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# Slower-chemical or faster-electrical signalling under stress in plants: Is it the hare and tortoise story of a slower signal winning the race?

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In stress physiology, one of the controversies related to root to shoot communication under stress, has been whether electrical signals from roots precede the chemical signal, represented by the predominant positive signal, abscisic acid (ABA) which accumulates up to 50 fold in the roots and xylem sap of stressed plants. Electric signals can be produced and transmitted to the shoots 18 cm away from the roots in 25 s when an osmotic stress is given to the roots. However a recent finding that ABA applied to the roots itself can generate electrical signals has only fuelled or exacerbated the controversy. In this paper

we have attempted to analyse the relative merits of a faster but apparently short distance intense signal, with the slower chemical signals. We have critically assessed what appears to be a 'deliberate strategy' of the plants to spatially separate two diverse but equally effective signals. The question we pose in this paper is, can a chemical signal still precede an electrical signal? If true, the plant must devise a different way to release an already available sequestered chemical signal. This is akin to resolving the classical dilemma of what comes first the chicken or the egg.

MAN has been concerned with plant stress adaptations since the first pre-historic cave dweller selected seed for propagation from plants that performed better than their neighbours. Physical and biochemical responses of plants to environmental stresses have been studied for over a century and a great mass of data is available. These responses embrace a fascinating spectrum of adaptation, ranging from the survival of the unicellular

algae *Dunaliella* in the harsh saline waters of the dead sea of Israel through a process called osmoregulation<sup>1</sup>, to the survival of *Opuntia*, the common cactus, in the Californian desert when the temperature of its shoot reaches 65°C, i.e. 17° above the air temperature<sup>2</sup>.

Although these two examples represent plant adaptations to a saline and high temperature stress respectively, the predominant abiotic stress affecting plant growth and development is by far drought or water deficits. This concern is reflected by the number of

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books devoted exclusively to the review of plant adaptations to water deficit<sup>3-6</sup>. These adaptations are classified as drought avoidance or escape, dehydration postponement, and dehydration tolerance. In the second category, the study of stomatal behaviour under stress should rank first amongst all the traits which have been studied during the last four decades particularly after the advent of porometry (a system designed to determine the stomatal resistance to gas exchange as a reflection of the rate of water loss from leaves) in the late sixties. As Rascke<sup>7</sup>, one of the pioneers in stomatal physiology, put it, 'Land plants are in a perpetual dilemma throughout their lives: Assimilation of CO<sub>2</sub> from the atmosphere requires intensive gas exchange and the prevention of excessive water demands that gas exchange be kept low'. Plants have learnt through evolution to strike some compromise between these two opposing objectives. Inherent in this strategy of compromise is to develop a system of communication, which will signal the stomata to restrict opening to avoid excessive water loss. In this system a 'message' synthesized by the roots which is the first to sense the water deficit, leaves the root, uses the xylem as a conduit, reaches the stomata and restricts water loss as a classical 'first line of defence'.

In the last decade there has been considerable interest in this novel signalling mechanism in different stresses; drought flooding and saline stresses<sup>8-10</sup>.

Several investigators including the authors of this paper, have been interested in root to shoot signalling under water stress. These signals can be either the slower chemical signal or the amazingly fast electrical signals. Recently this novel root to shoot communication has found a place in the second category of drought resistance mechanisms, that is those associated with postponement of stress<sup>11</sup> because these mechanisms are primarily meant to be a 'first line of defence' against stress. Thus a new area of plant stress research has emerged, targeting distress signal communication in plants. Whichever the signal, chemical or electrical, the consensus reached was that using this communication system, the shoots system of the plants have found an incredibly simple way to 'sense' perturbations in the soil environment, well before the shoot itself experienced it. However no consensus has been reached on the basic issue of which signal is more effective; the electrical (also called action potentials) signal or the chemical signal.

The latter has received more attention if the number of reviews during the last five years is any indication<sup>12-15</sup>. The exponents of the chemical signal have essentially used three systems

(a) Split-root systems, with half the roots in wet soil and the other half in dry soil<sup>16</sup>.

(b) Long soil columns with the upper roots in the top dry soil and the lower roots in wet soil, initially<sup>17</sup>.

(c) A balancing pressure given to the entire root to counter balance the decreasing water potential of shoots<sup>18</sup>.

All these had one thing in common, i.e. the whole root or a part of the root system was in dry soil, while the water status of the shoots was similar to that of non-stressed controls. Any change in a physiological process (either stomatal conductance or leaf expansion) can therefore be directly attributed to the production of a chemical signal produced by the loss of turgor of that part of the root system in dry soil. This signal was transported to the xylem and subsequently sent up to the epidermis of the leaf. Immunological quantification of the signal in the epidermis has shown convincingly that it is the signal arriving at the epidermis from the roots which causes the initial restriction of the stomatal opening<sup>16</sup>. This early warning signal coming from just a few roots which are the first to dry (in the natural field situation the top 10-15 cm of the soil profile is also the first to dry) is called the 'first line of defence'<sup>19</sup> as it helps to conserve water by reducing transpiration (restricting stomatal opening) and transpiration area (decrease in leaf expansion) so that some water is available at later stages of growth. Such a 'strategy' therefore enables the plant to balance its size with the availability of water.

The nature of chemical signal has received a lot of attention and has been reviewed thrice<sup>13-15</sup> during the last three years, with the stress hormone ABA receiving primary attention as the predominant positive or accumulating signal. Levels of this hormone have been shown to increase up to 50 fold in the xylem sap<sup>19-21</sup>, enroute to the shoots.

The electric or action potential has received more attention *vis-à-vis* the 'touch me not' syndrome in *mimosa*<sup>22,23</sup> since the pioneering work of Sir J. C. Bose<sup>24</sup> in 1913. Only recently were elegant experiments conducted to show that these faster signals (speed of transmission 7-14 mm/s) can be equally effective as a 'first line of defence' in distress signal communication under stress. Hebbar *et al.*<sup>25</sup> were able to show quite convincingly that when a sudden or snap osmotic stress was applied to the roots of sunflower, an electric signal originating in the roots reached the shoot in 25 s and manifested itself ultimately as a decrease in the stomatal conductance (the first line of defense). In another recent work, action potential was also shown to be involved in wound-induced signalling system of plants<sup>26</sup>. But taking the electric signalling work of Hebbar *et al.*<sup>25</sup> (Figure 1) under osmotic stress as a representative example and comparing it with any one of the examples of chemical signals; (say that of the Zhang and the Davies group<sup>19</sup> or our own work<sup>20</sup>), one realizes that it becomes extremely difficult to analyse the relative merits of either of these signals because of the following reasons.



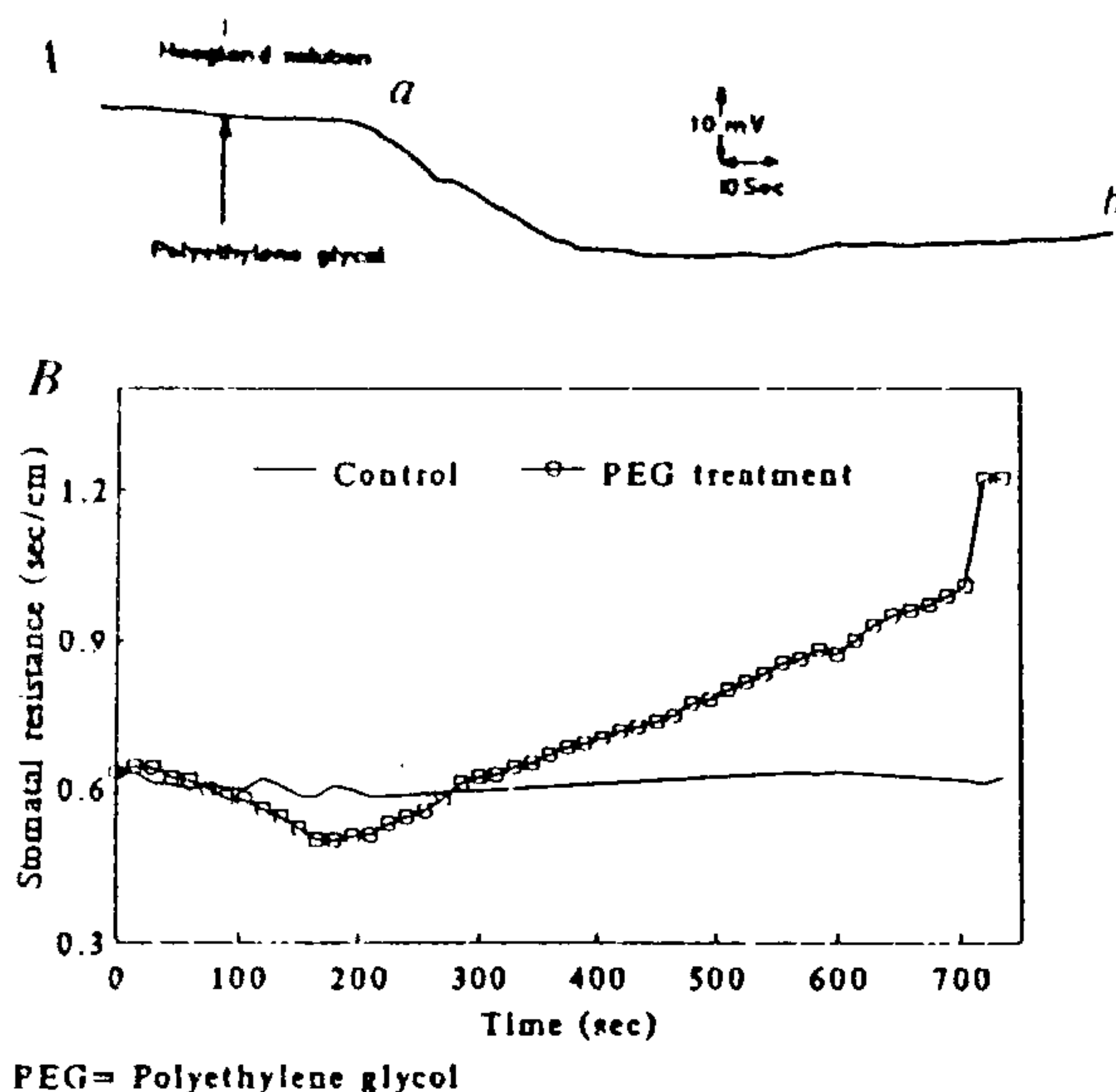


Figure 1. Influence of action or electrical potentials on stomatal or physiological processes under osmotic stress. (A) Effect of osmotic stress on surface electrical potential at the shoot apex of sunflower seedling. *a* = pattern of electric potential in control, *b* = pattern of electrical potential in response to osmotic stress. (B) Changes in stomatal resistance in control and PEG (25% w/v in Hoagland solution) treated sunflower seedlings with time. (after Hebbar *et al.*<sup>25</sup>)

(a) Hebbar's work involved a drastic osmotic stress, and therefore would be expected to convey the action potential in a few seconds.

(b) Our own work reported earlier or that of the Davies group involves a slow dehydration of the roots either naturally in a soil column or an artificial one where the lower, free hanging roots are allowed to lose water. This process even if accelerated involves a time frame of hours. For example, Neales *et al.*<sup>19</sup> utilizing this system showed a decrease in conductance of 30–40% in maize plants through a chemical signal ABA, in 5 h, by slow dehydration of the lower free hanging roots (Figure 2).

In all these cases, the root–water relations are mandatory (see Figure 2 of Neales *et al.*, 1989) and is useful to compare the extent of the ABA signal from different laboratories. A PEG stress, on the other hand, besides being a drastic stress has several shortcomings.

(a) Water relations of the root is extremely difficult to determine.

(b) The roots attain equilibrium with the water potential of the osmoticum only after several hours.

(c) Long before that the action potential has already been transmitted to the shoot.

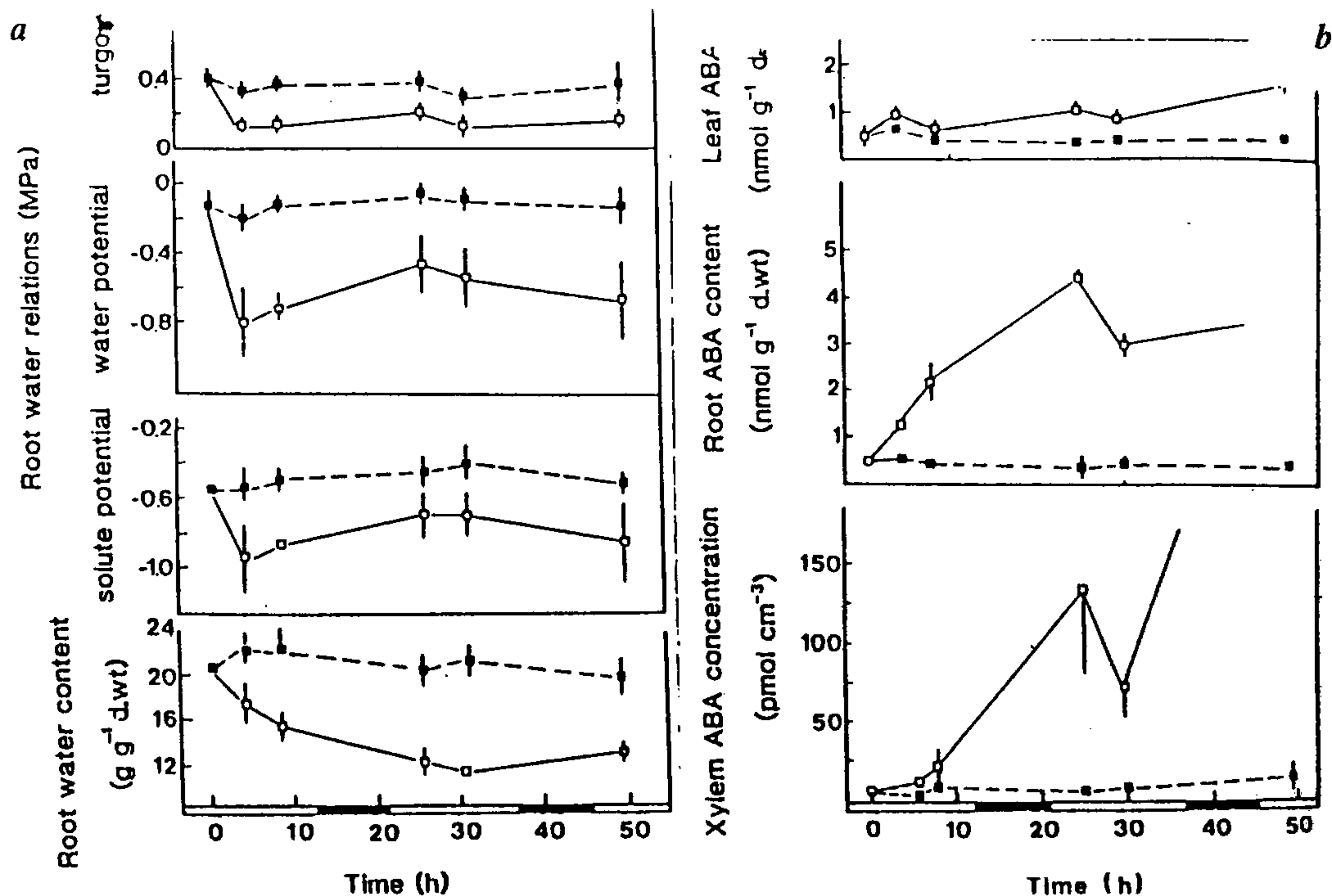


Figure 2 *a, b*. Influence of chemical signal on physiological process under water deficits simulated in the vertical split root system. *a*, Water relations of roots of *Helianthus* plants which protrude from soil, through the base of the pot. Roots in the nutrient solution after time 0 (controls, ■). Roots in moist air after a brief period of air drying (treatment, □). Each point is the mean ( $n = 3$ ) value with  $\pm$  s.d. bars. *b*, The ABA concentration in leaves, roots and xylem sap of control *Helianthus* plants (■) and in plants where protruding roots were dried (treatment, □). Points are means of 3  $\pm$  s.d. Root data shown are for protruding roots. (after Neales *et al.*<sup>19</sup>)



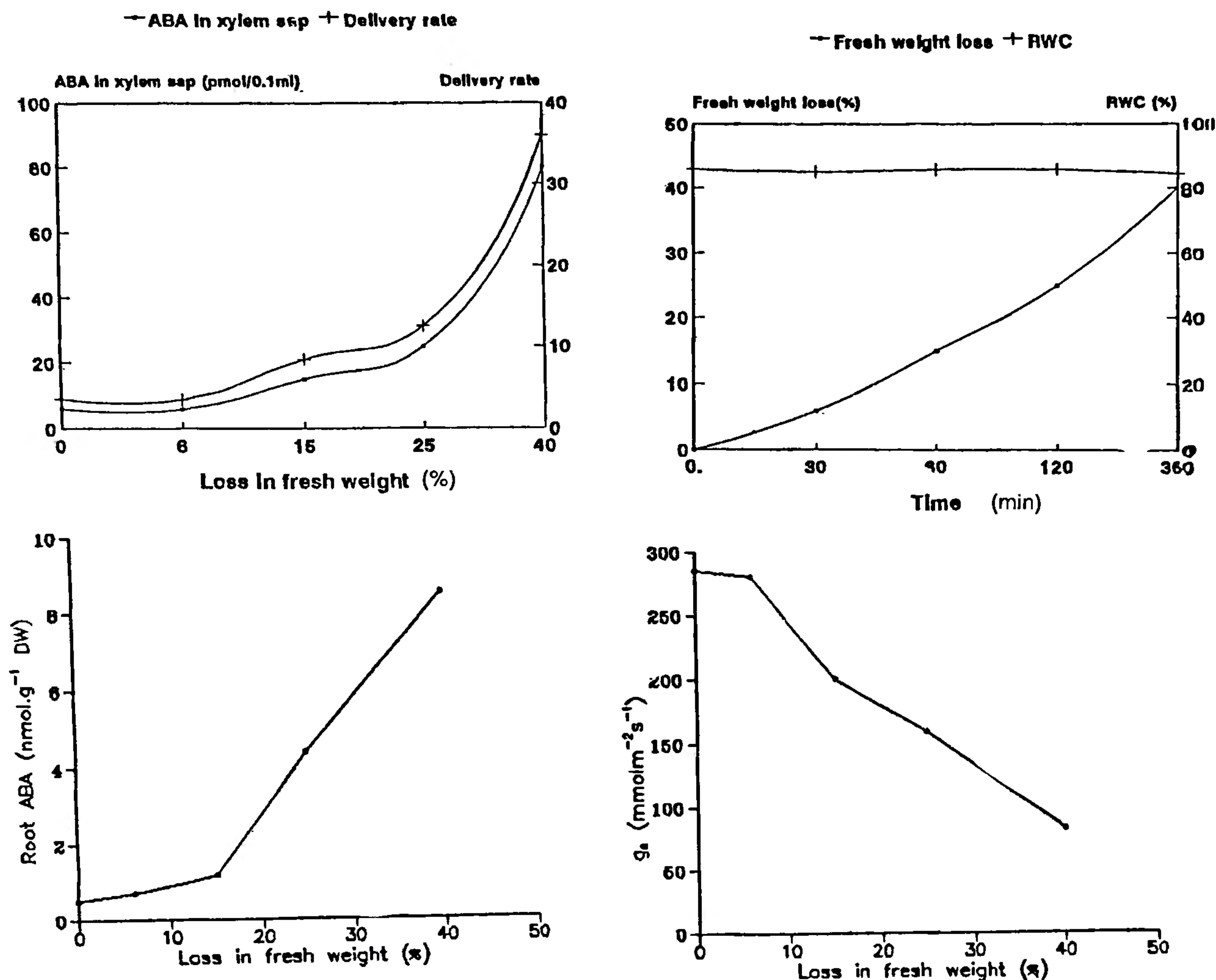


Figure 3. Changes in water relation, stomatal conductance and chemical signal ABA on rapid dehydration of free hanging roots in a vertical split system.



Figure 4. The vertical split root system developed by the Zhang and Davies group<sup>16</sup> (upper roots grow in wet soil while lower roots protrude through the base of the pot and hang freely. These roots can be dehydrated either rapidly (outdoors) or slowly (suspended in a humid cabinet).

(d) Comparisons between work of different workers are near impossible as equilibrium time varies with the morphology of the roots of different species.

How then does one analyse the relative merits of an electric signal and a chemical signal? The fact that the former is faster is obvious. Why then does a plant spend so much energy on producing a second chemical signal? The answer could hinge on resolving an entirely different issue.

(a) Is an electric signal only capable of being transmitted up to short distances (most work on these signals deals with distances of 18–20 cm, rarely more)? In fact Zawadzki<sup>27</sup> clearly showed the limitations of an electric signal with regard to the distance. The action potential, he observed, has three major limitations.

(a) Occurs only when the stem was electrically stimulated, not the leaves and roots,

(b) Does not spread to the neighbouring internode,

(c) Gets extinguished in the region of the stem apex and root neck.



The first one of course has been quite convincingly disproved by Hebbar's work, but the second and third suggest that an electric signal could fulfill only the initial need of transmitting a fast signal up to a short distance. Later a greater more intense long range signalling mechanism could operate (a chemical signal has also been shown to operate in the tree seedlings of apple<sup>28</sup>).

Resolving this issue has, however, become more complicated by the recent observation of Fromm<sup>29</sup> that the application of ABA (a chemical signal) was itself able to elicit an electric signal that travelled to the leaves within 2.5 seconds. The classical dilemma is what comes first – the chicken or the egg or to use a more scientific jargon, can a chemical signal precede an electrical signal. But since ABA accumulation has been shown to require transcription<sup>30</sup>, it is obvious that a chemical signal cannot be visualized in a time frame of seconds but only in hours. Very recently we specifically conducted experiments to resolve at least partly this question, i.e. can a time frame for a chemical signal to be sent from roots to shoot, be reduced at least to minutes. We fixed 30 min as the minimum time required for ABA accumulation after transcription and translation and artificially accelerated the process of dehydration of the roots using the split root system developed by Zhang and Davies<sup>16</sup> (Figures 3 and 4). When the lower free-hanging roots were dehydrated quickly and made to lose 6% of their fresh weight in less than 30 minutes, ABA increase in roots and xylem sap was still not significantly different from the control levels at 0 hours. Only a 15% loss in fresh weight in 1 h caused a significant increase in ABA which apparently in turn caused a significant decrease in conductance (Figure 3). Our data corroborates closely with that of Neales *et al.*<sup>19</sup> who also showed that a 20% drop in root water content in 5 h resulted in a significant increase in ABA. What we have done is merely shifted the time frame to 1 h instead of the 5 h by accelerating the process of dehydration. Once the required threshold loss in turgor is achieved, then current models are adequate to explain the mechanism of accumulation at the cellular and molecular level<sup>31</sup>.

It is impossible to visualize a time frame of seconds for a chemical signal like ABA to be detected in the roots even if it was caused merely by release of already sequestered ABA from organelles<sup>32</sup> due to ion gradients and not ABA synthesis. Since the latter requires transcription, one would have to visualize a time frame of at least 15–30 minutes for synthesis of ABA, even if one were to accelerate the dehydration process by whatever means to make the roots reach the threshold loss of turgor in just a few minutes.

Keeping aside these arguments, how does one explain Fromm's observation of an electrical signal being produced by ABA application to the roots in seconds. Clearly more work needs to be done at cellular levels using sophisticated neurophysiological techniques like

the patch clamp, while giving credit to Sir J. C. Bose for pioneering its use in the beginning of this century, exactly eighty-three years ago. Until then we would have to be satisfied merely by disagreeing with the moral of the hare and tortoise story, i.e. there is no way the tortoise (chemical signal) can win. The hare (electrical signal) will win all the time.

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## RESEARCH ACCOUNT

# Application of ion–solvent interaction parameter in interpreting the kinetic profiles of Diels–Alder reactions and thermal stability of DNA duplexes in ionic solutions

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**Rate enhancement in Diels–Alder reactions and thermal stability of a DNA duplex at several ionic concentrations are the main issues addressed in this account. The proposed quantity, internal pressure of a salt-solvent, which is also a measure of electrostriction effect together with the activation volumes of reactions can describe the impressive rate accelerations of the Diels–Alder reactions carried out in salt solutions. Similarly, obviation of high pressure conditions for the synthesis of organic molecules is described. More importantly, the application of the concept of internal pressure and volume has demonstrated that the thermal stability of several DNA duplexes in the presence of ions can be explained. The pressure dependence of thermal stabilities at constant ionic concentrations is linear in nature. The correlations exposed for the DNA duplexes have strong potential to unravel the forces responsible in stabilizing a DNA duplex or the formation of a duplex from its complementary strands.**

MOLECULAR interactions of a solvent molecule with nonionic and ionic solutes play vital roles in governing the static and dynamic behaviour of a system. Various thermodynamic, kinetic and other solution properties of the system represent specific behaviour of these molecules in a given environment. With regard to solvents,

Mother Nature discovered the secrets of water in different biological and other natural processes, whereas nonaqueous solvents continued to gain importance in a variety of man-made processes.

Considering the importance of the solute–solvent interactions in several processes, we have very recently embarked on a programme to investigate their role in controlling the kinetic profiles of the organic reactions, like Diels–Alder (D–A) reactions<sup>1,2</sup> and the association and dissociation processes involved in nucleic acids<sup>3</sup>. Some of these results along with the futuristic views have been described here.

## Definition of solute–solvent interactions in the present context

Cohesion among molecules in the liquid phase results from intermolecular forces. These forces, in general, include hydrogen bonding, dipole–dipole, multipolar, dispersion interactions and also interactions emerging from the repulsion between two molecules. The cohesion due to intermolecular forces gives rise to a 'pressure' which is experienced by the solvent molecules. This term for pure solvent was initially proposed by Hildebrand and Scott<sup>4</sup> and subsequently supported by the equation of state<sup>5</sup>. A liquid undergoing a small, iso-