000 g for 15 min. The supernatant was evaporated to dryness *in vacuo*. The residue was dissolved in lukewarm (38 °C) distilled water with HCl pH 3.5. A double extraction of the aqueous solution cytokinins with *n*-butanol (analytical grade) saturated wi distilled acidified water (pH 3.5) was accomplished. T acidified aqueous layers containing cytokinins were combin neutralized to pH 7 with KOH and then filtered throu Whatman No. 5 filter paper. A subsequent triple extraction this aqueous phase with an equal volume of alkaline buta (butanol: ammonia 9:1 v/v) transferred cytokinins into t organic phase and the aqueous phase, containing salts a other admixtures, was discarded. The combined organic ph was evaporated *in vacuo* and the final drops dried under stream of nitrogen. Further purification was achieved usi two 'Sep-Pak' C_{18} cartridge in series. The residue was th dissolved in 3 ml 50% (v/v) ethanol, centrifuged and p adjusted to 3.5 with NH_4-formate buffer (10 mM) and pass by gravity through two C_{18} 'Sep-Pak' cartridges in seri (Short-body, Waters, Milford, MA, USA) which were prim with 10 ml 80% ethanol and then 10 ml NH_4-formate buff (10 mM) at pH 3.5. The cartridges were washed with 10 ml the same buffer followed by 10 ml de-ionised water (DW Cytokinins were then eluted from the cartridges with 10 96% ethanol and the effluent evaporated to dryness *in vac* at 35 °C. The residue was then dissolved in a final volume 0.5 ml NH_4-formate buffer and filtered through Millex-HV 0.4 μm.

Cytokinin separation was performed using HPLC (Wate Associates). Solvent was delivered by two constant-flow r pumps (Model M-6000A) and controlled by a Model 6 automated gradient controller. Samples were introduc through an Intelligent Sample Processor Model 710B a absorbance of the column effluent was monitored using a 4 tunable absorbance UV detector. The filtrate was fraction using supersphere RP-select B analytical column (250 m long, 4 mm i.d; Merck, Darmstadt, Germany) eluted at 30 All solvents were HPLC-based on 70% acetonitrile (acetonitri water 70:30 v/v) as solvent B and 2% acetic acid (Merck) solvent A. A stepped linear gradient was performed based 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 continuo degassing with helium. The absorbance of the effluent w monitored at 268 nm.

Results and Discussion

Experiments were conducted to determine the cytokin

Table 1: Cytokinin content, determined by HPLC of dry seeds of *Rhazya stricta* collected on different dates. The symbols indicate as follows

Trans-Z = *trans*-Zeatin, DHZ = Dihydrozeatin *cis*-ZR = *cis*-Zeatin Riboside, ip = Isopentyladenine *trans*-ZR = *trans*-Zeatin Riboside. IpR = Isopentyl adenosine nd = not detected.

Sample's year of collection	Cytokinin content ng/gr dry weight.					
	Trans-Z	DHZ	*cis*-ZR	*Trans*-ZR	Ip	ipR
1982	48.3	11.3	60.2	662.0	97.0	nd
1998	200.4	5.6	268.6	482.7	1.9	nd
1999	2.8	26.7	91.8	248.4	1.3	11.1

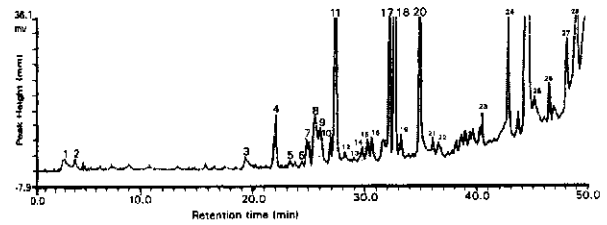

Fig. 1: HPLC profile for cytokinins in *Rhazya stricta* seeds (1982 collection). Peak numbers 5, 6, 10, 13, 24 show *trans*-zeatin (t-Z), dihydro-Zeatin (DH2), *trans*-zeatin rhiboside (t-ZR), *cis*-Zeatin riboside (Cis-ZR), and iso-pentyladenine (ip) respectively. Peak numbers 1-4, 7-9, 11-12, 14-23 and 25-28 show unidentified peaks

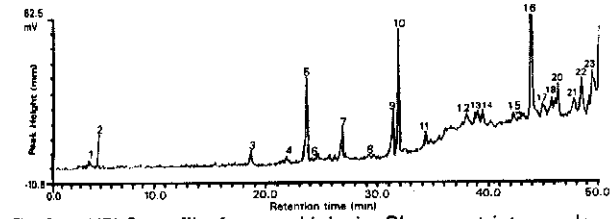

Fig. 2: HPLC profile for cytokinin in *Rhazya stricta* seeds (1998 collection). Peak numbers 5,6,7,8 and 16 show *trans*-Zeatin (t-Z), Dihydro-zeatin (DHZ), *trans*-zeatin riboside (t-ZR), *cis*-zeatin riboside (*cis*-ZR) and isopentyladenine (ip). Peak numbers 1-4, 9-15 and 17-23 show un-identified peaks

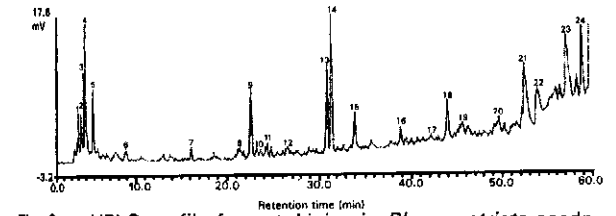

Fig. 3: HPLC profile for cytokinins in *Rhazya stricta* seeds (1999 collection). Peak numbers 10, 11, 12, 17 and 18 show *cis*-zeatin (*cis*-Z), Dihydro-zeatin (DHZ), *trans*-zeatin riboside (*t*-ZR), isopentyl-adenine (ip) and isopentyl adenosine (iPR). Peaks numbers 1-9, 13-16, 19-24 show un-identified peak

content in the quiescent seeds of *Rhazya stricta,* collected in 1999 and, previously, in 1982 and 1998.

Dry seeds were powdered and extracted to determine the cytokinin. HPLC profiles of extracted cytokinins, for three type of seed collections, were obtained, using appropriate internal standard (8- [^{14}C]-Benzyladenine). The result revealed the presence of *cis*-zeatin ribosides and dihydrozeatin ribosides which predominated other cytokinins in all the three types of seeds tested. Beside these ribosides, isopentyl adenine was detected but in small amounts. Trans-zeatin and dihydrozeatin content were very high in the 1998 collection followed by 1982. However, in the 1999 collection a very small quantity was detected. (Fig. 1-3; Table 1). The results obtained in the present study for Zeatin-ribiside (ZR) and dihydrozeatin riboside (DZR) are in close agreement with previous findings in *Zea mays* dry kernels where ZR and DZR have been unequivocally identified as endogenous cytokinins (Devlin and Witham, 1983; MacLeod *et al.,* 1976). In dicot seeds such as *Phaseolus vulgaris,* Zeatin and Ribosyl-Zeatin like cytokinins were detected (Hutton and Van Staden, 1982).

Hocart *et al.* (1988) have reported low levels of cytokinins present in mature seeds. They concluded that biosynthesis of cytokinin occurs during germination and early seedling growth. Hocart and Letham (1990) have explained the reason for the low level of cytokinin in dry caryopsis as immature caryopsis of maize have to mobolize cytokinins extensively by cleavage of side chain which in turn might explain the negligible level of zeatin in quiescent seed axis.

The endogenous cytokinin level in mature seeds are lower than in the developing seeds (Davey and VanStaden, 1979; Summons *et al.,* 1981). It is explained that between two and six weeks after anthesis (in *Lupinus albus*) the cytokinin of developing endosperm increased upto 80% of the cytokinins detected in the entire developing seed. As the embryo and the cotyledon develop, the cytokinin content of seeds of *Lupinus albus* decreased (Davey and Van Staden, 1979).

It was also observed in the present study that zeatin riboside contents were more in the stored seeds than in the non-stored seeds (Table 1). Horcasitas *et al.* (1998) determined similar types of results in *Zea mays* where stored seeds showed more ZR content than in the non-stored seeds.

Increase in zeatin ribosides is explained by different workers. Klemen and Klambt (1974) have shown that *de novo* synthesis of cytokinins could contribute to the internal pool of zeatin ribosides. Hydrolysis of ribosides of zeatin naturally occur in many plant tissues (Goodwin and Mercer, 1983). Devlin and Witham (1983) have explained an exchange between free bases, ribosides and ribotides of zeatin is known to occur in plant tissue. Another explanation is that considerable amount of cytokinin is released through breakdown of t-RNA (Klemen and Klambt, 1974; Smith and Bray, 1982). Numerous other reports claim that the hydrolysis of t-RNA is one of the main sources of natural cytokinins in plants (Barnes *et al.,* 1980; Mass and Klambt, 1981).

Acknowledgment

This research work has been done at Department of Biological Sciences, Wye College, University of London.

References

Abdul-Baki A.A. and J.D. Anderson, 1972. Physiological and Biochemical Deterioration of Seeds. In: Kozlowski, TT Seed Biology. Vol. 2. Academic Press, pp: 283-309.

Barnes, M.F., C.L. Tien and I.S. Gray, 1980. Biosynthesis of cytokinins by potato cell cultures. Phytochem., 19: 409-412.

Bewley J.D. and K.M. Black, 1982. Physiology and biochemistry of seeds in relation to germination. Vol 2. Viability, dormancy and environmental control. Springer-Verlag. Berlin, Heidelberg, New York.

Davey J.E. and J. Van Staden, 1978. Cytokinin activity in *Lupinus albus.* III. Distribution in fruits. Physiol Plant, 43: 87-93.

Davey J.E. and J. Van Staden, 1979. Cytokinin activity in *Lupinus albus.* IV. Distribution in seeds. Plant Physiol., 63: 873-877.

Del'Aquila A., 1987. Mean germination time as a monitor of the seed ageing. Plant Physiol BioChem., 25: 761-768.

Devlin R.M. and F.H. Witham, 1983. Plant Physiology. 4th Ed. Willard Grant Press. Boston, pp: 416-418.

Goodwin T.W. and E.I. Mercer, 1983. Introduction to Plant Biochemistry. 2nd Ed. Pergamon Press, PP: 508-509.

Hocart C.H., J. Badenoch-Jones, C.W. Parker, D.S. Letham and R.E. Summons, 1988. Cytokinins of dry *Zea mays* seeds: quantification by radioimmunoassay and gas chromatography-mass spectrometry. J. Pl. Growth Regul., 7: 179-196.

Hocart C.H. and D.S. Letham, 1990. Biosynthesis of cytokinins in germinating seeds of *Zea mays.* J. Exp. Bot., 41: 1521-1528.

Hocart C.H, D.S. Letham and C.W. Parker, 1990. Metabolism and translocation of exogenous zeatin riboside in germinating seeds and seedlings of *Zea mays.* J. Exp. Bot., 41: 1517-1524.

Horcasitas R.P, E.R. Escudaro, A.P. DeLa Cruz, E.S. De Jimenez and M. Albores-Velasco, 1998. Study of cytokinin content related to loss of viability of long term stored maize seeds. Seed Sci and Technology, 26: 169-178.

Hutton M.J. and J. Van Staden, 1982. Cytokinins in germinating seeds of *Phaseolus vulgaris* L. III. Transport and metabolism of 8[^{14}C] t-Zeatin applied to cotyledons. Ann Bot., 49: 701-706.

Khan A.A., 1971. Cytokinins: Permissive role in seed germination. Sci., 171: 853-859.

Klemen F. and D. Klambt, 1974. Half-life of RNA from primary roots of *Zea mays.* Physiol Plant, 32: 186-188.

Letham D.S., 1973. Regulators of cell division in plant tissue IV. Cytokinins from *Zea mays.* Phytochem., 12: 2445-2455.

MacLeod J.K, R.E. Summons and D.S. Letham, 1976. Mass spectrometry of cytokinin metabolites. Per (trimethysilyl) and permethyl derivatives of glucosides of zeatin and 6-benzylaminopurine. J Organic Chem., 4: 3959-3967.

Mass H. and D. Klambt, 1981. On the biogenesis of cytokinins in roots of *Phaseolus vulgaris* Planta, 151: 353-358.

Nandi S.K. and L.M.S. Palni, 1989. Transport and metabolism of dihydrozeatin riboside in germinating lupin seeds. J. Exp. Bot., 40: 615-621.

Perl M., 1988. Recent development in seed physiology and biochemistry. Seed Sci. and Tech., 135-138.

Perl M. and M. Kretschmer, 1988. Biochemical activities and compounds in seeds. Possibel tools for seed quality evaluation. Ann Bot., 62: 61-68.

Smith A.R. and J. Van-Staden, 1978. Changes in endogenous cytokinin levels in kernels of *Zea mays* L. during imbition and germination. J Exp Bot., 29: 1067-1075.

Smith C.A.D. and C.M. Bray, 1982. Intracellular levels of polyadenylated RNA and loss of vigour in geminating wheat embryos. Planta, 156: 413-421.

Summons R.E, D.S. Letham, B.I. Gollnow, C.W. Parker, B. Entsch, L.P. Johnson, J.K. MacLeod and B.G. Rolfe, 1981. Cytokinin translocation and metabolism in species of Leguminosae-studies in relations to shoot and nodule development. In metabolism and molecular activities of cytokinins. Eds. J. Guern and P. Peaud-Lenoel. Springer-Verlag, pp: 69-79.