

Seasonal changes of endogenous ABA and cytokinins in environmental adaptation of different ecotypes of reed plants*

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Abstract—The roots and leaves of four reed ecotypes (*Phragmites communis* Trinius) growing in the desert regions of northwest China were determined for the levels of abscisic acid (ABA) and cytokinins [isopentenyladenosine (iPA) and zeatin riboside (ZR)] using an enzyme linked immunosorbent assay (ELISA) throughout the whole of growing season. Three terrestrial reed ecotypes in drought and saline habitats contained more ABA than swamp reed during the main stages of the growing season. The ABA levels in the roots of these terrestrial reed ecotypes were higher in spring and autumn than in summer, an opposite situation was observed in their leaves. All reed ecotypes contained more iPA and ZR in the leaves than in the roots, and the decrease in iPA and ZR levels was found in both of tissues with the passage of the season. Compared to the swamp reed ecotype, three terrestrial reed ecotypes showed an increase of ABA and a decrease of cytokinins in the roots and leaves. These results were primarily discussed in relation to the environmental adaptation of reed plants.

Keywords: ABA; cytokinin; ecotype; environmental adaptation; *Phragmites communis* T.

1. Introduction

A rich literature has developed about the relationship between phytohormones and a plant's adaptation to soil drought and salinity. However, the regulation of hormones in the plant's responses to soil drought and salinity is complex and still incompletely understood (Davies, 1986; Munns, 1986). For many years of research attention, there seems to be a crucial role for abscisic acid (ABA) as a "stress hormone" and a chemical message transmitted from roots to shoots. In contrast, little information has been provided about other phytohormones and particularly about the simultaneous changes of various hormones in such cases (Davies, 1991; Zhang, 1989; Zhao, 1991). There is now interest in the effects on a plant's adaptation to soil drought and salinity produced in part by cytokinins. Perturbations in the root environments result in permanent decrease in cytokinin production and reduce cytokinin transport from roots to shoots. The possibility of cytokinins acting as root-to-shoot signals of plant in drying soil has been recently discussed (Amzallag, 1992; Bano, 1993; Thomas, 1993; Turner, 1986). Moreover, a considerable

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amount of studies under artificial stress manners show rather contradictory and even controversial results (Davies, 1986; Munns, 1986). The experiments using the materials which possess stable genetic tolerance/resistance to soil drought and salinity under natural conditions, therefore, are of great significance to gain much-needed data on the connection between phytohormones and a plant's adaptation to soil drought and salinity.

Reed plants are hydrophilous grasses of *Gramineae* whose typical habitats are the swamps, riversides and lakesides with shallow fresh and brackish. However, reed plants can adapt themselves to adverse terrestrial habitats, and evolve various ecotypes which exhibit genetic differences (Haslam, 1970; 1975; Matoh, 1988). There are three terrestrial reed ecotypes growing in the desert regions of northwest China (approximately 3.3×10^5 ha), we have found some stable variations occurring within their morphological and physiological characteristics in response to drought and saline habitats through our investigation for many years (Ren, 1992; Wang, 1993). These reed ecotypes are ideal materials for the research of environmental botany. For the above reasons the present paper describes seasonal changes of ABA and cytokinins in the tissues of the terrestrial reed ecotypes in comparison with that of the swamp reed ecotype. The discussions focus primarily on their environmental adaptation.

2 Materials and methods

2.1 Material and sampling site

Four reed ecotypes (*Phragmites communis* Trinius) were selected for studying at the Desert Research Institute of Chinese Academy of Sciences, Linzhe Research Area (39° N, 100° E). The mean annual precipitation is 118 mm, while the annual evaporation is 2392 mm. Air temperature is characterized by large daily fluctuation, the average annual maximum temperature being 39°C and the minimum -27°C. Frost season lasts 297 days. According to their habitat traits [water- and soluble salt contents of root zone (w. c. and s. c.)], they were named Swamp reed, Dune reed, Light salt meadow reed and Heavy salt meadow reed respectively (Table 1).

Table 1 Soil salt and water contents in root regions of four reed ecotypes

	Ecotype			
	Swamp reed	Light salt meadow reed	Heavy salt meadow reed	Dune reed
Water content	—	35.4	48.5	18.8
Soluble salt content	0.146	0.680	0.792	0.072
Ca ²⁺	0.020	0.024	0.019	0.006
Mg ²⁺	0.007	0.026	0.013	0.002
Na ⁺	0.016	0.156	0.253	0.026
K ⁺	0.003	0.015	0.017	0.002
Cl ⁻	0.027	0.059	0.121	0.014
SO ₄ ²⁻	0.034	0.017	0.094	0.028
HCO ₃ ⁻	0.040	0.048	0.081	0.030

Note: values present the mean of three measurements

The leaves and roots of the four reed ecotypes were individually sampled three times during

the 1991 growing season; May 12, July 15 and September 18. All the leaves and roots were frozen in liquid nitrogen immediately after harvesting. Samples were stored at -80°C until further processing.

2.2 Measurement of ABA

The leaf and root tissues were ground and extracted overnight at 4°C in 80% aqueous methanol containing 1 mg/L butylated hydroxytoluene (pH 8.0). The methanolic extract of tissue was passed over a Sep-Pak C_{18} cartridge (Waters, San Francisco, California, USA) to remove pigments and other lipophilic impurities. The methanol was removed by vacuum distillation and the aqueous residue was partitioned three times against ethyl acetate at pH 3.0 (HCl).

The combined ethyl acetate fractions were reduced to dryness under reduced pressure, the residue taken up in salt Tris buffer (pH 7.8), and analyzed by ELISA for ABA using commercially available ABA assay kits of Idetek (San Bruno, CA, USA). Assays were performed as per their instructions. Recovery of ABA after purification was between 94% and 98%.

2.3 Measurement of cytokinins

iPA (isopentenyladenosine) - like and ZR (zeatin riboside)-like cytokinins were extracted, purified and analyzed as described by Bollmark *et al.* (Bollmark, 1988). The leaf and root tissues were boiled for 5 min in saline phosphate buffer (pH 7.4) and centrifuged at 6000g for 30 min. The supernatant was filtered through a glass fiber filter, then the solution was further purified by PVP column at pH 2.7 and an immunoaffinity column chromatography at pH 7.4. Polyclonal antibodies against iPA and ZR were obtained from rabbits by established methods (Barthe, 1985; Weiler, 1980). For use in immunoaffinity chromatography, the antibodies were coupled to N-hydroxysuccinimide esters of derivatized cross-linked agarose (Affi-gel 10, Bio-Rad), as described by Davis *et al.* (Davis, 1986).

Details of competitive ELISA (enzyme linked immunosorbent assay) technique used for the determination of cytokinins in purified extracts have been described by Bollmark *et al.* (Bollmark, 1988). All results of immunoenzymatic cytokinin determination were to be understood as equivalents of cytokinin-like substances; a mass spectrometric analysis was not carried out.

3 Results

3.1 Seasonal changes in ABA levels

As shown in Table 2, Dune- and salt meadow reeds, which are subjected to drought and saline habitats, exhibited significantly higher ABA contents on May 11th and July 15th, compared to Swamp reed. On the 18th of September the leaves of Swamp reed appeared senescent and accumulated ABA and showed its higher level than that of Dune- and salt meadow reeds. The ABA contents of the latter were even lower on September 18th than on July 15th.

The seasonal fluctuation of the ABA contents in the roots and leaves of dune- and salt meadow reeds exhibited contrasting regular patterns, namely higher on May 11th and September 18th than on July 15th in the roots, and lower on May 11th and September 18th than on July 15th in leaves.

Table 2 Seasonal changes of ABA levels in roots and leaves of four reed ecotypes
 [nmol (g tissue DW)⁻¹]. Values are means ±SE from 4 independent replicates

Month	Swamp reed		Light salt meadow reed		Heavy salt meadow reed		Dune reed	
	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf
5	1.48 (±0.12)	1.02 (±0.08)	4.90 (±0.37)	3.21 (±0.05)	9.58 (±0.104)	7.05 (±0.68)	7.32 (±0.59)	2.53 (±0.34)
7	1.22 (±0.06)	1.26 (±0.11)	1.58 (±0.17)	7.96 (±0.62)	1.88 (±0.09)	9.03 (±0.74)	3.10 (±0.16)	9.87 (±1.13)
9	1.64 (±0.23)	8.33 (±1.06)	2.34 (±0.21)	3.28 (±0.14)	2.74 (±0.10)	3.79 (±0.31)	5.19 (±0.43)	1.26 (±0.08)

3.2 Seasonal changes in iPA and ZR levels

As seen in Table 3 and Table 4, the iPA contents were significantly lower than the ZR contents in the tissues of the four reed ecotypes. iPA are precursors of ZR syntheses, and the biological activities of ZR are far higher than that of iPA (Letham, 1983). Therefore, perhaps reed plants possessed an increased syntheses of ZR via the pathway which transforms iPA into ZR. The tissues of all the ecotypes exhibited decreases in iPA and ZR levels with the passage of the season, this apparently correlated with the seasonal growth and development of reed plants.

Table 3 Seasonal changes of iPA equivalent levels in roots and leaves of four reed ecotypes
 [μmol (g tissue DW)⁻¹]. Values are means ±SE from 4 independent replicated.

Month	Swamp reed		Light salt meadow reed		Heavy salt meadow reed		Dune reed	
	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf
5	11.56 (±0.88)	17.74 (±0.160)	1.85 (±0.06)	2.53 (±0.12)	1.01 (±0.13)	1.42 (±0.14)	1.61 (±0.09)	1.83 (±0.16)
7	6.44 (±0.38)	9.41 (±0.85)	0.89 (±0.02)	1.67 (±0.15)	0.76 (±0.07)	1.15 (±0.08)	1.37 (±0.12)	1.55 (±0.10)
9	1.08 (±0.04)	3.38 (±0.13)	0.61 (±0.06)	1.14 (±0.09)	0.16 (±0.00)	0.42 (±0.03)	0.36 (±0.02)	0.74 (±0.06)

Table 4 Seasonal changes of ZR equivalent levels in roots and leaves of four reed ecotypes
 [μmol (g tissue DW)⁻¹]. Values are means ±SE from 4 independent replicated.

Month	Swamp reed		Light salt meadow reed		Heavy salt meadow reed		Dune reed	
	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf
5	13.37 (±1.21)	25.12 (±2.34)	12.42 (±0.83)	20.72 (±1.79)	11.87 (±0.44)	16.81 (±1.67)	12.75 (±1.18)	21.08 (±2.01)
7	7.04 (±0.58)	14.62 (±1.44)	4.06 (±0.27)	6.45 (±0.62)	2.53 (±0.25)	3.07 (±0.26)	4.79 (±0.41)	8.48 (±0.63)
9	3.05 (±0.19)	5.55 (±0.61)	1.52 (±0.13)	1.95 (±0.20)	0.30 (±0.01)	1.61 (±0.12)	0.71 (±0.07)	2.64 (±0.21)

Moreover, the decreasing ranges of iPA were narrow. Whether this was responsible for maintaining of ZR syntheses or not should be further investigated. In May - September dune - and salt meadow reeds showed a lower level in iPA and ZR contents in comparison with swamp reed.

4 Discussion

The treatments of artificial drought and salinity can result in ABA accumulation in plant tissues, but it increases in either water-treated or salt-stressed plants only in extreme conditions. Thence, it has been still doubtful whether ABA metabolism is closely responsible for the plant long-term adaptation to soil drought and salinity (Davies, 1986). According to the data in Table 2, the tissues of dune- and salt meadow reeds maintained higher ABA levels during the main growth period (May - July), this seems to indicate that ABA accumulation makes a stable contribution to their habitat adaptation. Because ABA is a senescence-inducing substance, the leaf senescence of swamp reed in a favorable habitat was correlated with ABA accumulation in the leaves during the later period of the growing season. Dune- and salt meadow reeds may be insensitive to ABA senescence-inducing, and the higher ABA levels of their tissues contributed to improving the adaptability of the adverse habitats. Therefore, in September the decrease of ABA level in the leaves may be responsible for the leaf senescence of dune- and salt meadow reeds. This phenomenon needs to be further studied. In May and September the four reed ecotypes were all at the seedling and senescence periods respectively, and at these times the capability of ABA synthesis was limited in their leaves. However, the ABA levels of the roots were higher than that of the leaves in dune- and salt meadow reeds at both periods. For this reason it is quite evident that ABA accumulation in the roots cannot only result from ABA syntheses in the leaves of these reed ecotypes, while the higher ABA contents in their leaves than in their roots in July may be at least partly due to transport of root-sourced ABA to the shoots. Some researches have demonstrated that under water stress conditions ABA accumulation in the roots depends on ABA synthesis in the roots rather than downward leaf-sourced ABA transport from the shoots to the roots, and root-sourced ABA can be translocated in the xylem vessels to the stem and leaf tissues (Wolf, 1990; Zhang, 1989; Zhao, 1991).

Under soil drought and salinity cytokinins, which are synthesized in the roots, are strong candidates for chemical signals transmitted from roots to shoots, although in some instances they may have been masked simply because of inappropriate experimental conditions (Davies, 1986; Munns, 1986). According to the data so far, soil drought and salinity result in permanent decreases in the cytokinin levels of plant tissues (Amzallag, 1992). The present work also supports this idea. Some studies suggest that cytokinin levels of the shoot decrease, not as a result of reduction in cytokinin production by roots, but due to decrease in upward transport of cytokinins from roots to shoots (Davies, 1986; Turner, 1986). As seen in Table 3 and Table 4, dune- and salt meadow reeds show higher cytokinin contents in the leaves than in the roots. This indicates that the cytokinins synthesized in roots are efficiently transported to shoots. The lower cytokinin levels in their roots and leaves comparable to swamp reed are due to decreases in cytokinin syntheses of their roots. The ABA accumulation appears to be a reason why cytokinin production is reduced in the roots of dune- and salt meadow reeds since all the ecotypes exhibited a negative relationship between ABA and cytokinin contents in some extent. A wide variety of studies have reported that ABA inhibits the biosyntheses of cytokinins, and soil drought and salinity result in

the decreases of cytokinins and the increase of ABA (Back, 1972; Bourquin, 1990; Davies, 1986; Sondheimer, 1971).

Often only ABA actions are taken seriously in considering a plant's responses to soil drought and salinity. Perhaps ABA and cytokinins are of equal importance to the plant's stress - adaptating. For example, the water status influences the level of ethylene, cytokinin and ABA in the plant. Both an increase in ABA and a decrease in cytokinin can offset the stimulation of "stress ethylene" synthesis by induction of a low water potential (Davies, 1986). As far as reed plants are concerned, may be the interactions and specific balance between ABA and cytokinins in their roots and leaves are responsible for their adaptation to soil drought and salinity.

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References

- Amzallag GN, Lerner HR, Poljakoff - Mayber A. *J Exp Bot*, 1992; 43:81
Back A, Bittner S, Richmond AE. *J Exp Bot*, 1972; 23:744
Bano A, Dorffling K, Bettin D, Hahn H. *Aust J Plant Physiol*, 1993;20:109
Barthe GA, Stewart I. *J Agric Food Chem*, 1985; 33:293
Bollmark M, Kubat B, Eliasson L. *J Plant Physiol*, 1988; 132: 262
Bourquin M, Pilet P - E. *Physiol Plant*, 1990; 80:342
Davies WJ, Metcalfe J, Lodge TA, Costa ARda. *Aust J Plant Physiol*,1986; 13:105
Davies WJ, Zhang J. *Annu Rev Plant Physiol. Plant Mol Biol*, 1991;42:55
Davis GC, Hein MB, Chapman DA. *J Chromatog*, 1986; 366:171
Haslam SM. *Ann Bot*, 1970; 34:147
Haslam SM. *Ann Bot*, 1975; 39:881
Letham DS, Palni LMS. *Ann Rev Plant Physiol*, 1983; 34: 163
Matoh T, Matsushita N, Takahashi E. *Physiol Plant*, 1988; 72; 8
Munns R, Termaat A. *Aust J Plant Physiol*, 1986; 13: 143
Ren DT, Zhang CL. *Acta Boytanica Sinica*, 1992; 34; 698
Sondheimer E, Tzou DS. *Plant Physiol*, 1971; 47:516
Thomas JC, Bohnert HJ. *Plant Physiol*, 1993; 103: 1299
Turner NC. *Aust J Plant Physiol*, 1986; 13: 175
Wang HL, Zhang CL. *Acta Botanica Sinica*, 1993; 35; 533
Wolf O, Jeschke WD, Hartung W. *J Exp Bot*, 1990; 41; 593
Zhao KF, Munns R, King RW. *Aust J Plant Physiol*, 1991; 18:17

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