

# Root-shoot communication in drying soil is mediated by the stress hormones abscisic acid and cytokinin synthesized in the roots

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In drying soils, particularly during initial stages, stomatal conductance ( $g_s$ ) and leaf growth of plants may be controlled by a chemical message from the roots rather than by the water status of the leaves. The most likely mechanism involves a build-up of abscisic acid (ABA) in dehydrating roots, its transport through the xylem flow and regulation of  $g_s$  and leaf growth. We have developed different systems to monitor  $g_s$  and water relations of leaves during progressive soil drying, as well as to determine ABA and cytokinin content in roots at different depths of the drying soil in two species, sunflower (*Helianthus annuus*) and cowpea (*Vigna unguiculata*). The data suggest that the initial reduction in  $g_s$  under soil drying in both sunflower and cowpea could be exclusively attributed to root signals as there was no decrease in turgor of leaves. The hormonal basis of a novel communication mechanism between root to shoot for dehydration postponement is discussed.

In recent years, there has been concerted interest in the possibility that the root can communicate to the shoot some indication of the perturbation in the soil environment. Stomatal conductance may be influenced under drought conditions through changes in leaf water potential and metabolic changes in leaf. Evidence for a non-hydraulic root-to-shoot communication on soil water status, which causes stomata to close without changes in the water potential and the turgor of the leaf, had accumulated during the last decade<sup>1-5</sup>. Initial interest in root signals was generated by field observations<sup>6</sup> which showed that stomatal behaviour can be largely independent of changes in leaf water relations but may be more closely linked with soil water status. Later work<sup>1-7</sup> suggested that such a phenomenon results from roots sensing drying soil via the production of a chemical, 'root signal', which is transported to shoots in the transpiration stream to influence shoot physiology. The greatest advantage of such a response is that it can occur early in the soil drying cycle before any water deficit develops in the shoots and may, in fact, prevent or at least delay the onset of shoot water deficit. More recently, it has been suggested that root-sourced abscisic

acid (ABA) plays a major role in chemical signalling from roots to shoots<sup>8</sup>. It is apparently produced in roots which undergo only mild dehydration and appears shortly thereafter in xylem and in the leaf epidermis<sup>3,9-11</sup> and was therefore classified as a positive signal (Figure 1).

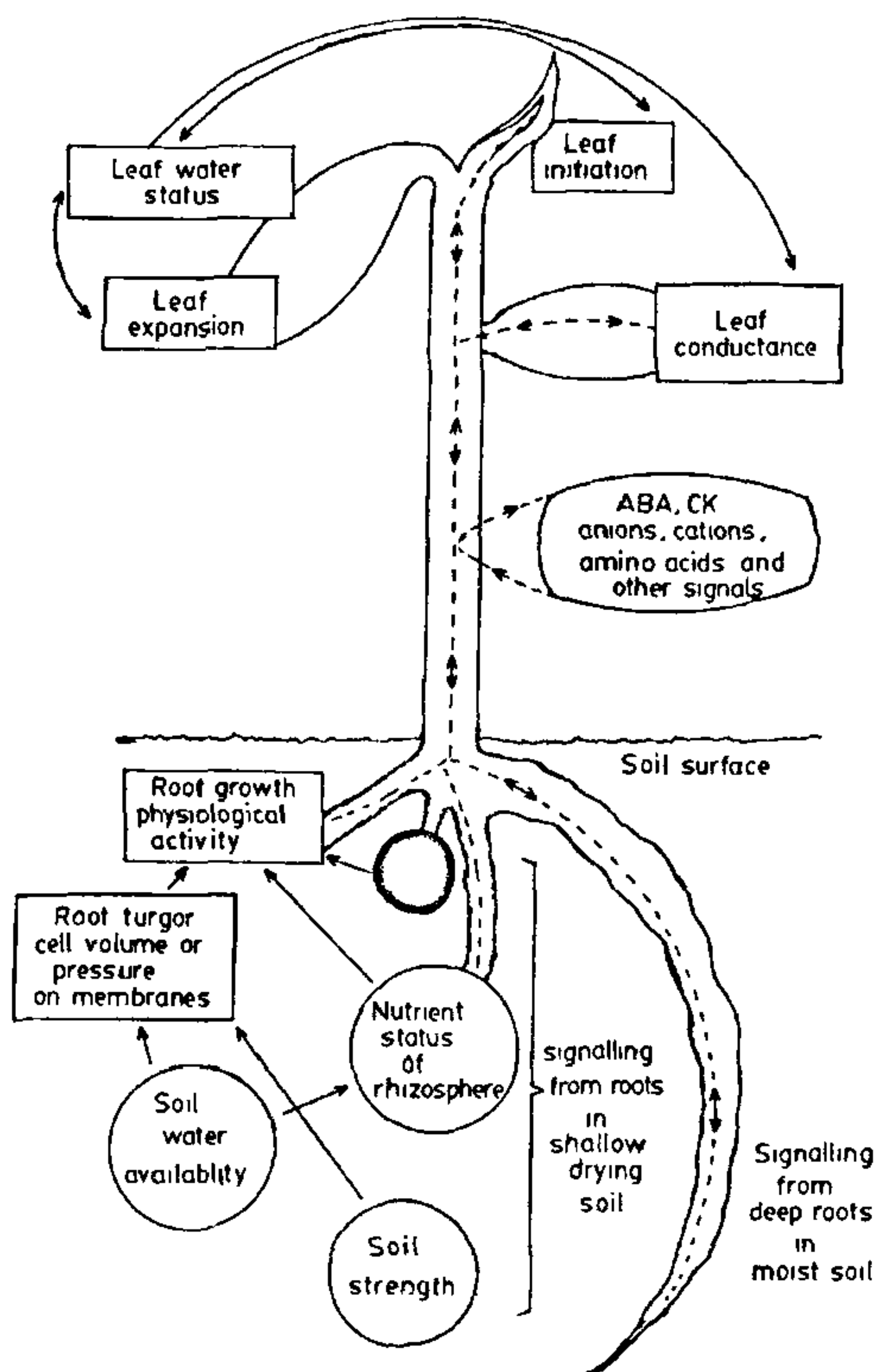


Figure 1 Diagrammatic representation of factors influencing the generation of chemical information (dotted line) in roots in drying soil, the transfer of this information to leaves and its effects on shoot processes (from ref. 8).

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Recent work has highlighted several other possibilities besides ABA which function as a signal in root-shoot communication which include cytokinins, ions and action potentials<sup>2-5</sup>. ABA and other chemical messengers from root are therefore thought to act as signals for the initiation of regulatory processes involved in adaptation.

Recently it was suggested<sup>12</sup> that if plants vary in sensitivity with which they 'anticipate' declining soil moisture, it would be possible to select this response by assaying ABA production in roots. This could result in an increase in water use efficiency in situations of drought.

The objectives of this investigation were to find out: (a) is signal-mediated response in stomatal conductance a function of leaf water status or independent of it? (b) is a single signalling system in the form of root ABA involved in root-to-shoot communication or is it a combination of negative and positive signals? To accomplish this, different systems were developed to determine the role of ABA (positive signal) and cytokinin (negative signal) in a plant growing in a progressively drying soil.

## Materials and methods

### *Experiment-I: Leaf conductance monitoring system (LCM)*

#### Approach-1

Water was withheld and sampling for  $g_s$  in the shoot and hormone content in the root and xylem sap were done when soil water potentials in the top 10 cm reached pre-determined degree of stress (mild, moderate, severe).

Sunflower plants cv. KBSH-1 were grown in battery containers and subsequently thinned to maintain two plants per container. When plants were 25 days old, a stress treatment was imposed by withholding water. Soil water potential (SWP) was determined using thermocouple psychrometer (WESCOR HP-115) for samples drawn from top root zone at 10 cm and  $g_s$  was recorded when the SWP reached -0.3, -0.6 and -1.2 MPa (hereafter referred to as mild, moderate and severe stress respectively). At this time the roots in the top 10 cm were sampled for ABA and cytokinins following solvent extraction procedure<sup>13</sup>. The two hormones were also determined in the xylem exudate collected following standard methods<sup>3</sup>.

#### Approach-2

$g_s$  was monitored continuously after initiation of soil drought and hormone content in roots and xylem sap determined at predetermined levels of reduction in  $g_s$  over non-stressed control.

Here two species of sunflower (cv. KBSH-1) and cowpea (cv. C-152) were interplanted in the same container. The pre-determined levels of reduction in  $g_s$  were

Stage-I:  $g_s$  decrease of 10-15% over non-stressed control

Stage-II: 40-45% decrease

Stage-III: 60-65% decrease.

### *Experiment-II: Vertical split root system*

The objective of this system was to see if allowing a portion of the roots to lose turgor while the rest maintain the transport of water to the shoots, would result in a chemical message being produced in roots which had lost turgor. If so, the leaves have to maintain a high leaf water potential, the chemical message synthesized in the roots which had lost turgor would be sufficient to cause a decrease in conductance. To achieve this, plants were grown with upper part of the root system in damp soil while the lower portion protrudes through the base of the pot.

### *Plant material*

The procedure of Neales *et al.*<sup>3</sup> was followed with minor modifications. Sunflower plants were grown with the upper part of the root system in damp soil of a 9.0 cm pot and the lower portion protruding through the base of the pot. This was achieved by replacing the base of each pot with a disc of rigid polythene mesh. This technique is thus analogous to the split root technique<sup>6</sup>, except that the root system is split vertically rather than horizontally.

### *Polyclonal antibodies for cytokinin and ABA*

Polyclonal antibodies were developed using standard procedures<sup>14</sup>. Both antigens (t-ZR for cytokinins and  $\pm$  cis-trans ABA; Sigma Chemical Co., USA) were conjugated to a carrier protein (BSA) before immunization in rabbits following established procedures<sup>15</sup>. Our previous studies (unpublished) had shown that zeatin type of cytokinins occurs up to 91-92% of total cytokinins in xylem sap. Estimation of zeatin group therefore gives a reasonably precise determination of total cytokinins. The presence of specific IgG against ABA-BSA and against t-ZR BSA was tested by standard immunodetection methods<sup>14</sup>. They included (i) Ouchterlony double immuno diffusion (ii) quantitative precipitin tests and (iii) dot immunoblot assays. A direct competitive binding, ELISA, as described by Weiler<sup>13</sup> was adopted for hormone determination. For hormone conjugation to alkaline phosphatase (enzyme label or tracer) the methods described by Weiler<sup>13</sup> were adopted.



Results

Experiment-I-Approach-I

The data on changes in leaf water potential and stomatal conductance at three soil water potential corresponding to (a) mild (b) moderate and (c) severe soil drying are presented in Figure 2. It is clear that during initial stages of soil drying (stage-I), the turgor or water status of the leaves had not changed, but decreased only under moderate stress (stage-II), and decreasing still further under soil drying. Interestingly,  $g_s$ , even during the early stages of soil drying, had decreased by as much as 20%. At this stage therefore the decrease in  $g_s$  was independent of the turgor of the leaf and could be attributed solely to a non-hydraulic signal from the roots. At later stages of soil drying, the turgor of the leaf decreases substantially and therefore decrease in  $g_s$  at that stage could be attributed to both (a) decrease in leaf water potential and (b) metabolic changes in the leaf in response to decrease in turgor.

The data on changes in ABA and cytokinin presented in Figure 3 reveal that there was a metabolic signal triggered by soil drying even during the early stages. The root and xylem ABA had increased substantially at stage-I accompanied by a decrease in cytokinin

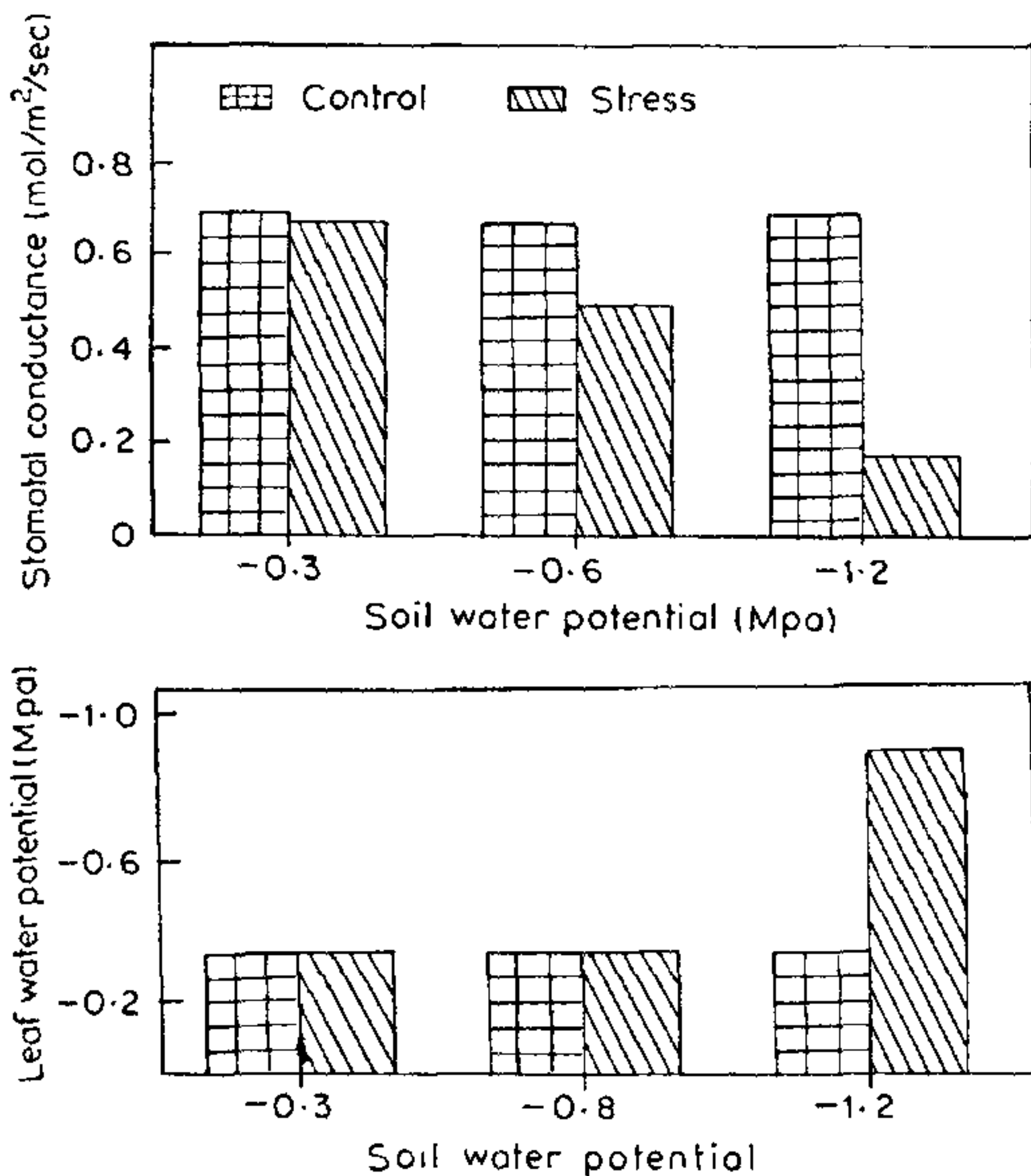


Figure 2. Leaf water potential and stomatal conductance of sunflower at different soil water potentials.

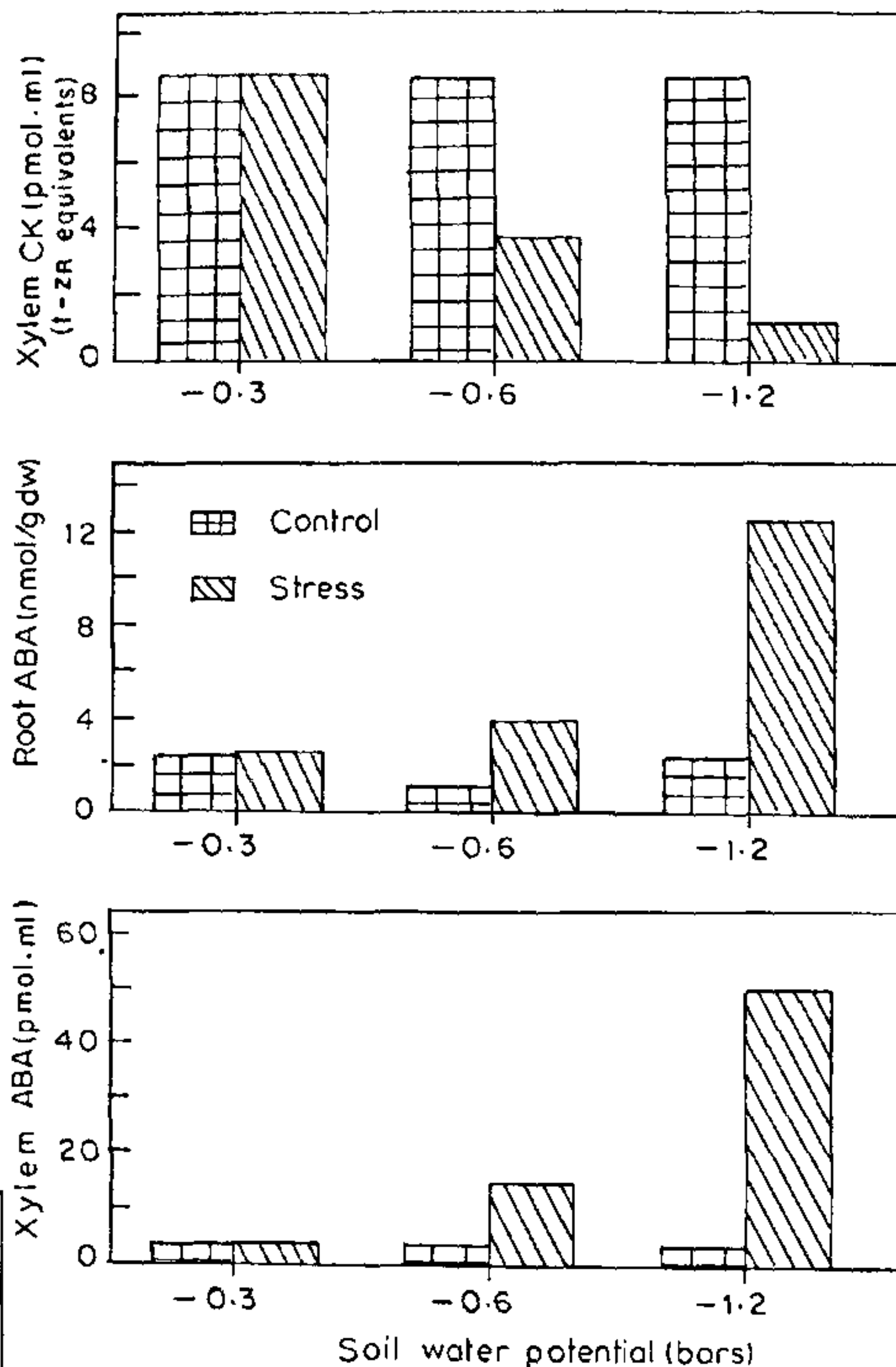


Figure 3. Abscisic acid and cytokinin content in the xylem exudate of sunflower.

Table 1. Leaf conductance monitoring system in sunflower. RWC and soil moisture content for different levels of decrease in conductance of leaves

Stage of stress	% decrease in $g_s$ over control	RWC (%)	Soil moisture content (%)	
			Top soil (0-10 cm)	Bottom soil (> 10 cm)
Control		95.0	25.3	*
Stage-I	10-15	91.0	5.9	18.3
Stage-II	40-45	63.1	5.5	9.2
Stage-III	60-65	53.0	*	*

\*Data not available.

content. The decrease in  $g_s$  at this stage could be attributable solely to the production of a chemical signal in the root, transduced subsequently to the shoot via the xylem.

**Table 2.** ABA content in xylem exudate and root extracts in L.C.M. system in sunflower

Stage of stress	Xylem sap ABA (pmol.ml <sup>-1</sup> )	Volume of exudates collected (ml <sup>-1</sup> .15 min <sup>-1</sup> )	ABA* delivery rate (pmol.15 min <sup>-1</sup> )	Xylem sap cytokinin (t.zr equivalents) (pmol.ml <sup>-1</sup> )	Delivery rate (pmol.15 min <sup>-1</sup> )	Root ABA (nmol.g DW <sup>-1</sup> )
Control	0.5	0.26	0.13	4.93	12.81	0.34
Stage-I	17.7	0.15	2.65	4.30	6.45	3.10
Stage-II	145.0	0.15	1.45	1.00	1.00	6.20
Stage-III	200.0	0.01	20.0	0.07	0.007	12.60
LSD 5%	2.4	0.04	1.24	1.23	-	2.3

\*Based on volume of exudate collected for 15 minutes at a pressure of -1.0 Mpa for stress plants and 0.5 Mpa for well-watered control.

**Table 3.** Vertical split root system: ABA content in roots and xylem exudate at different levels of soil moisture stress in sunflower

Treatment	Xylem sap ABA (pmol.ml <sup>-1</sup> )	Volume of exudate collected (ml <sup>-1</sup> .15 min <sup>-1</sup> )	ABA delivery rate (pmol.15 min <sup>-1</sup> )	Root ABA (nmol.gDW <sup>-1</sup> )	RWC (%)	% reduction in $g_s$
Control	0.37	0.30	0.11	0.112	93	-
SS-1	18.0	0.14	2.52	1.12	71	15-20
SS-2	100.0	0.08	8.0	7.00	65	40-50
LSD 5%	2.3	0.015	-	0.3	4.5	-

### Experiment-I-Approach-2

The results confirm that even during early stages of soil drying, despite the fact that high RWC and turgor had been maintained in the leaves,  $g_s$  dropped nearly 10-15% of the non-stressed plants (Table 1). The soil moisture content in the lower profiles was still relatively high (18.3%). This was probably adequate for the roots in this region to maintain high water transport to the leaves. Consequently the RWC in the leaves of both the crops was still very high (91 and 92.1 respectively) suggesting that the leaves have not lost the turgor at this stage of soil drying.

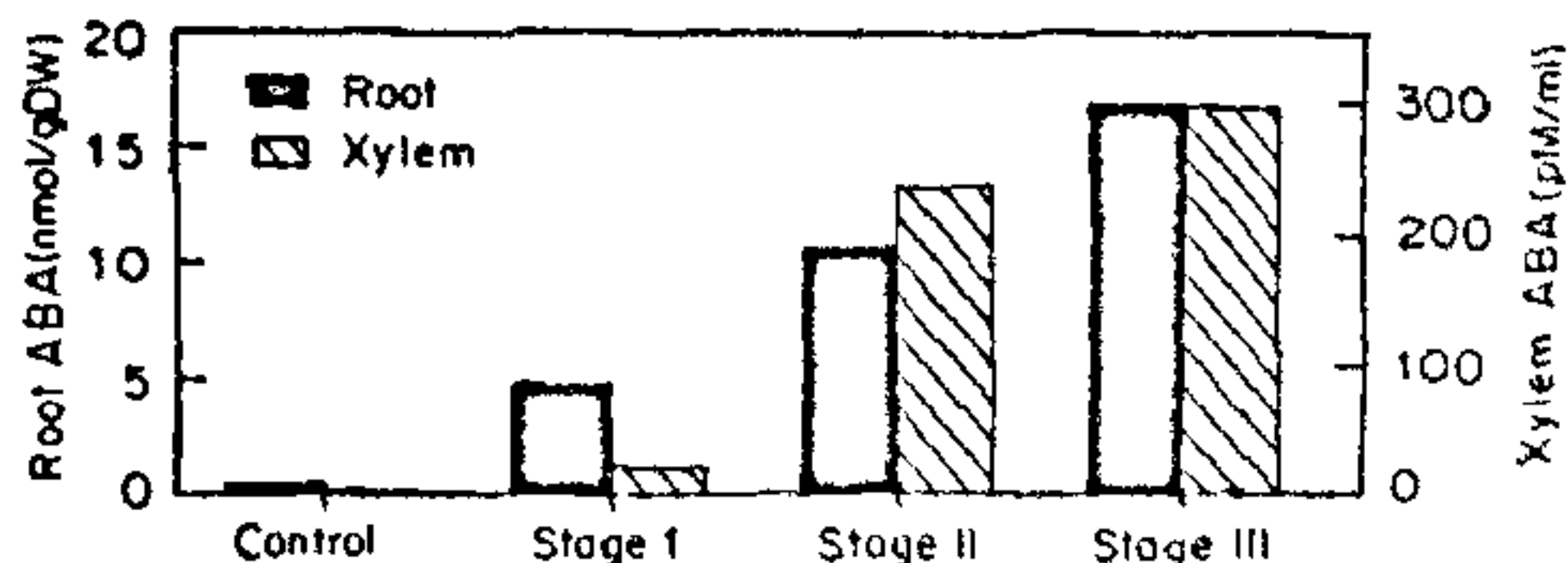
### Root and xylem ABA content

Root ABA content substantially increased even during the initial stages of soil drying. Although a similar trend was observed in cowpea plants at this stage, the extent of increase in ABA content was much lower. The same trend was also observed in moderate and severe soil stress (stages II and III).

Cytokinin levels in xylem sap and in roots decreased sharply even during initial stages of soil drying. Cytokinin levels decreased further as soil drying increased. The reduction was substantially higher in cowpea xylem sap.

### Experiment-II-Vertical split root system: ABA and cytokinin content in the root and xylem exudate

The ABA content in roots had decreased ten-fold (1.12) at the end of 24 hours of air-drying of the protruding roots (Table 3). Subsequently there was a substantial increase in ABA (7 nmol.gDW<sup>-1</sup>) after 48 hours by which time the conductance also showed a decrease of 40-45% (stage II). A similar trend was observed in the ABA content of xylem exudate, where the ABA increased nearly 48-fold at the end of stage-I and also under more prolonged stress (stage-III), at which point the conductance had decreased to 40-45%, the root ABA and xylem ABA to 7.0 pmol.g<sup>-1</sup> and 100 pmol.ml<sup>-1</sup> respectively.



**Figure 4.** Abscisic acid content in xylem exudate and roots of cowpea in the leaf conductance monitoring system.

## Discussion

The central tenet of root-shoot communication, since the work of Bates and Hall<sup>6</sup>, had been that at least during the early stages of soil drying, stomatal conductance was linked more closely to changes in turgor of whole root or part of the root, than the leaf turgor. While attempting to prove the veracity of this, several groups had used specific systems where turgor of the leaf could be kept constant during soil drying.

We tested this hypothesis in different systems where the two species (cowpea and sunflower) were either interplanted in the same container and subjected to a soil drying processes or subjected to separate treatment.

The data on RWC and moisture content when the conductance had dropped to about 15% in both the species did confirm that the initial decrease in conductance was independent of the leaf turgor.

Though our results largely confirm the core concept of root-shoot signals as espoused by Zhang and Davies<sup>4</sup>, several introspective questions based on our study are raised here which might be relevant to future work in this area: (a) Are root sourced positive signals like ABA effective as early warning system<sup>12</sup> only during the early stages of soil drying? (b) If so, only a 15–20% decrease in conductance can be exclusively attributed to root signals. (c) Beyond this, shoot water status has an over-riding effect, because once turgor of the leaves

decreases drastically, ABA content is much higher in leaves than in roots. (d) What is the right ratio or balance of these two important negative and positive signals represented by the two stress hormones, during the early stage of soil drying?

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