Moisture Stress

Plants are highly integrated and complex organisms with numerous metabolic controls. Water plays a crucial role in the existence and survival of plants as it is involved in the various cellular functions, ranging from photolysis of water in photosynthesis, to hydrophobic bonding of macromolecules. When plants are exposed to drought conditions, or to other water limiting conditions, they experience water stress. Provided the stress is strong and long enough, almost every physiological function may be affected. These effects may be reversed on rewathering, only if the stress is mild. Under moderate to severe stress conditions, the recovery may be partial or zero. Water stress is normally expressed in terms of the chemical potential of water $U_w$ (bars), using the chemical potential of pure water at atmospheric pressure and at some temperature, as the reference. With progressive loss of water, the cell experiences an increasing (positive) water stress, corresponding to a decreasing (negative) water potential. The effects of low water potential of the cell on the growth and the metabolism of plants are summarized below.

Growth and Development - The first measurable effect of water stress is the reduced cell expansion, caused by the decreased turgor of the cell wall (Doley and Leyton, 1968; Boyer, 1970).
In Nitella, a small drop in turgor caused a cessation of growth, which was readily reversible upon return of turgor (Green, 1968). The effect on turgor is normally followed by a decrease in the rate of cell wall synthesis. Cell wall synthesis was found to be substantially repressed in coleoptiles of oat (Cleland, 1967), and in leaves of other species (Plot and Ordin, 1964). Cell division is somewhat more tolerant and continues slowly until a severe water deficit develops (Gardener and Nieman, 1964; Doloy and Leyton, 1968). The cellular responses to water stress were seen to be reflected in a reduced growth of plants (Asana et al., 1958; Vaadia et al., 1961). One of the most rapid responses reported was that of corn leaf elongation, which was observed to resume within seconds after the stress was relieved (Acevedo et al., 1971). In general the leaf and stem growth are strongly influenced by water deficit (Asana et al., 1958; Jarvis and Jarvis, 1963a, b; Boyer, 1968). It was noted that those plant parts, which were growing most actively during the period of low water treatment, suffered the greatest check on growth (Williams and Shapter, 1955; Aspinall et al., 1964). The leaf initiation rate in sunflower (Marc and Palmer, 1976) and tobacco (Clough and Dilworth, 1975) and primordium initiation rate in barley (Aspinall and Hussain, 1970) were particularly susceptible to water stress. The stage of
growth of the plants was reported to influence the response of the plants to moisture stress. From the stage of spikelet initiation to fertilization of the ovules, the plants became very sensitive to water deficit, and if moisture stress was experienced in any of these phases of growth, a marked reduction in the grain yield resulted (Aspinall et al., 1964; Wells and Dubetz, 1966; Chinoy, 1962; Robins and Domingo, 1953).

The plants subjected to moisture stress experienced rapid senescence (Vaadia, 1961) especially when the tissues were already approaching maturity (Williams and Shapter, 1955; Gates, 1957). In fact, there had been numerous reports of protein breakdown accompanying drought stress, dating back to the 1920s and 1930s (Coates, 1928; Petrie and Wood, 1938). Wilson (1968) reported increased protein breakdown and accelerated leaf senescence in maize, as a result of increased water stress. A number of authors have reported enhanced rates of ethylene production by water-stressed plants, e.g., El-Dolaghy and Hall (1974), Guinn (1976), McMichael et al. (1973), etc.

Role of Endogenous Growth Regulators in Water Stress - Growth regulators have often been implicated in the water stress phenomenon. However, it appears that the changes observed in their levels during water stress are indirect and could
be merely the reflections of disturbed metabolism.

Cytokinins - The role of certain growth substances, particularly auxins, gibberellins, and cytokinins in, and the general similarity of many stress effects to, processes associated with senescence, has provoked interest in the role of growth substances in this phenomenon. Attention has been centered chiefly on cytokinins in this regard because of their well known role in retarding senescence and protein degradation (Richmond and Lang, 1957; Osborne, 1965). Water stress was shown to cause a decrease in the amount of cytokinins in the roots, as well as in the amount reaching the leaves (Itai and Vaadia, 1965); the latter, presumably because of the decreased synthesis coupled with decreased translocation from the roots (Itai et al., 1968). This decrease was reversible and upon termination of stress, cytokinin activity in the root exudate increased, initially exceeding the control. It was also observed (Benzioni et al., 1967), that the imposition of the stress on roots results in a decline in the protein synthesis potential of the leaves, as shown by the decreased 14C-leucine incorporation. This could be partially corrected by pretreatment of the discs by kinetin.

In their study, Itai and Vaadia (1965) could detect a loss in the cytokinin activity within 30 minutes of wilting, in the roots as well as in the excised leaves of
tobacco plants. However, work done by others (Misrahi et al., 1971) at the same institute, showed no decrease in the cytokinin content under similar conditions and in the same Nicotiana species. Moreover, the concentration of kinetin required by Benzioni et al. (1967) to alleviate the decrease in protein synthesis potential of the stressed leaves was rather high. Therefore, it was felt that the observed stress-induced reduction in the cytokinin levels could not be assigned a definite role.

Abscisic acid - It was observed that in the plants subjected to a moderate water stress (about 12 bars), there was a several-fold accumulation of the growth inhibitor, abscisic acid, in the leaf tissue (Wright, 1969; Zabadal, 1974). This accumulation has been shown to be due to an novo synthesis (Wilborrow and Nodde, 1970). It has been proposed that during water stress, the stomatal opening may be reduced by a concerted effect of a depressed cytokinin level and a rise in abscisic acid level (Livne and Vaadia, 1972; Boussiba and Richmond, 1976).

Apart from cytokinins and abscisic acid, the levels of some other growth regulators have also been found to change as a result of moisture stress. Thus, an increase in ascorbic acid content of cotyledons and germinating gram seedlings have been reported as a result of moisture stress (Vora et al., 1975), while a depression in the level was
observed in the embryonic axis. Certain growth inhibiting phenols have been detected in leaves following slight dehydration (Pustovoitova, 1972).

Nitrogen Metabolism under Water Stress - In 1957, Gates observed changes in the uptake and distribution of nitrogen and phosphorus in moisture-stressed plants. These changes were attributed to changes in nucleic acid and protein metabolism.

Nucleic acid metabolism - A reduction in the level and the rates of synthesis of DNA could be observed only under conditions of prolonged and severe stress (Shah and Loomis, 1965; Gardener and Nieman, 1964). The level and the rate of synthesis of RNA decreased at a much lower stress (Shah and Loomis, 1965; Maranville and Paulsen, 1972). Under prolonged stress, the degradation of RNA by the increased levels of RNase became important (Dove, 1967).

Protein metabolism - The total protein content of the water-stressed leaf tissue was shown to undergo a decrease by several workers (Mothes, 1928; 1931; 1956; Petrie and Wood, 1938). Chen et al. (1964) observed a cyclic pattern of increase, decrease and restitution in the protein levels, with increasing water stress in citrus seedlings. Barnett and Naylor (1966) found decreases in the protein levels when Bermuda grasses were subjected to water stress. In barley,
Singh et al. (1973a) reported an increase in the protein content in the first 20 hours of stress, no change in the next 20 hours, followed by decrease in the next 10 hours. Maranville and Paulsen (1972), however, reported a decrease in protein content of the leaves of maize, subjected to severe moisture stress, but no change under mild stress.

Several workers demonstrated that the decrease in the leaf protein, with increasing water stress, was due to accelerated hydrolysis (Lothée, 1928, 1956; Petrio and Wood, 1938; Kemble and MacPherson, 1954; Dovo, 1968). However, inspite of the observed proteolysis, the decrease in the protein content appears to be largely due to a decrease in the rate of protein synthesis (Shah and Loomis, 1965). This was partially confirmed when the incorporation of labeled amino acids was found to be reduced in previously stressed tissues (Bonzioni et al., 1967). Kemble (1970) observed that no $^{15}$N was incorporated into total nitrogen and into protein, under conditions of drought. Of more significance was the observation by several workers (Georke et al., 1967; Chen et al., 1968; Nir et al., 1970), that during water stress, polysome formation was slow and that polysome breakdown also occurred. At a water stress as small as 6 bars, Hsiao (1970) observed a marked and rapid drop in the ratio of polysomes to monosomes in maize seedlings; this was reversible upon rewattering. The
rapidity of the response of polyribosomes to stress, i.e., a decrease within 15 minutes of the initiation of stress (Hsiao, 1973), and also to the release of stress, suggested that the control may be at the translational level rather than the transcriptional level. The biochemical basis of this rapid response of polyribosomes is still obscure; indirect data argue against the involvement of RNase or cytokinin, at least during the early part of the stress (Hsiao, 1973). RNase may be involved under conditions of prolonged stress (Dovo, 1967).

**Enzyme level** - It appears that severe stress or desiccation generally lowers enzyme levels whereas moderate to mild stress often raises the levels of enzymes, which are involved in hydrolysis and degradation. Some notable examples of the latter were shown to be amylase in wheat leaves (Spoehr and Lillner, 1959) and catalase and reductase in sunflower and tobacco leaves (Goelovina, 1941). Viera-da-Silva (1970) found increases in the activities of catalase, acid phosphatase, RNase, invertase and C-amylase in water-stressed cotton. The increase in RNase activity of plants, subjected to desiccation, has been attributed to de novo synthesis of the enzyme (Tverus, 1970). The data with regard to peroxidases appears to be conflicting; a decrease was recorded in certain systems, e.g., in wheat (Todd and Yoo, 1964; Stutte and Todd, 1969) and an increase in others, e.g., in
maize (Potinov and Kalyashev, 1960; Weston, 1968; Sheon and Calvert, 1969). The functional significance of these enzymes still remain to be determined. Most of the other enzymes of the cell undergo a decrease in activity under moisture stress, though sensitivities were found to differ from enzyme to enzyme. Huffaker et al. (1970) reported a decrease in PEP carboxylase activity in barley, which was only half the extent of decrease observed in nitrate reductase activity. Nitrite reductase activity also registered a small decline under the same conditions while practically no change was observed in phosphoribulokinase and RuBP carboxylase. Among the enzymes examined, those that appear to be affected most readily are labile enzymes like nitrate reductase and phenylalanine- ammonia-lyase (Bardsik et al., 1971). On the other hand, under identical conditions of stress, a stable enzyme system like NADH oxidase complex was shown by these workers to be affected very slightly.

Nitrate reductase (NR) - The first observation on the activity of nitrate reductase at low leaf water potentials was made by Kattas and Pauli (1965), on a single corn genotype. The seedlings were subjected to 7 days of desiccation by withdrawal of irrigation, at a temperature of 38 C. Nitrate reductase activity was found to decrease significantly during the first 4 days, after which it remained constant at a low
Similarly, Bardzik et al. (1971) found that after a rapid initial decrease at 10 per cent water deficit, the level of NR activity in maize seedlings attained a low but steady value, even at water deficits as high as 50 per cent. However, Maranville and Paulsen (1972) noted that the NR activity in progressively desiccated maize seedlings was completely lost by the eighth day of stress. They showed that the stress-induced changes in NR activity could not be correlated with the decrease in the protein content. The decrease in the NR activity was rapid (38 per cent by day 2 after the initiation of stress) whereas the protein levels remained unaffected by mild stress and decreased only at severe water deficit, i.e., on day 6. It was demonstrated by Corilla et al. (1973) that the decrease in the NR activity of the desiccated maize seedlings was due to a decrease in the rate of enzyme synthesis. The polyribosome level of the stressed tissue declined even before the decrease in NR activity was apparent. On rewatering, the level of polyribosomes increased, followed by a recovery in the enzyme activity. These investigators also showed that the decrease in the NR activity at low water potentials, was neither due to increased rate of decay nor due to a direct inactivation of the enzyme. It was also shown that under such conditions, the nitrate content of the tissue was not limiting for induction of the enzyme.

The influence of moisture stress on NR activity has been shown in other plant species as well. In barley, the
NR activity was found to be inhibited by 58 per cent after 4 days of stress (Huffaker et al., 1970). The enzyme activity was not limited by the nitrate level. Within 24 hours of rewatering, the NR activity recovered with a simultaneous rise in the water potential. More recently, Anikiev and Kurmangasov (1975) demonstrated, that as compared to the main shoot, the lateral shoots of barley showed a differential response to drought and the latter were more sensitive. In wheat, a decrease in the NR activity of plants under drought stress was shown by Plaut (1973). It was found that this response was identical to that under salinity stress (Plaut, 1974). The enzyme activity recovered after irrigation, or by removal of sodium chloride in case of the salinity-stressed seedlings.

The earlier workers (Mattioc and Pauli, 1965; Baranville and Paulsen, 1972) had showed increases in the leaf nitrate levels, corresponding to decreases in the NR activity of the desiccated maize seedlings. However, Korille et al. (1973) showed that the leaf nitrate content of the maize seedlings decreased with the onset of desiccation, thus indicating a lack of correlation between the changes in NR activity and the nitrate content. It was subsequently established by Shancer and Boyer (1976 a) that the NR activity could be correlated more with the nitrate flux from the roots to the shoots, than with the nitrate content of the leaves.
Subsequently, the water stress-induced decrease in NR activity was shown to result from the concomitant decrease in nitrate flux to the leaf, rather than from a direct effect of water stress on protein synthesis (Shanor and Boyor, 1976b).

**Amino acid levels** - The hydrolysis of the existing proteins, as well as the inhibition of protein synthesis, result in profound changes in the levels of free amino acids in the water-stressed tissues (Barnett and Naylor, 1966; Routley, 1966; Saunder et al., 1968). Although the concentrations of some amino acids decline, as in the case of tryptophan (Barnett and Naylor, 1966), there is an overall increase in the level of soluble nitrogenous compounds which include amino acids, amides and soluble proteins. The number and the amount of the amino acids do not reflect uniform hydrolysis of the proteins of the cells. There is an especially marked accumulation of the amino acid proline (Chen et al., 1964; Komble and Raspherson, 1954; Barnett and Naylor, 1966; Routley, 1966; Stewart et al., 1966) and of certain amides e.g. glutamine, asparagine (Chen et al., 1964; Kothes, 1956). Under severe stress, proline may account for up to 30 per cent of the total soluble nitrogen or, as in case of halophytes, 10-20 per cent of the shoot dry weight (Stewart and Lee, 1974). When water stress was relieved, free proline level was observed to
decline rapidly in viable tissues (Singh, et al., 1973a; Stewart, 1972 b). The proline accumulation under moisture deficit has been shown to occur in the attached and detached leaves, under laboratory (Routley, 1966; Singh, et al., 1973a; 1973b; Waldren and Teare, 1974) as well as field (Palfi and Juhasz, 1971; Waldren, et al., 1974) conditions. These workers and those mentioned earlier have demonstrated proline accumulation of various degrees, in crops like sorghum, wheat, corn, barley, forage, grasses, soybeans, kidney beans, jack beans, turnip, radish, sunflower, tobacco and tomato.

It has been suggested (Stewart et al., 1966) that proline accumulation results from the inhibition of protein and polysaccharide synthesis and the consequent channelling of amino acid and carbohydrate metabolism into the synthesis of proline. Physiological and biochemical studies of detached leaves of barley, kidney beans, tobacco and turnip have indicated that during water stress, the synthesis of proline takes place from carbohydrates via α-keto glutarate and glutamate (Bogess et al., 1976a; Stewart et al., 1966; Corris et al., 1969; Singh et al., 1973a). These studies indicate that the regulation of the synthesis of α'-pyrrolino-5-carboxylate (PSC) - an intermediate product in the pathway - as well as the inhibition of proline oxidation, are involved (Bogess et al., 1976a; Stewart, 1972a). On relief of stress the decline in free proline level is probably due to both proline oxidation, and incorporation into protein
Singh et al., 1973a; Stewart, 1972b).

Apart from moisture stress, several other factors have been found to influence proline accumulation, e.g., low temperature (Chu et al., 1974), high light intensity, and high temperature (Kliwer and Lider, 1970) and salinity (Chu et al., 1976a; Falfi and Juhász, 1970). The proline accumulation of wilted leaf discs of *Zea mays* was stimulated by pretreatment with KCl but pretreatment with NaCl proved to be ineffective (Lukherjoe, 1974). Growth regulators have also been implicated in the proline accumulation. Thus, benzylationadino as well as gibberellic acid tended to reduce proline accumulation (Wample and Bewley, 1975; Singh et al., 1973d) while (2-chloroethyl) trimethyl ammonium chloride (CCC) promoted it under moisture deficit (Singh et al., 1973d). The effect of abscisic acid was not consistent. In wheat, it was found to induce proline accumulation (Singh et al., 1973d) while in sunflower it had no effect (Wample and Bewley, 1975).

Successive episodes of stress (drought hardening), applied on certain barley varieties, were found to increase successively, the amount of proline accumulation (Singh et al., 1973c). It was earlier suggested, from a comparison of the proline accumulating potential and the field performance under drought, that drought hardiness of plants was associated with a high potential for proline accumulation under the drought conditions (Singh et al., 1972).
Other Effects of Water Stress on Plant Metabolism - One of the most important effects of water stress is a progressive reduction in the photosynthetic capacity of the plants (El-Sharkawy and Hesketh, 1964; Slatyer, 1967; Boyer, 1970, 1971). It has also been demonstrated that dark respiration is generally suppressed, though not very markedly, by moderate to severe stress (Boyer, 1968, 1970, 1971; Brix, 1962) although substantial respiration may still take place after photosynthesis has ceased. Generally in water-deficient plants, a decrease in the starch level of the leaf has been observed (Iljin, 1957). Chlorophyll accumulation was found to be reduced (Virgin et al., 1965) when water-stressed etiolated seedlings were transferred to light. A decrease in leaf water potential also causes progressive stomatal closure (Hsiao, 1973). In addition to a reduction in photosynthesis, this effect results in a reduced loss of water due to a decrease in the transpiration (Beardsell, 1973).

The uptake of ions by the roots was shown to be inhibited at low water potentials, created by adding PEG or NaCl to the medium (Erlandsson, 1975). Alternatively, the transport of ions from the roots to shoots could be inhibited (Pitman et al., 1974). Severe water stress caused large tension in the xylem (Gillburn 1966) resulting in an increased
resistance to water flow. However, the transport of the assimilates in the phloem remained relatively unaffected by water stress (Wardlaw et al., 1967).

Heat Stress

When plants are exposed to a temperature higher than the range to which they are adapted, they experience heat stress. As a result, a number of physical and physiological parameters of the plants are affected. High temperatures were observed to cause significant reductions in the growth of the plants (Burleigh et al., 1964; Sunderman, 1964; Onwume et al., 1971; Chu et al., 1974). High temperatures could reduce the period of growth and of not dry weight production, thus affecting the crop yield, e.g., Asana and Williams (1965) reported a decrease in the grain yield of cereals which were subjected to high temperatures. Heat stress also resulted in marked reductions in photosynthesis (El-Sharkawy and Haseloff, 1964; Barbal'Chuck and Chernyabskaya, 1975). Chlorophyll accumulation in the leaves of barley and radish was reduced substantially as a result of heat injury (Chu et al., 1974).

An exposure to high temperature also brought about alterations in the membrane properties of the cell. Buttrose and Swift (1975) suggested that heating and desiccation denatured or disorganised the structural proteins in cell membranes of pea root tips. Skogquist (1974)
suggested disorganization of the membrane lipids as a result of heat injury of wheat roots.

**Nitrogen Metabolism**

High temperature stress exerts a significant influence on the overall nitrogen metabolism of plants. Burr (1961) found that increases in air temperature caused decreases in total nitrogen of leaves of sugarcane. Dickson and Lordo (1962) detected a temperature influence on the soluble leaf protein components of an inbred line of corn. Engelbrecht and Moses (1960, 1964) reported that proteolysis occurred in higher plants subjected to heat shock. They demonstrated rapid aging in the leaves of *Nicotiana rustica* exposed to a temperature of 49 to 50°C for 1 to 2 minutes. Treatment with kinetin prevented the aging by maintaining or increasing the protein content.

Corresponding to the proteolysis, the soluble nitrogen was shown to change in response to temperature (Fowden and Steward, 1957). Steward et al. (1959) concluded that night temperature and its interaction with photo-period was important in determining the quantity and composition of soluble nitrogen in the mint plant. Decreasing the night temperature from 18°C to 10°C caused a slight increase in the alcohol soluble nitrogen and some changes in the
concentrations of different free amino acids and amides in strawberry leaves (Boynton et al., 1961). Significant changes in relative concentrations of various free amino acids, and of the total amino acids in the soluble protein fraction, was reported by Younis et al. (1965). These changes indicated changes in the protein composition of maize seedlings with temperature. Some investigators have reported an accumulation of proline in response to high temperature e.g. Kliewer and Lider (1970), Chu et al. (1974).

\textbf{Nitrate reductase (NR)} - The effect of high temperature on NR activity was first noted by Rattas and Pauli (1965). However, in their experiments, the effects of high temperature and low moisture were not separated. The stress was, therefore, considered to be resulting from a combination of both the factors. Younis et al. (1965) studied the effect of temperature and its interaction with light and moisture on the nitrogen metabolism of another corn cultivar. They concluded that high temperature (successive 5°C increase in temperature from 20/15 to 30/25°C) was an important factor affecting nitrogen metabolism and in causing nitrate accumulation in corn seedlings through the effect on NR activity. Exposing barley seedlings to supra-optimal, but non-lethal, air temperatures of 41 to 43°C for 16 to 24 hours, Onwume et al. (1971) observed that NR activity decreased progressively during stress. When stress was removed, the activity was found to increase gradually, equaling the control
in 24 hours, and then increasing further to exceed the control. The loss of NR activity under heat stress was shown to result from an inhibition in the synthesis of the enzyme. In addition, these workers found that the reduced activity was neither due to limitation of nitrate nor due to production of inhibitors during the stress.

It has been shown that heat hardening, i.e., an increase in the resistance of the plants to heat injury, can be induced through prior exposure of the plants to lower temperatures for long periods or to critical temperatures for short periods (Yarwood, 1963; Englebrecht and Rose, 1964). This was found to result in an increase in the thermostability of certain enzymes, e.g., G-6-P dehydrogenases, acid phosphatases (Foldman et al., 1976), fraction I protein, crude carboxydismutases (Sullivan and Kinbacher, 1967) and malate dehydrogenases (Kinbacher et al., 1967). However, Enuweme et al. (1971) demonstrated that heat hardening did not alter the stability of nitrate reductase.

The activity of nitrate reductase at different day temperatures was correlated with leaf growth in soybean seedlings (Magalhães et al., 1976). At 32.5°C and 25°C, NR activity increased concurrently with leaf development and then decreased as leaf maturation progressed. At 40°C, no initial increase was observed and the activity decreased throughout the development of the leaf.