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Responses of ABA and CTK to soil drought in leafless and leafy apple tree*

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Abstract: The authors tested the contents of ABA (abscisic acid), ZR (zeatin riboside), DHZR (dihydrozeatin riboside) and iPA (isopentenyl adenosine) in leafless and leafy apple trees (*Red Fuji/Malus micromalus Makino*) during soil drought stress. ABA concentration in drought stressed leafless trees increased significantly compared to the controls. ABA both in roots and xylem rose steadily in the earlier drought stage, reaching a maximum of 1.46 ± 0.35 nmol g^{-1} FW and 117 nmol l^{-1} after the 8th day. Similar change patterns of ABA concentration was observed in the leafy trees during soil drought stress; ABA concentrations in roots and xylem sap increased and reached the maximum in the first three days; after 8th day, it decreased slightly, whereas leaf ABA concentration increased steadily in drought stressed plants throughout the duration of the experiment. Between drought stressed and control trees, no significant differences were observed in concentration of ZR and DHZR in both leafless and leafy trees; whereas iPA concentration of the drought stressed leafless and leafy plants decreased markedly in the later stage of drought. These results showed that endogenous ABA originated mainly from the roots in the earlier drought stage, and mainly from the leaves in the later drought stage. Total CTK showed no reduction in the earlier drought stage and decreased in the later drought stage.

Key words: Apple tree, Endogenous hormone, Soil drought, Relative water content, Water potential
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INTRODUCTION

It is generally accepted that soil drought can cause restriction of water uptake by drought roots, change of hormone concentration, reduce the tissue relative water content and water potential, and decrease stomatal conductance and leaf growth (Kramer, 1988; Davies et al., 1991), and that alteration in cytokinin and abscisic acid (ABA) concentration likely plays a major role as a positive signal in mediating stomatal conductance and leaf growth (Zhang et al., 1989; Shashidhar et al., 1996). However, whether endogenous ABA in drought stressed plant is originated from root or leaf and which is a debated problem (Neales et al., 1991; Slovik et al, 1995; Jokhan et al., 1996). The contribution of root-sourced ABA or leaf-sourced ABA to mediating stomatal conductance and leaf growth or other physiological courses is also not clear because root-sourced ABA that reached the guard cell could have originated not only from root biosynthesis (Zhang et al., 1989; Ali et al., 1996; Simonneau et al., 1998), but also from root soil environmental ABA secreted by soil microbions such as fungi and bacteria (Slovik et al., 1995). Leaf-sourced ABA may stem from not only synthesis of mesophyll cell or guard cell itself (Cornish et al., 1986), but from redistribution or recycling through the whole plant via phoem as well (Hubick et al., 1988; Wolf et al., 1990); a recent experiment provided evidence that stomatal closure is not directly related with ABA collection but with pH increase in xylem sap under drought stress condition (Daeter et al., 1995; Wilkinson et al., 1997).

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Not withstanding possible acceptance of ABA is a candidate of a positive signal born in droughted root, the controversy of cytokinin as a candidate of negative signal produced in droughted roots for communication between root and shoot in drying soil has lasted for several decades. Although cytokinins were generally considered to be an antagonist of ABA, there is still poor understanding of the precise role of cytokinins in stressed plants (Hare et al., 1997). Since Itai and Vaadia (1965) suggested that reduction of cytokinin in droughted plant was a sensitive signal for stressed root. Many attempts to verify their conclusion led to a lot of inconsistent result. Different researches focused on alteration of cytokinin concentration during drought condition led to different conclusions. The experiment of Blackman and Davies (1985) suggested it was necessary for a plant grown in drought soil to supply continuously enough cytokinin from roots in order to maintain maximal stomatal opening. Meinzer et al. (1991) showed that total cytokinins activity in the xylem sap of sugarcane decreased when the plant was under drought stress that resulted in closure of the stomatal opening. Subsequently, Bano et al. (1993) tested different kinds of cytokinins by using monoclonal antibody of trans zeatin riboside (t-ZR) and isopentenyl-adenosine (iPA), respectively; and observed significant reduction both in concentration and in delivery of two kinds of cytokinins (t-ZR and iPA) in xylem sap in plants under drought stress. However, both FuBeder et al. (1992) and Masia et al. (1994) obtained the different results, in that is there was no significant difference in cytokinin concentration in xylem sap as compared between control and stressed plants.

In this study, we quantified ABA concentration both in root and in xylem sap of leafless trees, as well as leafy trees grown in drying soil. Three kinds of cytokinins [involving ZR (zeatin riboside), DHZR (dihydrozeatin riboside) and iPA (isopentenyl adenosine)] concentrations were also tested. The contribution of root-sourced ABA and leaf-sourced ABA to the target cell were estimated by comparing ABA concentration in leafless tree with that in the leafy trees during soil drought stress. Changes in ZR, DHZR and iPA concentrations are discussed below.

MATERIALS AND METHODS

Plant material

Two-year-old apple trees (*Red Fuji/Malus micromalus Makino*) were transfered from the field in early spring and cultivated in pots ($H \times D = 45\ cm \times 30\ cm$) contaioing 17 kg soil. One plant was transcultivated per pot. Three pots are used for each experiment.

Experiment 1

After the tree lamina fell completely in winter, a group of leafless trees were subjected to soil drought stress by withholding irrigation. Root and xylem sap were sampled for ABA and CTK every three days throughout the duration of the soil drought stress. The control group was watered normally. Three replicates were tested for each test sample.

Experiment 2

In August, two groups of leafy apple trees were chosen as test materials. One group was subjected to drought stress by withholding irrigation; the other was the control group irrigated normally. Three replicates were set up for each sample.

Soil water potential (ψ_w)

After a 7 cm deep cavity was dug 5 cm below the surface of the pot soil of both the control group and drought stressed group, a probe was put into the cavity and covered with soil hermetically; after 8 hours equilibration, the soil water potential was measured with a pewpoint Microvoltmeter HR-33T (WESCOR, INC., LOGAN, USA).

Leaf water potential (ψ_w)

Six leaf discs harvested from six different laminas were transferred immediately to a hermetic chamber. After 4 h equilibration, measurement of leaf water potential was conducted on 3 replications per sample with Dewpoint Microvoltmeter HR-33T.

Relative water content (RWC)

RWC was estimated with the following equation: RWC (%) = (Fresh weight-Dry weight)/(Turgid weight-Dry weight). Fresh weight was determined directly with electronic scale. Then the sample was soaked to saturation in distilled water to get its for turgid weight; after which the dry weight of the sample was mea-

sured after the sample was baked at 65℃ in drying oven for 2 days.

Xylem sap collection

Xylem sap was collected with pressure technique of Neales et al. (1989). Apple tree was decapitated to leave a stump of about 10 cm above the soil surface. The original bleeding sap was removed with filter paper. Then the remaining stump with the whole root system was enclosed in a plastic bag (to prevent soil attached roots from scatting out) and transferred to a pressure chamber and pressurized at a 1.0 – 1.5 MPa. 0.1 – 0.5 ml xylem sap flowing from the cut stump was collected in the dark in an ice-cooled centrifuge tube with a 1 ml injector, and frozen in liquid nitrogen immediately for storing in a – 20 ℃ refrigerator for analysis of ABA and CTK.

Endogenous hormones extraction from roots and leaves

Roots and leaves samples were ground down in ice and extracted overnight at – 4℃ in 80% (v/v) aqueous methanol. The methanolic extracts were filtered and then passed through a Sep-pak C_{18} cartridge (eluted with 80% aqueous methanol) to remove the pigments and other lipophilic impurities. The eluates were reduced in vacuum to methanol and water with a rotary evaporator and adjusted to pH 3.0 with diluted ethyl acetate (Shashidhar, 1996). The coqueous residue was partitioned three times through diluted ethyl acetate at pH 3.0.

HPLC analysis for ABA and cytokinins

The total extract (20 μl) was processed by an HPLC (Beckman 322, USA) equipped with a reverse phase column (4.6 × 125 mm Lichrocart C_{18}, 5 μm). As solvent, 52% methanol containing 1% aq. acetic acid was used at a flow rate of 0.8 ml/min. The detection was run at 250 nm with a UV-detector. The results were quantified with a Hewlect-Packard 3396SA integrator using a relative calibration procedure by Eberle (1986) for benzyl derivatives.

ELISA analysis for ABA

Fresh leaf samples were extracted with 8 ml of 10 mmol/L $CaCl_2$ for 24 h at 4℃. The aqueous extracts were adjusted with 0.1 mol/L HCl to pH 3.0 and fractionized three times against ethyl acetate. The organic fractions were collected and reduced to dryness, taken up in TBS-buffer (Tris-HCl: 50 mmol/L Tris, 150 mmol/L NaCl, 1 mmol/L MgCl2, pH 7.8) and subjected to ABA assay by ELISA as described earlier (Weiler, 1986). The aqueous fractions containing ABA-conjugates were hydrolysed with NaOH (1 mol/L) for 1 h, adjusted to pH 3.0 with HCl and fractionated against ethyl acetate again. ABA released from ABA-conjugates was subjected to ELISA again.

In order to check the veracity of the HPLC assay, a linear regression between ABA concentrations in leaf was obtained by HPLC and ELISA, showing that the linear correlation was: $y = 0.26 + 0.88x$ ($n = 15$, $r^2 = 0.81$). x, value by ELISA; y, value by HPLC.

The data presented in the results were for single experiment with triplicate samples by HPLC. All analysis were performed by least significant difference ($P < 0.05$).

RESULTS

1. Change of soil water potential (ψ_{ws}) and soil relative water content (RWC_S) in leafless apple trees during soil drought stress

The soil water potential (ψ_{ws}) in leafless plant decreased gradually with the prolongation of drought. The average value reduced per day was about 0.12 MPa. (Fig. 1a). The soil relative water content (RWC_S) reduced at rate of 2.5% during the soil drought stress (Fig. 1b).

2. Change of leaf water potential (ψ_{wl}) and relative water content (RWC_l) in leafy apple trees during soil drought stress

A marked decrease of leaf water potential was shown in leafy apple trees during soil drought stress, the average value of reduction in leaf water potential per day being about 0.3 Mpa (Fig. 2a). Similarly, RWC_l reduced by about 5.5% per day during soil drought stress (Fig. 2b).

3. ABA concentration in root and xylem sap of leafless trees during soil drought stress

After irrigation was stopped, the ABA concentration in the roots increased steadily over that of the controls, reaching of the maximal level of 1.44 ± 0.08 nmol/g FW on the 8th day of soil drought, which was significantly higher ($P < 0.05$) than at of the control (0.1 ± 0.02 nmol/g FW), and remained constant thereafter (Fig. 3b). The change in ABA concentration in xylem sap was similar to that in the roots, and

both reached the maximum (118 ± 8 nmol/L, Fig.3a; 1.41 ± 5 nmol g FW, Fig.3b) after 8 days of drought. The obviously similar change pattern of ABA in roots and xylem sap of leafless trees under drought condition indicated that ABA in the xylem sap originated probably from the roots subjected to soil drought stress.

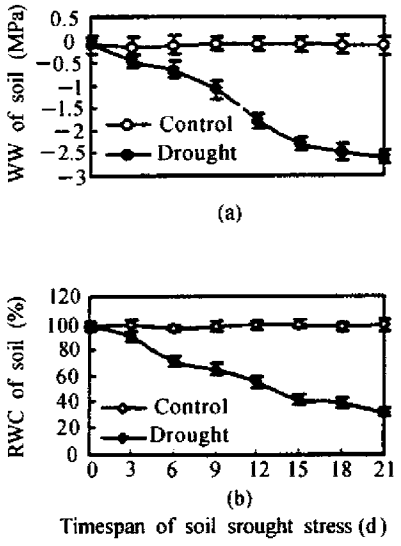

Fig.1 Soil water potential (a) and relative water content (b) of leafless apple tree changed with duration of drought during soil drought stress. Each point is the means (n = 3) value with standard deviation bars

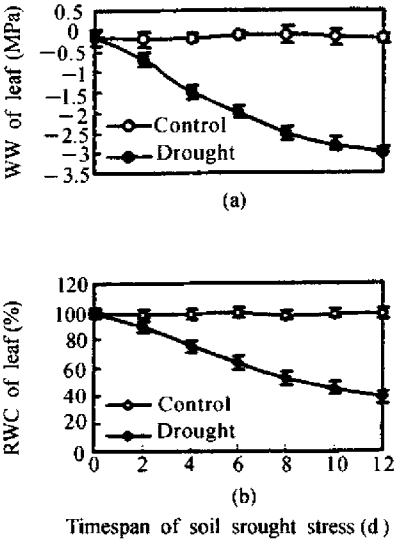

Fig.2 Leaf water potential (a) and relative water content (b) of leafy apple tree changed with duration of drought during soil drought stress. Each point is the means (n = 3) value with standard deviation bars

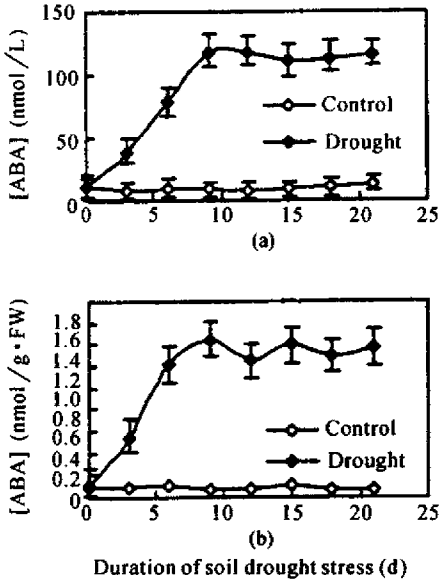

Fig.3 ABA concentration in xylem sap (a) and root (b) of leafless apple tree are influenced by soil drought stress during soil drought stress. Each point is the means (n = 3) value with standard deviation bars

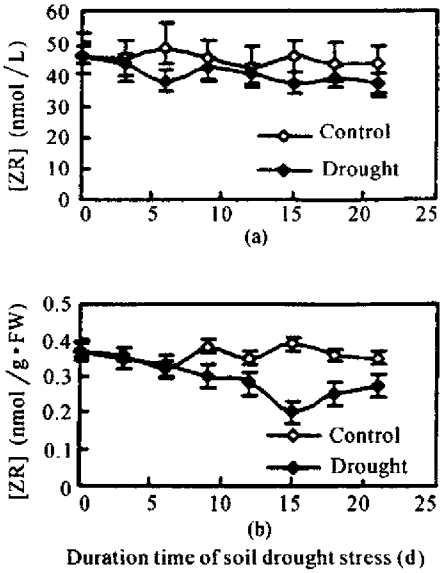

Fig.4 ZR concentration in xylem sap (a) and root (b) of leafless apple tree are influenced by soil are influenced by soil during soil drought stress. Each point is the means (n = 3) value with standard deviation bars

4. ZR DHZR and iPA concentration in xylem sap and root of leafless trees during soil drought stress

The ZR concentration in the xylem sap of the drought stressed and control trees were not significantly different ($P < 0.05$), but on the whole, the ZR concentration in the drought stressed plants at each point was lower than that in the controls (Fig. 4a). The ZR concentration in the roots began to decrease from the 6th day after withholding irrigation compared to the controls, but a little increase in [ZR] was observed from the 15th day of soil drought, which was still substantially lower than that of the control (Fig. 4b). DHZR changes had pattern similar to ZR. These results indicate that there was only slight decrease in endogenous ZR and DHZR of apple tree under soil drought stress.

Fig. 5a shows that in the earlier drought stage, the change of iPA concentration in the xylem sap of the drought stressed tree was similar to that of the control plant, but compared to the control plant, decreased significantly ($P < 0.05$) from the 12th day after the soil drought. Similar change pattern of iPA in roots was observed, too (Fig. 5b).

5. ABA concentration in leaf, xylem sap and root of leafy trees during soil drought stress

Compared to the controls, the leaf ABA concentration in the drought stressed trees increased significantly ($P < 0.05$) with the drought time throughout the experiment (Fig. 6a). The ABA concentration in the xylem sap increased rapidly after soil drought, reaching maximum (124.5 ± 7nmol / L) on the third day, and remained at maximum value during the drought until the 9th day (Fig. 6b). Similarly, the root ABA concentration in drought stressed leafy trees increased rapidly after irrigation was stopped, reaching and keeping the maximum value subsequently. During the later stage of the drought duration, a slight reduction of ABA concentration was observed (Fig. 6c). The similar change patterns, and the close values of the ABA concentration in

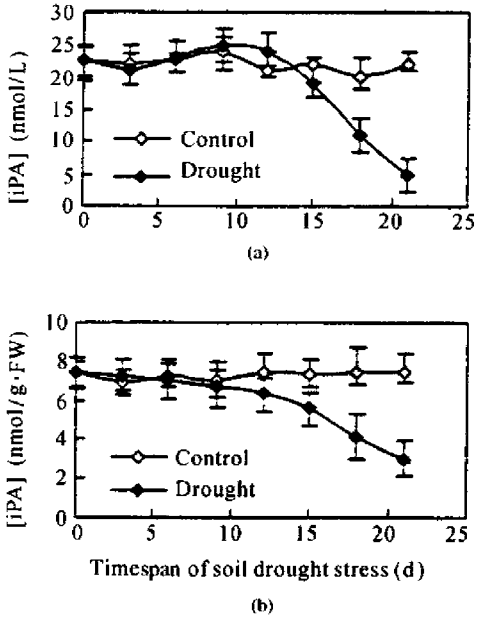

Fig. 5 iPA concentration in xylem sap (a) and root (b) of leafless apple tree during soil drought stress. Each point is the means ($n = 3$) value with standard deviation bars

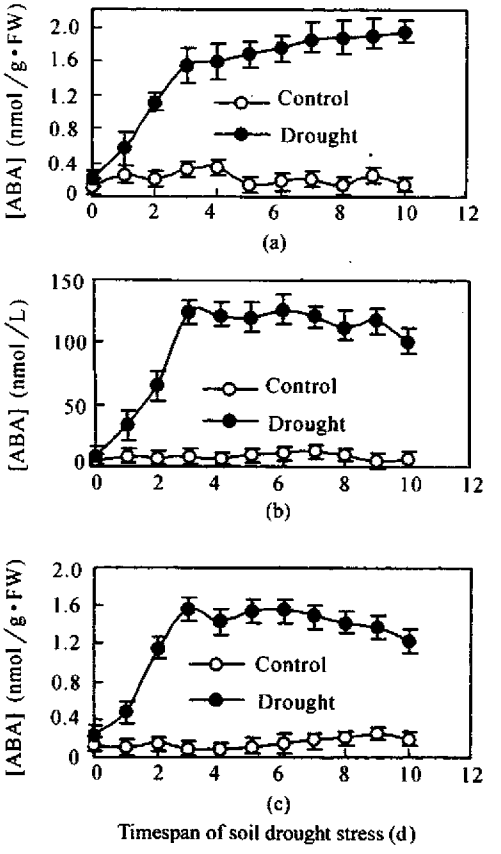

Fig. 6 ABA concentration in leaf (a) xylem sap (b) and root (c) of leafy apple tree are influenced by soil during soil drought stress. Each point is the means ($n = 3$) value with standard deviation bars

leaf, xylem sap and root under drought condition, together with combining the patterns and values of ABA in the leafless trees, suggested that the drought stressed roots were the main organs that produced the endogenous ABA in the apple tree in the earlier stage of soil drought; and that the xylem and leaf ABA came mainly from the roots. The xylem ABA as well as root ABA concentration decreased a little in the later stage of soil drought, probably because the roots that suffered from the heavy drought in the later stage were not able to produce ABA.

6. Cytokinin concnetration in leafy tree during the soil drought stress

No significant difference ($P < 0.05$) in ZR concentration in leaf, xylem sap and root of leafless apple trees was observed between the drought stressed and the control tree throughout the drought period (Fig. 7 a, b, c). Similar result was also obtained for DHZR. The iPA concentrations in leaf, xylem sap and root all remained constant compared to the controls in the first 6 days after withholding irrigation; started to decrease markedly in the drought stressed trees after the 6th day of drought (Fig. 8 a, b, c). Although there were no significant difference in the ZR, DHZR and iPA concentration in the earlier drought stage, we observed that the values of the total ZR and DHZR concentrations in drought stressed the plant were lower than those of the control. So the total cytokinin (involving ZR, DHZR and iPA) concentration in the drought stressed apple tree decreased significantly compared to the control tree. The similar changed level of CTK in the leaf, xylem sap and root showed that the endogenous CTK originated mainly from the roots during the whole period of drought stress.

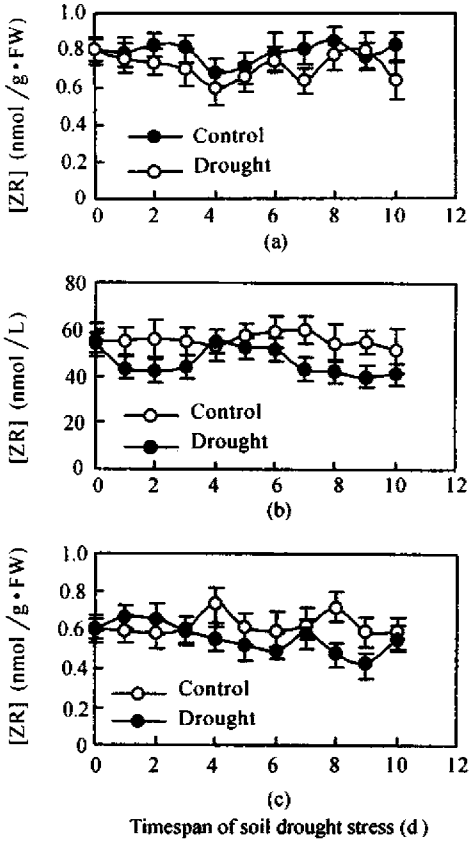

Fig. 7 ZR concentration in leaf (a) xylem sap (b) and root (c) of leafy apple tree are influenced by soil during soil drought stress. Each point is the means ($n = 3$) value with standard deviation bars

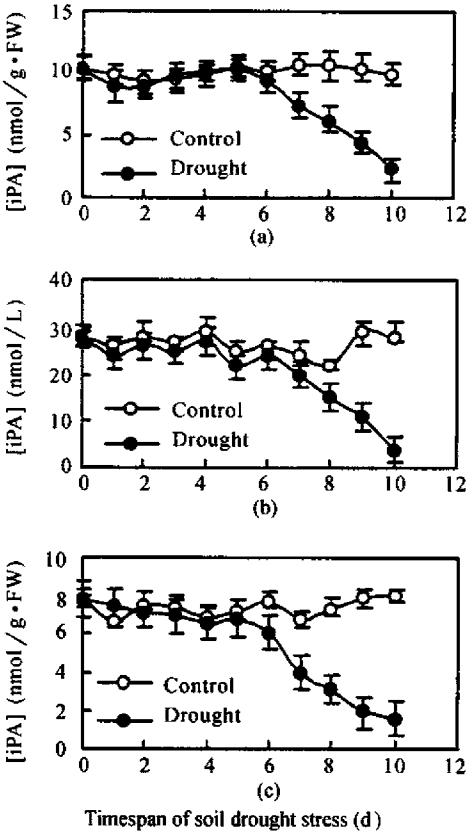

Fig. 8 iPA concentration in leaf (a), xylem sap (b) and root (c) of leafy apple tree influenced by soil during soil drought stress. Each point is the means ($n = 3$) value with standard deviation bars

DISCUSSION

The endogenous hormones are the first responsor of most plants to environmental water stress, Among all kinds of hormones, ABA and CTK were considered to the key hormones in responding to water deficit because they are very sensitive to environmental stress – ABA and CTK concentrations, or their delivery rate, which are changed substantially during water stress (Itai and Vaadia, 1965; Zhang and Davies, 1989; Jackson, 1994). In general, the experimental conclusions of most previous researcher were that endogenous ABA increased under drought conditions; the dehydrating roots were suggested to generate ABA throughout the whole drought period. Our experiments showed that ABA in leafless apple trees and leafy trees increased rapidly, reaching maximum in the earlier drought stage; and that the root ABA and xylem ABA had similar change pattern (Fig.3 a, b and Fig. 6 b, c). This result was similar to that of Shashidhar et al. (1996) who suggested that ABA concentration and delivery rate increased under the mild drought stress; and that root ABA contents mirrored those of xylem ABA. Ali et al (1998) observed that leaf and xylem ABA concentrations increased during mild soil drying in the field. Our results suggested that xylem ABA originated from the dehydrated roots, named root-sourced ABA, which was transported to the xylem and leaf, which confirmed further previous conclusions for other plants.

The roles and the changes of endogenous CTK in plant during dehydration had been concerned over the past decades. Most experiments showed that significant reduction of CTK concentration under drought condition, was caused by root-to-shoot communication signals mediating some physiological responses (Itai et al., 1968; Blackman et al., 1985; Incoll et al., 1987; Bano et al., 1993; Shashidhar et al., 1996). However other researches concluded that there was little difference in xylem sap CTK concentration in the control and the stressed plants (Masia et al., 1994; FuBeder et al 1992). To set detailed data on the response of CTK to water stress, Bano et al. (1993) measured the respective concentration and delivery rate of trans zeatin riboside (t-ZR) and isopentenyl-adenosine (iPA) by using monoclonal antibody. They found that both concentration and delivery rate of t-ZR and iPA in the xylem sap decreased significantly in the stressed plants; and were convinced that total CTK decreased during drought stress. This work results showing that the iPA concentration decreased markedly and the total CTK concentration reduced in response to the drought in the later stage supported firmly the conclusion of Bano et al. (1993). Our results (of no significant difference in both ZR and DHZR concentrations in stressed and control apple trees) were similar to those of Masia et al. (1994). So it was deduced that different alterations of each kind of phytohormones could possibly occur in different species during drought stress.

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