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Growth and water status responses of mung bean (Vigna mungo L.) and other dicot species to osmotic stress

Passos, Leônidas Paixão, Ph.D.

The University of Arizona, 1989
GROWTH AND WATER STATUS RESPONSES OF MUNG BEAN
(VIGNA MUNGO L.) AND OTHER DICOT SPECIES TO OSMOTIC STRESS

by

Leônidas Paixão Passos

A Dissertation Submitted to the Faculty of the
DEPARTMENT OF PLANT SCIENCES
In Partial Fulfillment of the Requirements
For the Degree of
DOCTOR OF PHILOSOPHY
WITH A MAJOR IN AGRONOMY AND PLANT GENETICS
In the Graduate College
THE UNIVERSITY OF ARIZONA

1989
As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Leonidas Paixao Passos entitled Growth and Water Status Responses of Mung Bean (Vigna mungo L.) and Other Dicot Species to Osmotic Stress.

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Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

Karen Mattson

Dissertation Director
STATEMENT BY AUTHOR

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SIGNED: [Signature]

[Name]
To my wife Yacyra and our sons Marcelo, Ricardo and Weber for their love and understanding.
ACKNOWLEDGMENTS

I am indebted to my in-laws Dr. Weber M. Batista and Mrs. Theresinha J. Batista for their help and friendship.

I would like to express my thankfulness to EMBRAPA (Brazilian Agency for Agricultural Research) for giving me the opportunity to pursue this training program at the University of Arizona and to its Head, Dr. Ormuz Freitas Rivaldo, for his friendship and support.

I wish to thank Dr. Kaoru Matsuda for his advice and patience in the course of this research.

Special thanks go to Dr. Robert E. Briggs for serving as my Minor Professor and committee member. His kindness and friendship are also greatly appreciated.

I am grateful to my committee members Dr. Paul G. Bartels, Dr. Frank R. Katterman and Dr. James W. O'Leary for their suggestions in the preparation of this manuscript.

I also thank my colleagues Euzebio Medrado da Silva, Ahmed Mohamed Rayan and Manoel de Castro Neto for their help and comments.
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<tr>
<td>°C</td>
<td>degree centigrade</td>
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<td>cm</td>
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<td>h</td>
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<td>M</td>
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<td>mg</td>
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<td>minute</td>
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<td>μmol</td>
<td>micromole</td>
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<td>μl</td>
<td>microliter</td>
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<td>ml</td>
<td>milliliter</td>
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<td>mm</td>
<td>millimeter</td>
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<td>MPa</td>
<td>megapascal</td>
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<td>mRNA</td>
<td>messenger ribonucleic acid</td>
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<tr>
<td>Ψπ</td>
<td>osmotic potential</td>
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<tr>
<td>PEG</td>
<td>polyethylene glycol</td>
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<td>percent</td>
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<td>RH</td>
<td>relative humidity</td>
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<td>s</td>
<td>second</td>
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<td>SD</td>
<td>standard deviation of series</td>
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<tr>
<td>Ψp</td>
<td>turgor potential</td>
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<td>Ψ</td>
<td>water potential</td>
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ABSTRACT

Intact dark- and light-grown mung bean (Vigna mungo L.), black bean (Phaseolus vulgaris L.), pea (Pisum sativum L.) cowpea (Vigna unguiculata (L.) Walp.) and squash (Cucurbita pepo L.) seedlings on hydroponic medium were osmotically stressed by exposing their roots to PEG 8000 of various concentrations (-0.2 to -0.6 MPa) to determine stress effects on growth and tissue water status. Growth of dark-grown mung bean hypocotyls ceases within 40 sec upon exposure to any level of stress, and resumes within 10 to 45 min. Growth of all other seedlings were measured usually after 3 to 24 h stress, and in 3 h, elongation is inhibited in dark-grown and is stopped in light-grown tissues. In dark-grown mung bean, black bean and squash hypocotyls and pea epicotyls, growth rates after 24 h stress were found to be proportional to the $\Psi$ of the medium. In mung bean hypocotyls, growth stopped before any change in $\Psi$ or $\Psi_n$ occurred in the growing region. In this tissue and also in dark-grown squash hypocotyls, pea epicotyls, and in light-grown cowpea hypocotyls, equivalent reductions in $\Psi$ and $\Psi_n$ were evident in the growing region after 3 h, so turgor remained constant. In other species, osmotic adjustment with turgor maintenance was evident after 24 h in both the growing and expanded regions. The results with mung bean
hypocotyls provided the first demonstration that stress causes an almost instantaneous stress-caused cessation of elongation in dicots. Since data from all plants showed that stress causes growth rate inhibition or cessation without a concomittant decrease in $\Psi_p$, it is concluded that turgor is not the factor regulating growth. More likely, stress-caused growth and water status changes are responses to an earlier signal, such as a stress-caused reduction in the apoplastic $\Psi$. 
INTRODUCTION

Water deficits restrict growth and productivity of crop plants, and this problem has serious social and economic implications in arid regions (Swindale and Bidinger 1981). As a result, intense efforts have been made to understand how water stress alters growth and other physiological processes. Nevertheless, large gaps still exist in our knowledge about water movement in plants and cellular responses to stress, and much of the literature remains conflicting.

Water stress is known to affect a variety of physiological responses, ranging from cell elongation to protein synthesis, hormone production, photosynthesis and amino acid metabolism (Hsiao 1973, Hsiao et al. 1976, Bewley 1981, Hanson and Hitz 1982). Hsiao (1973) assembled data from various sources and ranked processes according to water potential reductions required to effect changes. Using this criterion, he considered growth to be the most stress-sensitive process and he rated in order of decreasing sensitivity protein and cell wall synthesis, photosynthesis, respiration and sugar and amino acid metabolism. Further studies have confirmed that growth is inhibited by slight reductions in water status of the root environment (Matsuda and Riazi 1981, Bradford and Hsiao
1982, Aspinall 1986, Barlow 1986), and that photosynthesis is relatively insensitive to stress (Kriedemann and Downton 1981, Kriedemann 1986). However, it is now evident that the developmental state of tissues can greatly affect cellular sensitivity to stress, and some processes previously ranked as being insensitive must now be considered as responsive as growth to water deficits. For instance, in the growing region of barley leaves, tissue $\Psi$ and $\Psi_m$ (Matsuda and Riazi 1981) and polyribosome percentages (Mason and Matsuda 1985) were found to be directly related to the $\Psi$ of the external solution, whereas this relationship was inverse for proline levels (Riazi, Matsuda and Arslan 1985). In contrast, stress-induced changes in the above measures for the expanded tissues are minimal.

Studies with elongating tissues have also provided reasons for questioning some of the traditional ideas for explaining how water deficits regulate growth and metabolic processes. Growth has long been considered to be regulated by turgor (Acededo, Hsiao and Henderson 1971; Hsiao 1973; Hsiao et al. 1976; Cosgrove 1981, Taiz 1984, Cosgrove 1986, Cosgrove 1987a) but data obtained with growing tissues of various plants have shown that while positive turgor is required for cell growth, expansion may be limited by other factors. Stress reduces growth rates in barley leaves (Matsuda and Riazi 1981), etiolated soybean hypocotyls
(Cavalieri and Boyer 1982), corn leaves (Michelena and Boyer 1982, Van Volkenburgh and Boyer 1985) and corn roots and stems (Westgate and Boyer 1985), but in their growing regions, turgor remains relatively constant due to equivalent decreases in $\Psi$ and $\Psi_n$. Furthermore, in moderately (-0.8 MPa) stressed barley seedlings, leaves stopped elongating before any change in water status occurred in the growing region (Matsuda and Riazi 1981). However, reductions in $\Psi$ and $\Psi_n$ became evident after 0.5 to 1 h and osmotic adjustment with turgor maintenance continued for about 12 h. Also, the proportions of ribosomes present as polyribosomes were reduced significantly within 15 min (Mason and Matsuda 1985). Such data indicate turgor reductions are not controlling growth; rather, growth, water status and some metabolic alterations are responses to another rapidly transmitted controlling factor. Matsuda and Riazi (1981) suggested cells in the growing region may be responding to sudden stress-induced reductions in xylem $\Psi$. Anatomical, heat pulse and water labelling studies with barley seedlings have supported this view (Rayan and Matsuda 1988). More work, however, must be done with various plant species before this hypothesis can be accepted.

The objective of this study is to understand how water stress regulates growth and metabolism in growing and
expanded tissues of dark-grown mung bean and other dicots. Dark-grown dicots also undergo stress-induced reductions in their percentages of polyribosomes (Mason and Matsuda 1985), but little is known about the relation of growth changes to alterations in water status.
LITERATURE REVIEW

Current Hypotheses Describing the Relationship of Water Status to Growth

Plant growth is considered to be mostly caused by cell enlargement (e.g. Boyer, Cavalieri and Schulze 1985). Mechanistically, it is believed that cell enlargement occurs because there is a metabolically driven wall loosening which is followed by wall yielding due to hydrostatic pressure within the protoplast (Cleland 1971, 1981). Wall yielding then gives rise to wall relaxation (Cosgrove 1987b), which lowers $\frac{\psi}{\psi}$ and therefore $\psi$, leading to water absorption and subsequent expansion (Boyer 1985). If enlargement occurs steadily, the above mentioned processes will occur continuously and $\frac{\psi}{\psi}$ and $\psi$ will be lower in growing cells (due to wall relaxation) than in non-growing cells (Boyer et al. 1985). Once expansion is initiated, there must be continuous solute uptake so that $\psi_n$ is kept low enough for $\frac{\psi}{\psi}$ maintenance and subsequent wall yielding (Boyer 1985).

Equations relating growth to tissue water status were developed originally by Lockhart (1965), who studied the expansion of the giant cells of the algae Nitella when immersed in solutions with varying $\psi_n$. Further studies with Nitella (Green, Erickson and Buggy 1971) and oat
coleoptile segments (Ray, Green and Cleland 1972), have led to modifications of Lockhart's views, and the following equation has become universally accepted as an expression of how plant cell growth is regulated:

\[ G = m(\Psi_p - Y) \]

where \( G \) is growth rate (cm.s\(^{-1}\)), \( m \) is cell wall extensibility (cm.s\(^{-1}\).bar\(^{-1}\)), \( \Psi_p \) is turgor potential and \( Y \) is the yield threshold, a minimum turgor that must be exceeded before wall extension occurs (Cleland 1959). Normally, \( m \) and \( Y \) are considered to be constant for a tissue, and it is assumed that water transport does not limit growth (Barlow 1986). Growth, therefore, is regarded as being directly proportional to tissue turgidity. Further arguments for this view and analysis of various growth equations can be found in reviews by Taiz (1984), Boyer (1985), Cosgrove (1986), Boyer (1987), Cosgrove (1987a, 1987b), Hsiao and Jing (1987) and Ray (1987).

Several problems can be encountered in trying to determine the components of the growth equation. Values of \( Y \) have been obtained by either soaking tissue segments in osmotic solutions (e.g. Cleland 1967a) or supressing water supply to determine the turgor at which growth will stop (e.g. Boyer 1968). Boyer (1985) noted that \( Y \) values may be altered in the several hours of incubation used in their
determinations and this has been confirmed in more recent studies (Matyssek, Maruyama and Boyer 1988). Matyssek and co-workers combined the use of a pressure probe, which permits rapid determination of turgor changes that occur in individual cells, and a "guillotine" psychrometer, which excises tissues within the measuring chamber, and measured $Y$ in growing regions of pea and soybean seedlings. They concluded that cell walls relax to $Y$ in a matter of minutes and were therefore not surprised at the wide variations in threshold turgor values which were obtained in previous reports.

Values for "$m$" have been estimated as the slope of the curve when excised sections are grown in osmotic solutions of different concentrations and growth rates are plotted against turgor potential (e.g. Cleland 1986). This method requires initial growth rates and $Y_F$ to be constant, and "$m$" reflects an average over a period of hours. Wall extensibility can also be estimated by a turgor relaxation method (Cosgrove, Van Volkenburgh and Cleland 1984; Cosgrove 1985) which assumes that if water supply to growing sections is increasingly restricted with osmotic solutions, biochemically driven wall loosening will continue until a threshold turgor, $Y$, is reached where loosening no longer occurs. Values for $m$ are obtained from the half times for pressure relaxation as measured by the
pressure probe (Husken, Steudle and Zimmerman 1978). Alternatively, Boyer et al. (1985) determined the \( \Psi \) of the growing regions of intact plants, then continuously measured the decline in \( \Psi \) that occurred inside a guillotine psychrometer chamber. They next froze and thawed the tissue to determine \( \Psi_m \) and calculated turgor. They assumed that turgor will decrease until \( Y \) is reached, and \( m \) is the slope of the curve when steady state growth is plotted as a function of turgor. This method relies on the unverified assumption that \( \Psi_P \) will change but \( m \) and modulus of elasticity will not.

Values of wall extensibility have also been obtained using dead tissues. In the Instron method (Cleland 1967b) constant tension is applied to methanol-killed cell walls and a value for plastic extensibility (DP) is estimated from the rate at which the tissues expand. Even though DP does not directly reflect \( m \), Cleland (1984) concluded, based on a variety of results, that DP is proportional to \( m \) and can be used to detect changes in \( m \). A stress relaxation procedure (Yamamoto, Shinozaki and Masuda 1970; Zarra and Masuda 1979), in which walls of killed tissues are rapidly extended to a pre-determined level and maintained at this state, has also been used to estimate \( m \). The time lapse before the decrease in tension can be detected is assumed to be a direct
measurement of $m$ (Masuda 1978). Errors can occur, however, because killed tissues are used and cell walls receive only a unidirectional vector instead of the multidirectional stress induced by turgor. Also, changes in wall properties that would occur naturally due to continuous wall synthesis are excluded.

**Auxins and the Acid Growth Hypothesis**

Hormones participate in wall loosening (Cleland 1986) and while several (auxins, gibberellins, and cytokinins) are known to promote growth, the role of auxins have been studied most extensively. There is general agreement that auxin increases $m$ in hypocotyls of Lupin (Penny et al. 1972), Helianthus (Mentze et al. 1977), soybean (Boyer and Wu 1978, Courtney and Morre 1980) and mung bean (Prat and Goldberg 1984), in epicotyls of pea (Yoda and Ashida 1960; Tanimoto and Masuda 1971; Cosgrove et al. 1984; Cosgrove 1985), and in coleoptiles of oat (Cleland 1959, 1967b; Yamamoto et al. 1970; Zarra and Masuda 1979).

In auxin responsive tissues, polarly transported auxins (Bandurski and Nonhebel 1984) are believed to bind to receptive proteins to form conjugates (Schneider and Wightman 1974; Bandurski 1979, 1980; Sembdner et al. 1981; Firn and Kearns 1982). The conjugates then trigger enzymatic reactions (Chock, Ree and Stadtman 1980) that
result in wall acidification and also production of proteins required for growth (Bandurski and Nonhebel 1984). Even though the mechanism of wall acidification remains unknown, H⁺ ions are considered to be the long-hypothesized wall loosening factor (WLF) that causes alterations in m (Rayle and Cleland 1977, Taiz 1984, Cleland 1986).

Early work established that H⁺ can induce cell wall loosening in oat coleoptiles and can also partially reproduce auxin effects (Rayle and Cleland 1970). Subsequently, two research groups (Cleland 1971; Hager, Menzel and Krauss 1971) published the "Acid Growth Hypothesis", which proposes that auxin stimulates an H⁺-ATPase to extrude H⁺ which causes wall loosening (Rayle and Cleland 1972). Further work with corn roots (Edwards and Scott 1974), soybean stems (Perley, Penny and Penny 1975; Vanderhoef, Shen Lu and Williams 1977), oat coleoptiles (Pope 1978, Tepfer and Cleland 1979) and bean leaves (Van Volkenburgh and Cleland 1980) supported this hypothesis, but it was also demonstrated (e.g. Perley et al. 1975) that protein synthesis is required for elongation as well. According to Taiz (1984), the acid growth hypothesis is backed by the following lines of evidence: (a) tissue response to auxin is rapid (which suggests a membrane mediated process), (b) acidic buffer induces elongation in living segments and rapid wall loosening of frozen-thawed
sections, (c) neutral buffer blocks auxin effects, and (d) other proton extrusion promoters such as fusicoccin also cause elongation. Additionally, the ability of ATPases to promote acidification has been demonstrated in sealed membrane vesicles (Sze 1985), suggesting they play a role in growth-caused acidification and metabolically driven solute uptake. More recently, Cleland, Cosgrove and Tepfer (1987) identified two phases for the acid-extension response: a burst (exponential) and a constant rate of extension (linear), and concluded that the latter is likely to be the type of extension walls undergo during normal auxin-induced growth.

Turgor as the Possible Regulator of Plant Growth

The origin of the widely accepted view that turgor regulates plant growth rates actually began before the Lockhart equation (Lockhart 1965) was formulated. In 1959, Cleland hypothesized that growth of osmotically stressed oat coleoptiles was directly proportional to turgor. However, supporting evidence for this view was not presented until 1967 when he (Cleland 1967a) showed that in the presence of auxins, growth of oat coleoptile segments in osmotic solutions was proportional to $W_p$. Subsequently, Green et al. Buggy (1971) grew the algae Nitella in solutions of various $W_m$s and noted a clear proportionality
of growth rate to turgor; further, in elegant studies that influenced thinking for many years, they observed that slight reductions in $V_p$ caused growth to stop within minutes. Many other studies with intact higher plants provided additional reasons for suggesting cell turgidity was controlling plant growth. A proportionality of growth to turgor was found in roots of water stressed pea (Gracen and Oh 1972) and in osmotically stressed radish cotyledons (Kirkham, Gardner and Gerlaff 1972). In corn seedlings, Acevedo et al. (1971) demonstrated that sudden osmotic stress also causes almost immediate cessation of leaf elongation. To explain this phenomenon, Hsiao (1973) noted the above correlations of growth to $V_p$ and based on results obtained with Nitella, reasoned that only changes in turgor could cause such rapid growth changes. Subsequently, he and others demonstrated that growth is proportional to turgor in water-stressed leaves of sorghum (Hsiao et al. 1976), prairie grass (Chu and McPherson 1977), soybean (Bunce 1977), sunflower (Takami, Rawson and Turner 1982), wheat (Eastham, Oosterhuis and Walker 1984) and in salt-stressed leaves of bean (Neumann, Van Volkenburgh and Cleland 1988).

A critical examination of the literature, however, shows that it is inappropriate to rely solely on correlations to make a blanket assumption that growth
should be proportional to turgor. In the references cited above, for example, comparisons of time course changes in turgor and growth were made only in the study involving Nitella. In all other studies, $V_p$ determinations were performed several hours or even days after stress was initiated, and no evidence was presented to show that $V_p$ changes in higher plant tissues precede or occur simultaneously with growth changes. Additionally, whole organs or fully expanded tissues rather than growing tissue usually were sampled for water status determinations, and it is known now that stress responses of growing tissues may differ considerably from those of expanded tissue (Matsuda and Riazi 1981). In other cases such as in studies with excised oat coleoptiles, auxins were added and their use precluded the possibility that changes in wall extensibility could be a factor regulating elongation. Finally, clear exceptions to the "growth proportional to turgor" hypothesis are present in the literature. In unstressed corn plants (Acevedo et al. 1979), daytime growth rates of leaves were higher but turgor was lower than those at night. In contrast, in unstressed rice plants, nightly growth rates of leaves were higher but turgor was lower than in the daytime (Cutler et al. 1980). In sunflower, Takami et al. (1982) found that nocturnal growth rates of leaves were reduced by water stress while
turgor was either increased or remained unaltered. More recently, Barlow (1986) compared stress responses of expanding and expanded leaves of wheat and noted that correlations between \( \Psi_p \) and growth occurred only in expanded leaves.

More direct evidence for questioning the role of turgor in regulating growth was provided by comparing stress-caused growth and water status responses of young barley leaves (Matsuda and Riazi 1981). As shown earlier in corn (Acevedo et al. 1971), osmotic stress at various levels caused immediate cessation of barley leaf elongation. In barley, this growth cessation occurred before there was a measurable change in any water status measure of the growing or expanded tissues. Under continuous stress, however, barley leaves will resume growth after a lag period of a few min for the mildly stressed plants (-0.3 MPa), and 1 to 2 h for the more severely stressed plants (e.g. -1.2 MPa), and the new growth rates are proportional to the \( \Psi \) of the external solution. Stress also reduces \( \Psi \) and \( \Psi_n \) of the growing region, but the changes are not abrupt and do not coincide with growth alterations. In plants stressed with PEG of -0.8 MPa, for example, detectable reductions in \( \Psi \) are often evident in 0.5 to 1 h, but \( \Psi \) values will continue to decrease for nearly 12 h before stabilization occurs.
Additionally, because $Y_n$ and $Y$ decrease by equivalent amounts, turgor remains constant. Also, when plants are exposed to different levels of stress, the magnitude of reductions in stabilized values of $\Psi$ and $\Psi_n$ of the growing region is nearly the same as that applied in the external solution. In contrast, $\Psi$ and $\Psi_n$ in the expanded blade (i.e. the tissue most often sampled in water status measures) are usually only slightly altered by stress.

Because of the long held belief that growth is proportional to turgor, the accuracy of $\Psi$ values obtained psychrometrically for cut sections of growing tissues was questioned (Cosgrove et al. 1984). It was reasoned that since wall relaxation will occur during the required equilibration period, observed values of $\Psi$ and therefore $\Psi_p$ will be lower than in-situ values. However, subsequent studies have shown that $\Psi$ values obtained psychrometrically with cut sections of growing tissues of barley and wheat leaves, and hypocotyls and epicotyls of dark-grown squash and pea, respectively, were identical to values obtained when $\Psi$'s were determined with the dye method using conditions ($4^\circ$C, 8 min incubation) which restricted the possibility of wall relaxation (Mason and Matsuda 1985). Additionally, Cavalieri and Boyer (1982) and Westgate and Boyer (1984) noted that $\Psi$ obtained psychrometrically with cut sections of growing regions were only slightly lower
(less than 0.1 MPa) than values obtained with in-situ psychrometers. In later studies with cut sections that contained growing cells plus attached expanded cells, Boyer's group found that the attached expanded cells delay excision-induced wall relaxation (Boyer et al. 1985) and that the greater the amount of attached expanded tissue, the slower the relaxation (Matyssek et al. 1988), suggesting that the slowing growing or expanded tissue acted as a source of water for the growing cells.

Further studies have confirmed $\Psi_p$ maintenance and parallel reductions in growing region $\Psi$ and $\Psi_H$ occur in many other plant tissues following stress-induced growth cessation. Mason and Matsuda (1985) found stress reduced growth in leaves of wheat and in dark-grown pea epicotyls and squash hypocotyls, but $P$ of the growing region remained relatively unaltered, showing that growing regions of both dicots and monocots share the same trend in the initial response to osmotic stress. Turgor maintenance was also found to occur in growing regions of hypocotyls of dark-grown soybean (Cavalieri and Boyer 1982), leaves of corn (Michelena and Boyer 1982, Van Volkenburgh and Boyer 1985), and stems and roots of corn (Westgate and Boyer 1985) when stress was imposed by withholding water.

Studies with stress effects on polyribosomes have provided independent support for the view that responses of
growing tissues differ significantly from those of expanded tissues, and they have also provided information about how stress may alter developmental processes.

Protein synthesis is required for growth (Key and Vanderhoef 1973) and one might expect that protein synthetic rates will change in close association with growth rate alterations in stressed tissues. An estimate of the extent of protein synthesis can be inferred by measuring the proportion of ribosomes associated with mRNAs (polyribosome percentages). Early studies have shown that stress reduces polyribosome levels within 30 min in corn coleoptilar nodes (Hsiao 1970) and pumpkin cotyledons and pea shoots (Rhodes and Matsuda 1976). Polyribosome reductions also occur in stressed wheat floral apices (Barlow et al. 1977) and black locust seedlings (Brandle, Hinckley and Brown 1977). Moreover, reductions in polyribosome levels are proportional to the degree of stress (Hsiao 1970) and polyribosome percentages were shown to correlate with the growth rates of stressed squash fruits (Cocucci, Cocucci and Poma-Trecanni 1976) and light-grown pumpkin and pea seedlings (Rhodes and Matsuda 1976).

More recent studies have demonstrated that stress effects on polyribosome percentages of growing regions differ significantly from those observed on expanded tissues. Stressed growing wheat leaves show decreases in
polysome levels along with growth cessation, but fully expanded leaves are unresponsive (Scott, Munns and Barlow 1979). Stress also causes polyribosome reductions in growing regions of corn mesocotyl (Bewley and Larsen 1982). Growing regions of barley leaves (Mason and Matsuda 1985) and dark-grown soybean hypocotyls (Mason, Mullet and Boyer 1988) also exhibit decreases in polyribosome/total ribosome ratio, with relatively little effects on expanded tissues.

Kinetic studies provided further insight into the relation of polysome levels to stress. Stress initiates rapid reduction in polyribosome percentages of growing tissues, but reductions in percentages will continue over the course of several hours even after new growth rates in osmotic solutions are established. Mason and Matsuda (1985) exposed barley seedlings to solutions containing PEG of -0.8 MPa and found detectable reductions in polysome percentages in 15 min, but levels continued to decline for about 4 h before stabilized values were obtained. Expanded tissues, on the other hand, showed no reduction in polyribosome percentages for 2 h. These workers also stressed barley, wheat and dark-grown squash and pea seedlings with various concentrations of PEG and compared growth rates of leaves (wheat, barley), hypocotyls (squash) and epicotyls (pea) to stabilized polyribosome percentages and water status values of the growing regions.
obtained after 0.5 to 1 day. Tissue polyribosome percentages and $\Psi$ and $\Psi_m$ were directly correlated with growth rates of all organs; additionally, they found that when polyribosome percentages and growth rates were expressed as a fraction of that present in unstressed tissues, data from all plants fit a single curve which showed that when polyribosome percentages are reduced 50%, growth rates are reduced by 90%.

The Possible Rapid Stress Signal Sensed by Growing Cells

In an attempt to explain the fact that osmotic stress applied to roots of intact young barley seedlings effected an almost immediate leaf growth cessation without a concomittant change in turgor or any other water status measure of the growing region of leaves, Matsuda and Riazi (1981) hypothesized that sudden restricted water availability together with ongoing transpiration will cause a reduction in the transpiration stream's $\Psi$, and cells in the growing region are able somehow to respond to those changes. Because the expanded tissues were found to be relatively insensitive to stress, they reasoned that cells in the blade were in some way uncoupled from stress effects on the transpiration stream.

As a further test of the hypothesis that growing cells were likely responding to changes in $\Psi$ of the transpiration
stream, Rayan and Matsuda (1988) performed anatomical, heat pulse and labelling studies to define the transpiration stream and to test whether stress effects a reduction in its $\Psi$. Anatomical studies showed that mature vessels passed through the basally located growing regions, and these vessels were all clustered in five vascular bundles which were separated by about 20 to 30 closely packed mesophyll cells. The anatomical suggestion that water moves in vessels was confirmed by heat pulse transport studies which demonstrated that water moved at rates expected for passage through lumens of vessels alone. Anatomical examinations also provided a basis for explaining the uncoupling of most mesophyll cells in the expanded blade from possible effects of stress on the xylem's $\Psi$. As in the growing region, vessels functional for water transport in the expanded blade are also confined to five vascular bundles. Since water loss is likely to occur through the closest stomata (which are only two or three cells from the bundles capable of transporting water) rather than through the more distal ones (which are eight or more cells away from the functional vessels), it seems reasonable to assume that stress responses of the few cells immediately around the functional vessels will differ considerably from most mesophyll cells. Finally, as a test of the view that osmotic stress would rapidly effect a reduction in the
xylem's \( \Psi \), they found that sudden stress induced a reduction in xylem water transport within 1 min, while transpiration rates were not affected for at least 5 min.

The suggestion that stress acts by first reducing xylem's \( \Psi \) is also compatible with other anatomical and physiological data obtained with barley leaves. Anatomical results indicate the amount of water in the xylem is about 1 percent of the total water in tissues, so reductions in its \( \Psi \) would not be detectable in stressed tissues (Rayan and Matsuda 1988). In addition to effecting sudden growth cessation, stress also rapidly initiates osmotic adjustment (Matsuda and Riazi 1981; Riazi et al. 1985) and reductions in polyribosome percentages (Mason and Matsuda 1985) in the growing regions of barley leaves. It is possible that the same signal might regulate all three measures.

**Water Stress Studies with Mung Beans**

Several attempts have been made to understand the basis for stress-affected growth responses in soybean (e.g. Cavalieri and Boyer 1982), but comparable, comprehensive studies have not been performed with most other dicot species. For example, in the case of mung bean, that has features (e.g. uniform germination and growth) which are desirable for studying stress responses, workers have
attempted to link growth responses to changes in osmotic concentrations of solutes, or to turgor, but there appears to be no effort made to link growth responses simultaneously to changes in all water status components.

Dark-grown mung bean seedlings have been shown to reduce both hypocotyl growth and $\Psi_H$, when stressed by either withholding water (Zhao, Kamisaka and Masuda 1983) or by use of osmotica (Itoh et al. 1986). In contrast, light-grown plants exposed to 0.2 M (-0.5 MPa) mannitol exhibited only slight reductions in epicotyl elongation, but $\Psi_H$ was reduced in both growing and expanded regions (Zhao, Kamisaka and Masuda 1985). Stress promoted increases in epicotyl (Zhao et al. 1985) and hypocotyl (Zhao et al. 1983) contents of soluble sugars and amino acids, and these solutes accounted for nearly 80% of the osmotic adjustment. Since solute increases are minimized by cotyledon removal, the cotyledons are considered to be the major source of solutes for osmotic adjustment. In these studies, data were obtained after 12, 24 or 48 h stress and short-term kinetic results were not presented.

In more recent studies with roots of etiolated plants, water status and growth measurements were initiated 1 h after application of osmotic stress (Itoh et al. 1987a). Although root growth was not affected by stress, significant reductions in $\Psi_H$ occurred within 3 h and
potassium, chloride, free amino acids and sugars (mainly fructose), accounted for about 80% of the intracellular $\psi_m$. In another approach, short-term time courses were conducted to verify if osmotic adjustment is triggered by changes in turgor (Itoh et al. 1987b). Stress caused immediate growth cessation, and pressure probe-measured epidermal $P$ dropped from 0.65 to 0.14 MPa within 5 min. However, while growth fully recovered within 1 h, it took 6 h for $P$ to peak to a stable value (about 0.5 MPa), showing that $P$ does not regulate osmotic adjustment. Despite these efforts, many questions remain. Although studies on stress effects on root growth and $\psi_m$ have been performed, little is known about the total water status and growth responses of the hypocotyl and other aerial parts. In addition, while the precise location of the hypocotyl growing region has been well defined (Prat 1985; Goldberg, Morvan and Roland 1986), many attempts to relate stress to hypocotyl growth and water status have not focused on the rapidly expanding area. Finally, there is a clear need to know the kinetics of stress-induced growth alterations in all water status components to better understand how stress initiates changes in growth and other processes.
Work Needed to Improve Our Knowledge About How Water Stress Regulates Growth and Other Physiological Processes in Plants

There are currently two views on how water stress regulates growth in plants. The traditional one states that growth is regulated by the turgidity of the growing tissues (e.g. Hsiao 1973) and assumes that water availability to the cells in the growing tissues is not limiting (e.g. Hsiao et al. 1976). The belief is supported by a clear demonstration of the relation of P to growth in the algae Nitella (Green et al. 1971) and by various correlation data (e.g. Gracen and Oh 1972; Eastham et al. 1984) which showed that growth of tissues is proportional to turgor. In the turgor hypothesis, however, there is no attempt to link stress caused growth changes to alterations in other plant processes.

The more recent view assumes that growth in complex tissues is usually not controlled by turgor (Rayan and Matsuda 1988); rather, stress-induced changes in growth and several other processes (e.g. protein synthesis as estimated by polyribosome percentages, osmotic adjustment and alterations in tissue water status) are likely to be responses to Ψ or Ψ_P shifts in the apoplast, which is a minute fraction of total tissue water. This hypothesis also
requires that water movement into growing cells must somehow be restricted, and assumes that anatomical features of tissues will greatly alter water movement and thereby cellular responses to stress. The hypothesis is consistent with data obtained from both short- and long-term stress studies performed with barley and from relatively long-term studies with several dicot species; yet extensive studies must be conducted with other species before statements can be made about its general applicability to higher plants. Accordingly, it was felt that the following studies should be carried out:

A. Several dicot species must be examined to further test whether plants are likely responding to water stress-induced alterations in mesophyll cell turgor or to apoplastic water status. Dicots were selected for this study because much less is known about many of their stress responses.

B. In all cases, a specific effort must be made to distinguish responses of growing from expanded regions. The need for using this approach became apparent when it was shown that stress responses of growing regions may differ greatly from those of expanded areas. The lack of this distinction has led to considerable conflict regarding the mechanism by which plants respond to stress.

C. Whenever possible, both short- and long-term stress
responses will be examined to see how stress-caused growth responses compare with changes in the water status of both growing and expanded regions. This approach should provide a better basis for suggesting possible cause/effect relationships that might exist between water status and growth alterations.
MATERIAL AND METHODS

Plant Material

Dark-grown Plants

Seed were germinated and maintained in darkness, but all manipulations were performed under dim green light, which does not induce opening of plumule hooks. Germination and initial growth were conducted in vermiculite, at 20±1°C and 58±5 %RH for black bean (Phaseolus vulgaris L. purchased locally), mung bean (Vigna mungo L. Burpee's cv. 'Berken') and pea (Pisum sativum L. Burpee's cv. 'Alaska'), and 33±2°C and 75 %RH for cowpea (Vigna unguiculata (L.) Walp. Ca CIA's cv. 'California #5') and squash (Cucurbita pepo L. Joseph Harris' cv. Zucchini 'Elite'). Plants were carefully removed from vermiculite after either 3 (cowpea and squash), 2, 3, 4, or 5 (mung bean), or 4 or 5 days' (black bean and pea) growth, and transferred to racks (Bhola 1978) with roots immersed in aerated modified Hoagland's medium (Hoagland and Arnon 1938), which consisted of 1 ml each of 1 M MgSO₄, 1 M KNO₃, 1.5 M Ca(NO₃)₂, 1 M KH₂PO₄: 1 M K₂HPO₄, micromix (3.75 g H₃BO₃, 2.25 g MnCl₂.4H₂O, 75 mg CuCl₂.2H₂O, 75 mg MoO₃, and 0.33 g ZnSO₄, made to 3 liters), and 15 mg Chel 138 (Geigy) per liter of solution.
Light-grown Plants

Seeds were germinated in vermiculite at either 25±2°C (black bean, mung bean and pea) or 33±2°C and 75 %RH (cowpea and squash), under 13 h (200 μmol m⁻² s⁻¹) light: 11 h dark conditions. Plants were transferred to aerated modified Hoadland's medium after either 2 (squash), 3 (cowpea), 5 (black bean) or 6 or 7 day's (mung bean and pea) growth.

Stress Application

In all cases, plants were allowed to recover from transplantation for 20 h in Hoagland's medium before osmotic stress treatments were initiated. Racks containing the plants were then transferred to new Hoagland's medium (0 MPa) or were stressed by exposing their roots to medium containing PEG 8000 (-0.2, -0.4, -0.6 or -0.8 MPa). In cases when rapid growth responses were measured, the nutrient solution was drained and replaced.

Growth Measurements

Rapid stress effects on growth of dark-grown mung bean seedlings were performed in a blackened light-tight chamber of approximately 0.4 X 0.4 x 0.4 m ID. In addition to being fully accessible from the front, the chamber was constructed with a slot on the top to allow passage of threads for growth measurements, and it was also equipped
with a system of tubes that permitted external draining and refilling within 0.5 min of the 0.5 L aerated nutrient solutions that were used to grow seedlings. Internal air circulation was provided by two slots located at the bottom of the chamber.

Four uniform seedlings, transferred from vermiculite trays onto shortened wooden racks were allowed to grow in the chamber for 16 h. A loop of black thread was then positioned around the hook of each seedling, and the other end of the thread, which was connected to a sewing needle (0.25 g) via a washer as an added weight (0.3 g), was gently passed over a horizontally placed teflon tube that was notched slightly to prevent lateral movement. The length of the thread was such that it was possible to position the tip of the needle so that it rested slightly on the side of a horizontally placed 1.27 cm wide ruler, and growth was determined as the distance the tip moved with respect to the width of ruler. Hypocotyl growth was determined by time-lapse photography with a Pentax K-1000 camera equipped with close-up lenses (Tiffen +1, +2 and +4) using a laterally placed green light source. The film (Kodak Plus-X) was developed and magnified by projection to a total of 15.7 times. Growth rates refer to change in length that occurred in the time interval from preceding photograph (the 1st photo was taken 4 h prior to seedling
exposure to stress solution).

Since growth rates measured in this way were identical to values obtained when elongation was determined with a ruler after 2 or 24 h otherwise undisturbed growth, it was concluded that looping threads around hooks had no detrimental effect on mung bean hypocotyl growth.

This procedure was, however, effective only for detecting growth cessation and resumption, because growth rates' SDs were relatively high and mean contrasts could not be rigorously compared. Therefore, elongation rates were routinely measured with a ruler for dark- and light-grown plants of all studied species. Seedlings were transferred from vermiculite to racks and allowed to grow for 16 h, and then lengths of organs were measured. After 4 h, roots were placed into either fresh (control) or osmoticum-containing medium, and organ lengths were measured immediately. After 3 and 24 h, and in some cases other periods of stress, lengths were redetermined. Stated growth rates represent changes in length that occurred in the time interval from the preceding measurement of length.

**Determination of Growing Regions**

Growing and expanded regions of shoot parts were determined by rolling a stamp pad blotted plastic screw onto the surface of the organ to mark equally separated
(0.2 cm) horizontal lines (Mason and Matsuda 1985). After 24 h, the areas where line spacings increased the most were designated the growing region, and those where spacings remained unaltered were considered to be the expanded region. Our so-called growing region was actually that with rapidly elongating cells, since regions exhibiting slower growth were also detected, especially in hypocotyls.

Since roots are fragile, they were marked in an alternative way. They were gently blotted with tissue paper and equidistant marks were made with a permanent ink-blotted pin. Immediately after the marks dried, roots were immersed into nutrient medium and reexamined after 24 h. Well defined growing and expanded regions were found for the main roots of all species except squash. In squash, the growing region was considered to be the most basal region, whereas the expanded area was the uppermost tissue.

Despite differences in seedling age, the positions of growing (rapidly elongating) and expanded regions generally matched those obtained by others and were as follows: (a) Dark-grown hypocotyls: growing regions in the uppermost 0.5 cm for mung bean (Prat 1985; Goldberg et al. 1986) and 1.0 cm for squash (Mason and Matsuda 1985), black bean and cowpea; expanded regions at basal 1 cm for all species; (b) Dark-grown epicotyl (pea): growing region at the 3rd internode apical 1.0 cm and expanded region at
basal 1.0 cm (Mason and Matsuda 1985); (c) Dark- and light-grown roots: growing region at the tip (0.5 cm), and expanded region at the basal 0.5 cm for black bean, cowpea, mung bean (Itoh et al. 1986, Oka et al. 1987) and pea; (d) Light-grown leaves: growing region at basal 0.8 cm and expanded region at the apical 0.5 (mung bean), 0.8 (black bean and squash) cm (Barlow 1986); (e) Light-grown epicotyl (mung bean): growing region at the apical 0.6 cm and expanded region at basal 0.7 cm (Zhao et al. 1985); and (f) Light-grown hypocotyls (cowpea): growing region at apical 1.0 cm (just below the cotyledons) and expanded region at basal 1.0 cm.

**Water Status Measurements**

For water status determinations and extraction of soluble osmotica, samples of plant organs were taken from their rapidly growing and fully expanded regions (unless otherwise noted), and consisted of discs 0.6 cm in diameter (for light-grown leaves) or segments 0.5 cm long (for either light- or dark-grown hypocotyls, epicotyls and roots). Samples were obtained from stressed and unstressed seedlings, after 0, 3 and 24 h of continuous stress, unless otherwise noted. Water status values were obtained from single tissue sections (except for root growing region, where three sections were taken) using calibrated Merrill
75-13 psychrometers and a Wescor MJ-55 microvoltmeter. Samples from nutrient medium were collected with Whatman #1 filter paper discs 0.6 cm in diameter, and those from tissues consisted of excised 0.5 cm segments of hypocotyls, epicotyls and roots, or 0.6 cm discs of leaf blades. Determinations of $\Psi$, $\Psi_n$ and $\Psi_p$ were performed according to Mason and Matsuda (1985).

**Extraction of Soluble Osmotica**

Hypocotyl samples of 10 sections from stressed and unstressed 5-day-old dark-grown mung bean seedlings were collected after 2, 3, 4, 10 and 24 h of stress and weighed (fresh weight), lyophilized overnight, and reweighed (dry weight), and % dry weight and sample water content were calculated. Subsequently, soluble osmotica were extracted from samples taken after 2, 3 and 4 h stress using overnight extraction at room temperature with 80% ethanol (Castro Neto 1988), and 2 aliquots (10 ul) were taken per sample for psychrometric $\Psi_n$ determinations.

Statistical analyses were performed for all data and means were compared by the t test. However, for reasons of clarity, many comparisons are presented using SDs.
RESULTS AND DISCUSSION

Measurement of Rapid Growth Responses of Dark-grown Seedlings to Osmotic Stress

Attempts to measure elongation of dark-grown mung bean hypocotyls photographically quickly revealed that the procedure is suitable primarily for detecting major changes in growth rates. In Fig 1, for example, growth cessation caused by stress of -0.6 MPa was readily detectable but while mean values for growth rates of unstressed plants of different ages tended to decrease in the order from 6- to 5- to 4- day-old seedlings, significant age-related differences were not found, even though 12 or more replicate plants were studied.

The data of Fig 1 are in agreement with previous results obtained with Nitella (Green et al. 1971), dark-grown corn (Acevedo et al. 1971) and light-grown barley (Matsuda and Riazi 1981) seedlings in that osmotic stress caused an almost immediate cessation of mung bean hypocotyl elongation. As was noted in barley leaves, hypocotyls of continuously stressed mung bean seedlings resumed growth after an initial lag period (Fig 1), and stabilized growth rates were lower than those of unstressed plants. Also, the latent period increased with seedling age. In the case of 4-day-old seedlings, growth resumed in 10 min, but rates
Figure 1. Rapid stress-induced hypocotyl growth responses of dark-grown mung bean seedlings of 3 ages. Growth rates were determined photographically and means are from 12 (4- and 5-day-old) or 20 (6-day-old) plants.
from 15 to 45 min stress were higher than those after 1 h, when growth rates stabilized. In contrast, hypocotyls of 5-day-old seedlings began to grow in 15 min and stabilized rates were achieved in 20 min, whereas growth of 6-day-old seedlings began after 30 to 45 min and rates gradually increased for about 2 h.

These response differences suggest several conditions required for growth are altered by stress. For instance, stress-induced immediate stoppage of hypocotyl growth indicate that all cells involved in growth can respond to reduced water availability in the root environment. Cells capable of growing adjust to reinitiate elongation and since the latent period before expansion resumes increases with seedling age, it is possible that the relation of seedling size to available storage materials present in the cotyledon is important. Additionally, visual observations revealed that excision of tissue sections results in exudation of water, with amounts decreasing in the order from 4- to 5- to 6-day-old plants. This may mean that translocation of water to growing cells may also be a factor.

Growth responses of seedlings stressed with other concentrations of PEG were also photographically determined, and they showed that exposure to mild (-0.2 to -0.4 MPa) or relatively severe (-0.7 to -0.8 MPa) stress
invariably caused growth to cease within 1 min. However, growth responses obtained upon continuous exposure to stress were difficult to reproduce. For example, when 4-day-old seedlings were mildly stressed, growth resumed in less than 10 min, but growth rates varied widely and stabilization seldom occurred. When severe stress (-0.8 MPa) was applied, 4-day-old plants resumed growth in 20 min, but growth of 5- and 6-day-old seedlings was blocked for at least 24 h.

Several attempts were made to determine if the duration of the lag period or the growth rates under stress could be altered by changing the environment surrounding roots or hypocotyls. Again, because of high variability in data, specific curves showing growth response are not presented. In one group of studies, 4-day-old seedlings were stressed with PEG of -0.6 or -0.8 MPa, and growth responses following relief of stress after 5 or 15 min were compared to responses obtained with continuously stressed plants. Unlike the situation observed in barley leaves, where removal of stress resulted in immediate growth resumption (Matsuda and Riazi 1981), stress-relieved mung bean plants showed no alteration in the latent period or in stabilized growth rates. In one experiment, for example, unstressed 4-day-old seedlings grew at 1.5 ± 0.6 mm/h. When stressed with -0.6 MPa, they resumed growth after 10 min and the
mean stabilized growth rate (reached after 30 min) was 1.1 ± 0.2 mm/h. Plants relieved of stress after 5 min showed the same latent period and a mean stabilized growth rate of 0.8 ± 0.3 mm/h. If stress was relieved after 15 min, the mean growth rate was 0.9 ± 0.1 mm/h. Such results indicate osmotic stress does more than limit water supply in mung bean; rather it rapidly initiates changes in one or more additional factors (such as limiting the ability to produce wall loosening factor, or causing an increase of growth inhibitor).

Since the mung bean plant is auxin-responsive and H⁺ is the presumed wall loosening factor (Prat and Goldberg 1984), attempts were made to see if hypocotyl growth responses of 5-day-old plants under stress conditions could be altered by spraying the growing region with acidic solution (Sorensen's citrate I Buffer, pH 3.0). Plants sprayed with water 5 min after exposure to -0.6 MPa took an additional 10 min to resume growth. In contrast, plants sprayed with acid at the same time had a 5 min shorter latent period, but growth rates 10 min after spraying (0.4 ± 0.3 mm/h) were similar to control plants (0.5 ± 0.2 mm/h).

Although photographically measured growth rates of unstressed plants were similar to values obtained by determining length increases over longer intervals with a
ruler (e.g. compare Figs 1 and 2), the photographic technique showed that individual plants appeared to grow and then shrink. To resolve this apparently anomalous behavior, undisturbed plants were allowed to grow in fresh medium and their hypocotyls were photographed under safe dim light at short time intervals (0, 1, 5, 10, 15, 20, 25, 30 and 35 min). Magnified pictures revealed that considerable swaying occurs during growth, and it was therefore concluded that this growth pattern was the major cause of variability.

Because of this variability and the fact that looping a thread around the plant hook was found to inhibit growth of dark-grown pea epicotyls and black bean hypocotyls, the photographic procedure was abandoned. All subsequent growth measurements were performed with a ruler, which normally requires a minimum of 1 h to detect significant changes in growth rates.

Stress-induced Alterations in Growth Rates and Water Status

Values of Dark-grown Mung Bean Seedlings

Stress-induced growth responses were evaluated for all emergent seedling age groups which had measurable growth rates. A representative study (Fig 2) showed that, in general, unstressed growth rates were highest and stress-caused reductions the most pronounced in 5-day-old
Figure 2. Growth responses of dark-grown mung bean seedlings of various ages to osmotic stress. Hypocotyl heights were measured with a ruler at -4, 0, 3 and 24 h following transfer into fresh aerated solution containing 0, -0.2, -0.4 or -0.6 MPa PEG. Means (±) are from 10 observations.
seedlings. Since plants of this age also reached stabilized growth rates in osmotic solutions rapidly (Fig 1), they were used in most subsequent studies.

Hypocotyl growth data from a highly replicated study with 5-day-old seedlings (Fig 3) were virtually the same as shown in Fig 2. Growth rates tended to decrease in proportion to the level of applied stress, but differences between treatments receiving -0.2 and -0.4 MPa stress were not significant. Effects of moderate to severe stress (-0.6 MPa) were significantly less pronounced when plants were grown under 100 %RH. In yet another study (Fig 4), stress effects on hypocotyl growth were detectable within 2 h, and the response differences observed in the two levels (-0.2 and -0.6 MPa) of stress were maintained for up to 24 h. Stress (-0.4 MPa) also caused growth inhibition of roots (Fig 3), which was more pronounced than that observed in hypocotyls.

Selected regions of mung bean seedlings were analyzed for their water status values (Fig 5) to determine the relationship of this parameter to growth. In unstressed plants, \( \Psi \) and \( \Psi_m \) values were highest in the expanded region of the root (-0.36 and -0.52 MPa, respectively), lower in the expanded region of the hypocotyl (-0.61 and -0.75 MPa, respectively) and the lowest in the growing region of the hypocotyl (-0.77 and -0.91 MPa, respectively). In
Figure 3. Effects of osmotic stress on hypocotyl and root growth of dark-grown 5-day-old mung bean seedlings. Means (±) are from 40 observations.
Figure 4. Effects of short duration stress on hypocotyl growth responses of dark-grown 5-day-old mung bean seedlings. Means (+) are from 50 observations.
Figure 5. Water status alterations in selected regions of dark-grown 5-day-old mung bean tissues in response to osmotic stress. Means (±) are from 15 observations.
particular, the $\Psi_m$ values confirmed previous work with hypocotyls of dark-grown mung bean (Zhao et al. 1983; Itoh et al. 1986). These rankings in water status were not changed by stress (-0.6 MPa), but $\Psi$ and $\Psi_m$ values of all tissues were reduced in the course of 24 h, and the most pronounced reductions (about 0.4 MPa) were observed in the expanded region of roots (Fig 5). In contrast, $\Psi_p$ values were similar (about 0.1 MPa) for all tissues and were not significantly altered by stress, but a tendency of reduction was observed in the expanded region of roots.

To test whether water status values of the growing region of hypocotyls will change as stress-caused growth cessation occurs, measurements were made 5 min before, at, and 5 and 10 min following first exposure to stress. No difference in any water status measure was found (Table 1), even in the growing region of the root. Roots are very sensitive to any level of stress and they undergo desiccation and loss of turgor within 24 h under -0.4 MPa or greater stress (data not shown).

Several steps were taken to improve knowledge of the basis for changes in water status that occurred in stressed tissues. In an effort to infer whether reductions in $\Psi_m$ are due to water loss or to solute accumulation, water and dry weight contents were determined for hypocotyl segments of unstressed and stressed plants (Fig 6). Exposure to -0.6
Table 1. Short-term water status values (MPa) of selected regions of osmotically stressed (-0.6 MPa) tissues of dark-grown 5-day-old mung bean seedlings. Means are from 13-20 observations, and were compared by the t test (5% probability).

| MINUTES OF CONTINUOUS STRESS | HYPOCOTYL REGION | | ROOT REGION |
|-------------------------------|------------------|------------------|
|                               | GROWING          | EXPANDED         | GROWING          | EXPANDED         |
| Y                             |                  |                  |                  |
| -5                            | -0.76 a          | -0.47 a          | -1.17 a          | -0.56 a          |
| 0                             | -0.76 a          | -0.54 a          | -1.30 a          | -0.54 a          |
| 5                             | -0.80 a          | -0.57 a          | -1.40 a          | -0.65 a          |
| 10                            | -0.90 a          | -0.57 a          | -1.28 a          | -0.65 a          |
| Yn                            |                  |                  |                  |
| -5                            | -0.83 a          | -0.58 a          | -1.26 a          | -0.75 a          |
| 0                             | -0.81 a          | -0.70 a          | -1.46 a          | -0.70 a          |
| 5                             | -0.87 a          | -0.73 a          | -1.45 a          | -0.77 a          |
| 10                            | -0.93 a          | -0.72 a          | -1.33 a          | -0.80 a          |
| Yp                            |                  |                  |                  |
| -5                            | 0.07 a           | 0.11 a           | 0.09 a           | 0.19 a           |
| 0                             | 0.04 a           | 0.14 a           | 0.05 a           | 0.12 a           |
| 5                             | 0.06 a           | 0.16 a           | 0.05 a           | 0.12 a           |
| 10                            | 0.03 a           | 0.16 a           | 0.05 a           | 0.11 a           |
Figure 6. Stress-caused changes in dry weight and water content in different regions of dark-grown 5-day-old mung bean tissues. Means (±) are from 2 or 12 groups containing 10 hypocotyl segments.
MPa induced increases in dry weight within 3 h in both the expanded and growing regions, and for the first 10 h, those increases were greater for growing than for expanded tissues. Water content (Fig 6) of unstressed and stressed hypocotyls changed considerably over the course of the study and were highest in the growing region at 4:00 PM, but no water content differences were found between unstressed and stressed tissues except in the growing region after 24 h stress. These data show that initial reductions in $Y_n$ and $Y$ cannot be due to the loss of water and are more likely the result of accumulation of osmotic solutes.

To better understand the nature of substances involved in osmotic adjustment, tissue segments were immersed in 80% ethanol, the extracted solutes were dried and an amount of water equivalent to that found originally in the tissues was added. Since $Y_n$ values obtained through the reconstituted solutes were generally the same as those determined for tissues by the usual freezing and thawing procedure (Table 2), it was concluded that accumulated, osmotically active solutes were responsible for changes in $Y_n$ of stressed tissues.

An estimate of the average molecular weight of solutes responsible for osmotic adjustment was obtained by using the water content (Fig 6), the decrease in $Y_n$ (Fig 5) and
Table 2. Osmotic potential values (MPa) of different regions of dark-grown 5-day-old mung bean hypocotyls as measured by tissue (T) or soluble osmotica (S) samples. Means are from either 5-10 (T) or 20-40 hypocotyl segments (S), and were compared by the t test (5% probability).

<table>
<thead>
<tr>
<th>TYPE OF SAMPLE/STRESS LEVEL (MPa)</th>
<th>HOURS OF CONTINUOUS STRESS</th>
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<tr>
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<td>2</td>
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<tr>
<td>GROWING REGION</td>
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<tr>
<td>F</td>
<td>-0.87 a</td>
</tr>
<tr>
<td>S</td>
<td>-0.86 a</td>
</tr>
<tr>
<td>EXPANDED REGION</td>
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<tr>
<td>F</td>
<td>-0.80 a</td>
</tr>
<tr>
<td>S</td>
<td>-0.98 a</td>
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</table>
the increase in dry weight (data not shown) that occurred in the growing region of hypocotyls after 3 h stress. If the solutes are assumed to be non-ionized substances, the calculations indicate compounds averaging about 200 daltons (what is a MW similar to that of hexoses) may account for the observed osmotic adjustment.

Short- and long-term growth and water status responses observed in this study and those of others have provided some basis for suggesting the relationship that might exist between growth and tissue water status. In the experiment reported here (Fig 1), and in studies with barley leaves (Matsuda and Riazi 1981), sudden osmotic stress effected growth cessation before noticeable changes occurred in any water status measure of the growing region. Furthermore, stress-induced rapid growth cessation in roots of dark-grown mung bean seedlings was accompanied by a reduction in $V_p$ of growing tissues (Itoh et al. 1987), but growth resumption was initiated without $V_p$ increments. Such results suggest that turgor loss or changes in any other water status measure are unlikely to be the cause of immediate growth stoppage.

When plants are stressed continuously in mild (-0.2 to -0.4 MPa), moderate (-0.6 MPa) or relatively severe (-0.7 to -0.8 MPa) osmotica, growth has been shown to resume but at rates lower than those in unstressed seedlings. In the
experiments reported here (Figs 1, 2, 3 and 4) and in other studies with hypocotyls of dark-grown mung bean (Zhao et al. 1983; Itoh et al. 1986), growth rates after about 24 h continuous stress have been found to be proportional to the $\Psi$ of the nutrient medium. Relatively to other species, this fact has been shown to apply to leaves of barley (Matsuda and Riazi 1981, Mason and Matsuda 1985), wheat (Mason and Matsuda 1985) and to dark-grown epicotyls of pea and hypocotyls of squash (Mason and Matsuda 1985). Finally, our results suggest that short- (Fig 1) and long-term (Fig 2) growth responses to stress vary with seedling age.

Since stress causes sudden growth cessation in dark-grown mung bean with apparently no change in water status, this response is likely to be regulated by other factors. Moreover, a specific regulation of growth by $\Psi_p$ even for long-term stress-induced inhibition may not occur because $\Psi_p$ is maintained due to nearly parallel reductions in $\Psi$ and $\Psi_n$. It appears possible that stress-induced growth cessation, along with other subsequent alterations (e.g. resumed growth at lower rates, solute accumulation as major cause for early osmotic adjustment) are responses for a common signal, to be quickly transmitted from the stressed roots to the growing aerial tissues. This could be a reduction in hydrostatic pressure in the xylem, as proposed by Rayan and Matsuda (1988).
Stress-induced Alterations in Growth Rates and Water Status Values of Light-grown Mung Bean Seedlings

Osmotic stress was applied to light-grown plants at noon, and at that time mean blade lengths of 7- and 8-day-old seedlings were 2.8 and 3.5 cm, respectively, and epicotyl lengths were 1.5 and 2 cm. Because preliminary tests with different concentrations of PEG showed that -0.2 MPa did not stop growth and -0.6 MPa resulted in plant death within 24 h, -0.4 MPa was used routinely in these studies.

Stress effects on growth of 7-day-old seedlings were complicated because of large variation in growth rates of both blades and epicotyls of unstressed plants (Fig 7). Osmotic stress caused epicotyl and leaf blade growth to stop within 2 h, and growth essentially did not resume for 24 h. The pronounced changes in growth rate of both leaf blade and epicotyl may have resulted from handling because growth rates of unstressed plants at 24 h (Fig 7) were just a fraction of those observed in the first measurement of 8-day-old seedlings (Fig 8).

Growth rates of unstressed 8-day-old seedlings were more uniform (Fig 8). Stress did cause epicotyl growth to stop between 2 to 4 h, and growth resumed by 10 h (Fig 8). A more diverse response was seen in blades, where growth
Figure 7. Growth responses of light-grown 7-day-old mung bean seedlings to osmotic stress. Means (±) are from 10 observations.
Figure 8. Growth responses of light-grown 8-day-old mung bean seedlings to osmotic stress. Means (±) are from 8 (epicotyl) or 16 (leaf blade) observations.
stopped between 2 to 3 h, resumed for 1 h, stopped again, and eventually resumed in the interval between 10 to 24 h.

To test whether growth changes could result from handling of the seedlings, growth rates of additional sets of 8-day-old seedlings receiving minimal manipulation (i.e. leaf blade and epicotyl lengths were measured only at 0 and 24 h stress) were compared with normally handled plants. In this study, blade growth rates (±SD) for unstressed and stressed seedlings were $0.80 ± 0.07$ and $0.70 ± 0.12$ mm/h, and epicotyl growth rates were $0.83 ± 0.18$ and $0.74 ± 0.21$ mm/h. Those values did not differ significantly from those obtained in the interval from 10 to 24 h (Fig 8), and also in plants that were handled several times during 24 h (data not shown). The results contrast sharply with those obtained by Zhao et al. (1985), who observed only slight decreases in epicotyl growth rates of 5-day-old mung bean seedlings stressed with mannitol of $-0.5$ MPa for 48 h. As indicated by the comparison between 7- and 8-day-old seedlings, differences in plant age and time of growth measurement may have been responsible for this discrepancy.

Because of their overall higher growth rates, leaf blades were analyzed for water status. In unstressed plants of both ages, $\Psi$ and $\Psi_n$ values of the growing region of blades were about 0.1 MPa lower than those of the expanded
region (Figs 9 and 10). Stress caused no alteration in water status of expanded regions of both 7- and 8-day-old plants, but it reduced $\Psi$ of the growing regions by 0.1 MPa within 2 h without affecting $\Psi_m$ (Figs 9 and 10). In this study no osmotic adjustment was detected in 24 h in the growing region of 7-day-old plants. However, osmotic adjustment occurred in 24 h in 8-day-old plants, as observed by Zhao et al. (1985) in epicotyls of light-grown mung bean after 48 h of stress.

**Stress-induced Alterations in Growth Rates and Water Status Values of Dark-grown Black Bean Seedlings**

Studies were performed with 5- and 6-day-old seedlings because younger plants were devoid of well defined growing and expanded regions, and older plants had very low growth rates.

Hypocotyl growth rates of unstressed 5-day-old seedlings (Fig 11) were about twice those found for mung bean plants (Fig 3), and all stress levels caused elongation to decrease within 3 h. At 24 h, growth rates were proportional to the $\Psi$ of the nutrient solution.

Root growth rates were about half those found for dark-grown mung bean seedlings (Fig 3), and growth of only the treatment receiving -0.6 MPa stress was reduced after 3 h. At 24 h, those rates were proportional to medium $\Psi$ in
Figure 9. Changes in water status of selected regions of light-grown 7-day-old mung bean leaf blades in response to osmotic stress. Means (+) are from 8-15 observations.
Figure 10. Changes in water status of selected regions of light-grown 8-day-old mung bean leaf blades in response to osmotic stress. Means (±) are from 20 observations.
Figure 11. Effects of osmotic stress on hypocotyl and root growth of dark-grown 5-day-old black bean seedlings. Means (±) are from 8 observations.
plants stressed with mild osmotic solutions, and growth was absent in plants exposed to -0.6 MPa.

Wide variations in $\Psi$ and $\Psi_n$ were occasionally found in both the growing and expanded region of the hypocotyl but with few exceptions, all osmotic solutions (-0.2, -0.4 and -0.6 MPa) caused similar reductions in $\Psi$ after 3 h (Fig 12). Further reductions in $\Psi$ were obtained after 24 h, but for reasons which are not clear, $\Psi$ increased after 48 h stress. Additionally, because $\Psi_n$ reductions equalled reductions in $\Psi$, turgor was maintained in the growing and expanded regions of the hypocotyl of stressed seedlings. It was not possible to obtain water status values for the growing region of roots, but interestingly, $\Psi$ of the expanded region of roots were lower than those of the hypocotyl sections, and all stress levels also caused $\Psi$ and $\Psi_n$ to change by similar amounts.

Hypocotyl growth rates of dark-grown 6-day-old black bean seedlings, following transfer to fresh nutrient medium (3.44 and 2.54 mm/h after 3 and 24 h, respectively) were again about twice those found for mung bean plants (Figs 1 and 2). Growth rates were not reduced when seedlings were exposed to -0.2 or -0.4 MPa, but growth ceased within 3 h following exposure to -0.6 MPa and showed only slight recovery (0.48 ± 0.5 mm/h) after 24 h.

Measurements of water status of hypocotyls of 6-day-
Figure 12. Changes in water status of selected regions of dark-grown 5-day-old black bean tissues in response to osmotic stress. Means (+) are from 10 observations.
old seedlings showed that $\psi$ and $\psi_p$ values of unstressed plants tended to be slightly lower in the growing region than in the expanded region (Fig 13). Stress (-0.6 MPa) caused about a 0.2 MPa reduction in $\psi$ of the hypocotyl growing region within 1 h and also after 3 h. Because of virtually equivalent reductions of $\psi$ and $\psi_p$, $\psi_p$ was not altered in the first 3 h of stress, although hypocotyl growth clearly stopped.

To determine if reductions in $\psi_p$ are caused by solute accumulation or water loss, dry weight and water contents were determined for hypocotyl and root segments from unstressed and stressed 6-day-old plants (Table 3). Growing regions of both hypocotyls and roots increased in DW and also lost water when plants were stressed with -0.6 MPa for 2 h. In contrast, DW and water contents of the expanded region of hypocotyls were not affected (Table 3). In the hypocotyls, these alterations preceded the onset of stress-induced decreases in $\psi_p$, which observed in 3 h for the growing region and in 24 h for the expanded one (Fig 13). These data show that, at the level of precision of our methods, initial loss of water and solute accumulation do not cause detectable osmotic adjustment in hypocotyls of stressed dark-grown black bean plants. These responses contrast with those observed in hypocotyls of dark-grown mung bean (Fig 6), where water loss in stressed plants was
Figure 13. Changes in water status of selected regions of dark-grown 6-day-old black bean hypocotyls in response to osmotic stress. Means (±) are from 5 observations.
Table 3. Changes in % dry weight and water content of selected regions of dark-grown 6-day-old black bean tissues. Means are from 12 segments, and were compared by the t test (5% probability).

<table>
<thead>
<tr>
<th>PLANT ORGAN</th>
<th>ORGAN REGION</th>
<th>STRESS LEVEL (MPa)</th>
<th>HOURS OF CONTINUOUS STRESS</th>
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<td>1</td>
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<td></td>
<td></td>
<td></td>
<td>11.1 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.2 a</td>
<td>11.8 b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.2 c</td>
<td>5.0 e</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.6 c</td>
<td>5.9 e</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.9 b</td>
<td>14.9 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.4 a</td>
<td>8.9 d</td>
</tr>
<tr>
<td>% DRY WEIGHT</td>
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<tr>
<td>Hypocotyl</td>
<td>Growing</td>
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<td></td>
<td></td>
<td>-0.6</td>
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<td>0.0</td>
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<tr>
<td>Root</td>
<td>Growing</td>
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<td>WATER CONTENT (µl/sample)</td>
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<tr>
<td>Hypocotyl</td>
<td>Growing</td>
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<tr>
<td>Root</td>
<td>Growing</td>
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seen only in the growing region after 24 h.

Stress-induced Alterations in Growth Rates and Water Status of Light-grown Black Bean Seedlings

Osmotic stress was applied to 6-day-old light-grown plants at noon, and at that time mean blade and epicotyl lengths were 5.7 and 2.7 cm, respectively.

In unstressed seedlings, growth rates of blades were higher than those of epicotyls (Fig 14), but both decreased during the course the study. Exposure to either -0.2 or -0.6 MPa stress caused growth to virtually stop within 3 h, and no growth was observed after 24 h stress. As in mung beans (Figs 7 and 8), the results show that stress-sensitivity is greater in light-grown than in dark-grown plants (Figs 11 and 14).

Analysis of the water status of the blades of unstressed plants (Fig 15) showed that \( \psi \) and \( \psi_n \) values of the growing and expanded regions were similar. In stressed plants, \( \psi \) and \( \psi_n \) of the growing and expanded regions were not altered for 3 h, were significantly reduced in 24 h, and \( \psi_p \) remained unaltered throughout the study (Fig 15). As was found for mung beans, stress-caused growth cessation preceded any change in water status of the growing region. In light-grown black beans, turgor remained positive and constant after 24 h despite the fact that growth did not
Figure 14. Growth responses of light-grown 6-day-old black bean seedlings to osmotic stress. Means (+) are from 8 observations.
Figure 15. Stress-induced alterations in water status of different regions of light-grown 6-day-old black bean leaf blades. Means (±) are from 5 observations.
Stress-induced Alterations in Growth Rates and Water Status of Dark-grown Pea Seedlings

Stress-caused growth responses were evaluated for the two seedling age groups which had measurable growth rates. A representative study (Fig 16) showed that epicotyl growth rates of unstressed plants were higher and stress responses generally more pronounced in 5-day-old rather than in 6-day-old plants. The latter apparently were affected by the transfer to nutrient solution, since their initial growth rate was half that seen in 5-day-old plants after 24 h (Fig 16).

Epicotyl growth data from a highly replicated study with 5-day-old plant (Fig 17) were basically the same as shown in Fig 16, except that significant stress-caused growth rate reductions were not observed in the initial 3 h. Interestingly, effects of moderate to severe stress (−0.6 MPa) were significantly more pronounced when plants were grown under 100 %RH. Stress also reduced root growth rates drastically in 3 h (Fig 17) but some recovery was observed after 24 h.

In order to determine whether water status changes are related to growth, selected tissues of dark-grown pea seedlings were sampled and analyzed (Fig 18). Unstressed
Figure 16. Effect of osmotic stress on epicotyl growth of dark-grown pea seedlings of 2 ages. Means (±) are from 10 observations.
Figure 17. Epicotyl and root growth responses of dark-grown 5-day-old pea seedlings to osmotic stress. Means (±) are from 40 observations.
Figure 18. Water status alterations of selected regions of dark-grown 5-dayold pea tissues in response to osmotic stress. Means (+) are from 5 observations.
tissues underwent some variations in water status over the course of the day. Except in the growing region of epicotyls, where stress for 3 h led to equivalent reductions in \( \Psi \) and \( \Psi_n \) and therefore turgor maintenance, osmotic stress had no effect on any water status measure from those seedlings.

**Stress-induced Alterations in Growth Rates and Water Status**

**Values of Dark-grown Cowpea and Squash Seedlings**

In unstressed cowpea plants, growth rates of roots remained constant throughout the duration of the study, but growth rates of hypocotyls at the start of the experiment were about half those found in later measurements (Fig 19). Osmotic stress (-0.4 MPa) for 3 h transiently reduced growth of both hypocotyls and roots but growth recovery was complete after 24 h (Fig 19).

In unstressed cowpea seedlings, \( \Psi \) and \( \Psi_n \) of the growing regions of hypocotyls and roots were 0.1 to 0.3 MPa lower than those of the expanded region of hypocotyls during the initial 3 h of study (Fig 20) but this difference disappeared between 3 to 24 h. Stress (-0.4 MPa) for 3 h reduced the \( \Psi \) of the hypocotyl growing region and since correspondent reduction in \( \Psi_n \) was not observed, a transient drop in turgor occurred (Fig 20).

In squash, stress (-0.4 MPa) caused hypocotyl growth
Figure 19. Growth responses of dark-grown 4-day-old cowpea and squash seedlings to osmotic stress. Means (+) are from 10 observations.
Figure 20. Water status alterations in selected regions of dark-grown 4-day-old cowpea tissues. Means (±) are from 5 observations.
to stop within 3 h and only a slight recovery was observed in 24 h (Fig 19). Values in unstressed and stressed (between 3 to 24 h) squash plants were about half those obtained by Mason and Matsuda (1985) after 20 h stress of 3.5-day-old squash seedlings.

As in cowpeas (Fig 20), $\Psi$ and $\Psi_H$ values of the expanded region of hypocotyls of unstressed squash plants were initially higher than those of the growing region (Fig 21) but both measures decreased progressively in the course of the day to levels similar of those of the growing region. In 3 h, osmotic stress (-0.4 MPa) caused $\Psi$ and $\Psi_H$ to decrease nearly in parallel in both hypocotyl regions (Fig 21). Hence, $\Psi_p$ was not altered.

**Stress-induced Alterations in Growth Rates and Water Status**

**Values of Light-grown Cowpea and Squash Seedlings**

In light-grown 4-day-old cowpea seedlings hypocotyl, epicotyl and root lengths at the time stress was applied were 8.4, 2.2 and 8.8 cm, respectively and leaf blades were not yet unfolded. In unstressed plants, hypocotyl growth rates increased during the course of the day (Fig 22) whereas root growth rates peaked in 3 h and subsequently decreased. Epicotyls showed measurable growth rates after 24 h. Stress (-0.4 MPa) caused significant hypocotyl and root growth reductions in 24 h. Epicotyl growth was not
Figure 21. Stress-caused changes in water status of different regions of dark-grown 4-day-old squash hypocotyls. Means (±) are from 9-25 observations.
Figure 22. Growth responses of light-grown 4-day-old cowpea seedlings to osmotic stress. Means (+) are from 10 observations.
affected.

In cowpea seedlings, neither epicotyls nor leaf blades were sufficiently developed to show defined growing and expanded regions. Consequently, water status measurements were conducted only on the growing and expanded regions of the hypocotyl and on the growing region of roots. \( \Psi \) and \( \Psi_n \) values of unstressed plants were similar for all tissues (Fig 23) and, particularly in the root growing region, tended to decrease during the course of the study. Stress (-0.4 MPa) caused equivalent reductions in \( \Psi \) and \( \Psi_n \) in the growing region of hypocotyls within 3 h, but recovery occurred after 24 h (Fig 23). The expanded region of the hypocotyl was not affected. The growing region of roots also showed equivalent reductions in \( \Psi \) and \( \Psi_n \), but this response was seen after 24 h. In all cases \( \Psi_p \) was maintained.

In light-grown 3-day-old squash plants, growth and water status determinations were performed on leaf blades, which were 4.7 cm in length at the time stress was applied. Growth rates were reduced equally within 3 h in plants exposed to -0.4 or -0.6 MPa (Fig 24), whereas no alteration was seen when stress of -0.2 MPa was applied.

In unstressed squash plants, \( \Psi \) and \( \Psi_n \) values tended to be lower in the growing than in the expanded region (Fig 25), and increased in both tissues during the course of the
Figure 23. Effect of osmotic stress on water status of selected regions of light-grown 4-day-old cowpea tissues. Means (±) are from 5 observations.
Figure 24. Leaf blade growth responses of light-grown 3-day-old squash seedlings to osmotic stress. Means (±) are from 10 observations.
Figure 25. Effects of osmotic stress on water status of different regions of light-grown 4-day-old squash leaf blades. Means (±) are from 5 observations.
experiment. Only the most severe stress level (-0.6 MPa) caused reductions in $\Psi$ and $\Psi_m$ of the growing region in 3 h. By 24 h, however, $\Psi$ and $\Psi_m$ values tended to be lowered in proportion to the level (-0.2, -0.4 or -0.6 MPa) of applied stress (Fig 25). $\Psi_p$ values of growing and expanded tissues were not affected by stress.

**General Aspects of Growth and Water Status Alterations in Response to Stress**

The experiments reported here were designed to broaden the basic understanding about how water stress regulates growth and water status of dicot plants, and to resolve how these different measures might be related. To gain this understanding, dark- or light-grown seedlings of mung bean, black bean, pea, cowpea and squash were grown in hydroponic medium and water stress was simulated by exposing the roots of intact seedlings to PEG of various osmotic concentrations. These studies differed from those of most previous reports in dicots in that a specific attempt was made to measure stress-caused responses in usually 3 h or less, as well as for longer intervals. Additionally, a specific effort was made to measure the water status of the regions involved in organ growth. The responses obtained with the diverse plant species have shown many similarities to those observed with dark-grown mung bean seedlings,
which were most extensively studied.

In dark-grown mung beans, it was possible to study immediate effects of stress on hypocotyl growth and the results provided the first demonstration that osmotic stress will cause an almost instantaneous cessation of elongation by dicot tissues (Fig 1). In addition to showing that growth stops within 40 sec, these studies have demonstrated that when stress of -0.6 MPa is applied, growth will resume after 10 to 45 min, with lag periods increasing with seedling age. The growth responses of mung bean hypocotyls, however, differed from those of barley (Matsuda and Riazi 1981) leaves in that relief of stress does not cause an instantaneous resumption of growth. Such data suggest that stress-caused growth cessation in mung beans is probably not due simply to loss of water by growing cells. Possibly, a restriction in another critical factor required for growth may become limiting in mung bean hypocotyls. Since the latent period for growth resumption to occur increases with age, it may be possible that amounts of that factor will be reduced as seedlings age.

As was found in young barley leaves (Matsuda and Riazi 1981), growth cessation occurred before there was a change in any water status measure of the growing region. Also, confirming results obtained with leaves of barley (Matsuda and Riazi 1981) and corn (Michelena and Boyer 1982, Van
Volkenburgh and Boyer (1985) and dark-grown epicotyls of pea and hypocotyls of squash (Mason and Matsuda 1985) and soybean (Cavalieri and Boyer 1982), reductions in $\Psi$ equalled decreases in $\Psi_H$. Hence, turgor was maintained at a positive and nearly constant value, despite the fact that growth ceased and subsequently resumed. In accord also with observations in other dark-grown dicot seedlings (Mason and Matsuda 1985) and cereal leaves (Matsuda and Riazi 1981, Mason and Matsuda 1985), growth rates of dark-grown mung bean seedlings measured over a 24 h period were reduced as stress intensities were increased (Figs 2 and 3). The overall results with the short-term mung bean studies are in agreement with but do not prove the hypothesis of Rayan and Matsuda (1988) that growth and water status values are likely regulated by a reduction in hydrostatic pressure of the xylem and not by turgor reduction of the cells in the growing region.

As was observed with dark-growth mung beans, studies with light-grown mung beans and light- or dark-grown plants of the other four studied species showed that -0.2 to -0.6 MPa stress will effect growth inhibition regardless of age. Those changes were normally detected within 3 h following application of stress, and were more pronounced in light-than in dark-grown plants, and by 24 h, growth rates were often related to the $\Psi$ of the nutrient solution.
Except in the case of the expanded region of 6-day-old dark-grown black bean hypocotyls, where turgor loss occurred after 2 h stress, and in leaf blades of 7-day-old light-grown mung beans, where no change in water status was evident in plants stressed for 24 h, osmotic stress effected significant and equivalent reductions in tissue $\Psi$ and $\Psi_n$. However, when subjected to the same level of stress, species varied widely in the extent of water status change and the time required before significant alterations were observed. When stressed for 24 h, equivalent reductions in $\Psi$ and $\Psi_n$ occurred in the growing region of light grown cowpea hypocotyls and light-grown 8-day-old mung bean leaf blades; both the growing and expanded region of dark-grown black bean, mung bean and squash hypocotyls, and light-grown black bean and squash leaf blades; and in the expanded region of dark-grown cowpea hypocotyls. Because stress causes growth changes without a concomittant reduction in turgor, it can be reasonably concluded that growth rate in the various dicot species that were studied is not directly controlled by the turgidity of the mesophyll cells involved in elongation.
REFERENCES CITED


