9

Ion Transport and Nitrogen Metabolism

INTRODUCTION

Plants require inorganic salts that are absorbed from solutions in contact with the plant. The management of their uptake and use is an important part of agriculture and is treated in several books (Epstein, 1972; Marschner, 1986; Nye and Tinker, 1977). This chapter provides an overview of salt uptake with emphasis on how plant water status affects it and how the mineral nutrients act as signals for enzyme change during dehydration.

The salts are required in growth because they contain chemical elements that together with the products of photosynthesis become part of the structures, enzymes, and metabolites of the cell. The elements are needed in various amounts and those used in greatest quantity are N, P, K, Ca, Mg, and S and are termed macronutrients while others used in minor quantity are Fe, B, Mn, Cu, Zn, Mo, and Cl and are termed micronutrients. Each element plays a particular role that determines its requirement by the plant. Some provide elemental constituents for macromolecules that function as enzymes or part of cell structures. Others provide cofactors for enzyme activity and thus play a regulatory role. In every case, an inadequate supply reduces growth and can prevent reproduction if the deficiency becomes severe.

For primitive plants in the marine environment, the surrounding water provides a ready supply of most salts, and the dissociated ions are simply absorbed from the surrounding solution by crossing the plasmalemma. The surrounding solution generally is kept uniform by the stirring from water currents. On land,

salts are in the soil solution, and uptake and transport require specialized organs, the roots, to supply the rest of the plant. In general, the soil restricts ion movement compared with the marine environment because the soil structure prevents stirring. Large gradients in concentration can form next to the root. The ions diffuse from place to place and because roots often absorb large quantities of water, there is some bulk flow of soil solution toward roots. Some ions such as phosphate form insoluble complexes with soil minerals and others such as potassium bind to soil particles.

The concentrations of ions are markedly different in the ocean and in soil (Epstein, 1972). Sodium and chloride dominate in the ocean (nearly 500 m*M* for each), but Fig. 9.1 shows that the macronutrients calcium, magnesium, potassium, and sulfate also are present in considerable quantity (10–56 m*M*). In the soil, these nutrients are normally present at concentrations below 10 m*M* (Fig. 9.1). In contrast, nitrate and phosphate are barely detectable in most marine environments but occur in higher concentrations in soil solutions. Thus, plants in marine environments enjoy higher availabilities of most nutrients than land plants do except for phosphate and nitrate where the reverse is true.

The ability of roots to supply nutrients is enhanced by various symbioses, in particular by the mycorrhizal relationships that form between roots and fungi (see Chapter 5) and by nitrogen-fixing bacteria (see later). Mycorrhizal fungi surround roots or penetrate them and extend out into large soil volumes. The root/fungus system is particularly important for solubilizing phosphate from its insoluble complexes with iron, calcium, and organic matter, allowing transport to the plant (Gerdemann, 1968; Gerdemann in Torrey and Clarkson, 1975; Harley and Smith, 1983). Nitrogen fixation is carried out by free-living bacteria and by symbiotic bacteria such as in legumes. The N_2 of the atmosphere serves as the substrate and the bacteria supply reduced N to the plant. Together these symbioses further enhance the supply of phosphorus and nitrogen to land plants.

The supply of salts is more stable in the ocean than in soil solutions. Marine concentrations become variable mostly in coastal waters where they are affected by runoff from the land. Soil concentrations depend on the parent material, degree of weathering, and amount of water and organic matter. This complexity in the soil environment is illustrated by the different soil types occupying the global land area. Each can be classified according to its suitability for growing plants (Dudal, 1976). Table 9.1 shows that 22% of the world land area has soils with mineral nutrient problems. Another 28% is too dry and 24% too shallow (subject to frequent drying). Flooding affects 12% and 3% is too rocky. About 15% of the land area is too cold. Only 10% of the global land area is relatively free of unfavorable characteristics and those soils are our richest farmlands.

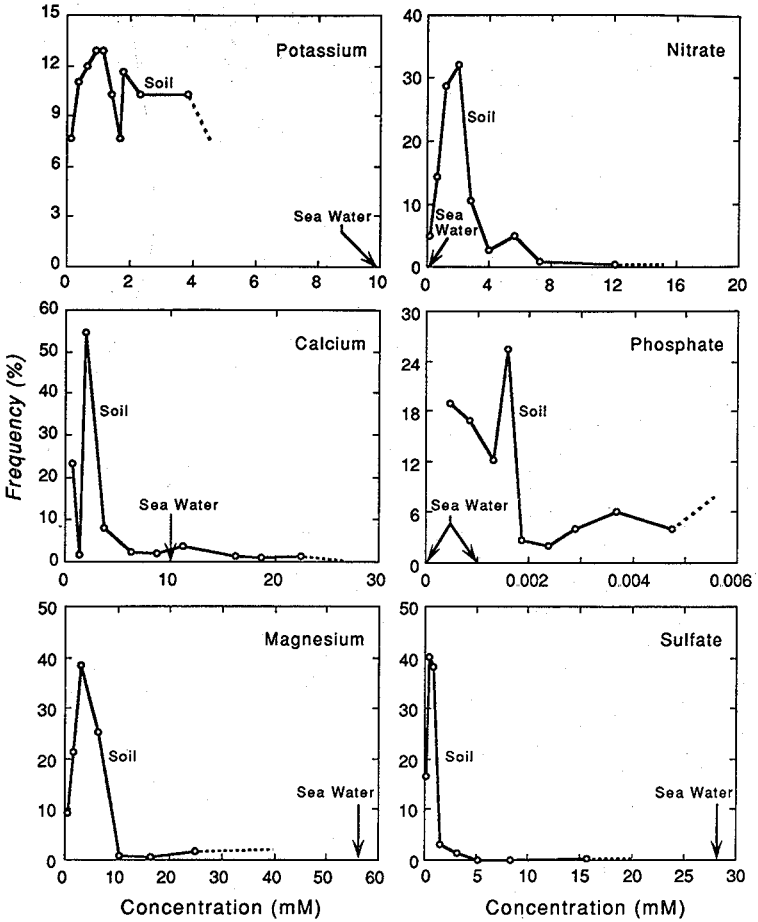

Figure 9.1 Concentration of mineral nutrients in soil solution and seawater. The frequency with which various concentrations occur is shown for each range of concentrations. The number of soils sampled was 149 to 979 depending on the element. The dashed lines indicate that a few soils had concentrations above those shown. Seawater concentrations are stable except for phosphate and nitrate, which vary in the range shown. After Epstein (1972).

ION UPTAKE AND TRANSPORT

Optimum Conditions

Almost any ion can enter plant cells in small quantity, but nutrient ions are accumulated selectively by specific mechanisms, often against an electrical or concentration gradient. Metabolic energy often must be spent for some aspect

Table 9.1 Area of Total World Land Surface Subject to Environmental Limitations of Various Types[a]

Environmental limitation	Area of world soil subject to limitation (%)
Drought	27.9
Shallow soil	24.2
Mineral excess or deficiency	22.5
Flooding	12.2
Miscellaneous	3.1
None	10.1
Total	100.0
Temperature	14.8

Note. Area affected by unfavorable temperatures overlaps with other classifications and is shown separately.

[a]From Dudal (1976).

of their uptake, and the expenditure can be large. For example, Pate *et al.* (1979) reported that roots of plants deprived of nitrate salts for 10 days respired at only 71% of the rate for roots supplied with nitrate, presumably because less energy was used for ion uptake in the starved roots. Transport across the plasmalemma and tonoplast often involves specific energy-driven, molecular carriers. The energy is consumed primarily by ATPases that pump protons across the membrane. The resulting proton gradient is then dissipated when the protons return to the original side of the membrane through carriers coupled to the transport of another ion such as potassium. Because the carrier is specific for the other ion, uptake selects for each ion separately. The cells are thus actively involved in accumulating the ions that provide elemental nutrients, and the rate of uptake depends not only on the concentration of the ion in the extracellular solution, but also on the carriers and energy available for transport.

Plants containing a vascular system dump the ions into the root xylem where they are swept along in the transpiration stream at concentrations that depend on the rate of water entry into the xylem. When water enters rapidly, ion concentrations can become quite low. However, the rate of delivery to the shoot is determined strictly by the rate of delivery from the roots, and thus by the ability of the roots to unload ions into the xylem. Shoot tissues absorb the ions from the nearby transpiration stream and the plant experiences a steady supply of mineral nutrients as long as the roots unload ions steadily.

The selective uptake results in a regulated supply of ions to the cells. The cells contain each element in a particular range of concentrations based on dry weight. Table 9.2 shows that a mature maize plant contains 1.46% of its dry mass as nitrogen, 0.20% as phosphorus, 0.92% as potassium, and so on. As a

Table 9.2 Elemental Composition of a Maize Plant as a Percentage of Dry Matter

0	44.43	P	0.20
C	43.57	Mg	0.18
H	6.24	S	0.17
N	1.46	Cl	0.14
Si	1.17	Al	0.11
K	0.92	Fe	0.08
Ca	0.23	Mn	0.04

[a]From Miller (1938).

first approximation, the requirement for these elements is proportional to the rate that dry mass accumulates, and inorganic ions generally are absorbed most rapidly during the most rapid phases of growth.

The ions are carried to the root surfaces by bulk flow and diffusion (Barber, 1962). Bulk flow occurs mostly because ions are swept along in the water absorbed by roots. Diffusion occurs because absorption during periods of low transpiration lowers the concentration at the root surface and creates a concentration gradient extending from the soil to the root surfaces, and the ions diffuse down the gradient toward the root. Occasionally, ions are delivered by bulk flow faster than they are taken up and they accumulate at the root surface, forming a gradient for back diffusion away from the root.

As mineral nutrient requirements increase with increased growth rates, transpiration increases and more nutrients move toward the roots by bulk flow. For some nutrients, it is possible to account for all nutrient delivery to the roots by this means (S. A. Barber *et al.*, 1962a,b; Renger *et al.*, 1981). The supply of nutrients can be calculated from the concentration in the soil solution multiplied by the flow of the solution to the root. Table 9.3 shows that the calculated supply was adequate to account for the calcium and magnesium requirement

Table 9.3 Supply of Elements to Maize Roots by Bulk Flow Caused by Transpiration

Element	Percent of plant dry matter	Concentration of soil solution needed if transpiration is 500X dry matter (m*M*)	Concentration of soil solution in 145 Maize soils (m*M*)
Ca	0.22	0.11	0.83
Mg	0.18	0.15	1.15
K	2.0	1.02	0.10
P	0.20	0.13	0.002

[a]After S. A. Barber *et al.* (1962a).

but not the potassium or phosphorus requirement in maize. Renger *et al.* (1981) reached a similar conclusion for sugar beet, spring wheat, and spring barley and found that the nitrate requirements often were met as well.

If the inorganic ions are absorbed at a relatively greater rate than bulk flow can provide, as with phosphate and potassium, the concentration in the soil solution will decrease next to the root. In response, ions are released from the soil particles and tend to buffer the concentration. Nevertheless, there is a lowering at the root surface and ions will tend to move into the depletion zone by diffusion in addition to bulk flow. Figure 9.2A shows that the depletion of phosphorus around an onion root decreased the concentration at the root surface to less than half the concentration in the bulk soil solution. The depletion zone extended about 2 mm into the soil. Root hairs can extend 1 to 4 mm from the root and increase the depletion zone to 6 mm or so (Bhat and Nye, 1974). Rhodes and Gerdemann (1975) showed that mycorrhizal fungi extend as much as 10 cm beyond this depletion zone and supply phosphorus to the root from great distances. This appears to be one of the main benefits of mycorrhizae (Gerdemann, 1968; and in Torrey and Clarkson, 1975; MacFall *et al.*, 1991b,c; Sanders and Tinker, 1971).

On the other hand, if the ion is absorbed at a relatively slow rate compared to the bulk flow to the root, the concentration in the soil solution may build up next to the root. Sulfate is an example because its uptake is moderate in plants but the ion is highly mobile in the soil (S. A. Barber *et al.*, 1962b). Figure 9.2B

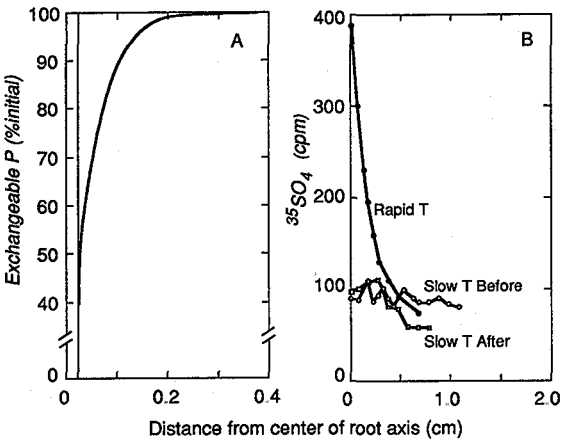

Figure 9.2 (A) Depletion zone for phosphate and (B) accumulation zone for sulfate extending radially from the root. For (B), transpiration (T) was varied, and the accumulation zone was measured before, during, and 16 hr after exposing the plant to rapid T for 18 hr. After Nye and Tinker (1977).

shows an example from Wray (1971) who observed that transpiration caused sulfate to accumulate around onion roots, with the amount depending on how rapidly transpiration occurred. Rapid rates caused an accumulation in a zone extending about 5 mm into the soil. No accumulation was evident when transpiration was slow either before or 16 hr after the rapid transpiration, indicating that the accumulation zone dissipated by back diffusion when transpiration was slow but could quickly reform when transpiration resumed.

Thus, depending on the interaction of the two processes by which soil solutes move—bulk flow and diffusion—solutes can be depleted or accumulated in the soil next to the root. Over the life of the plant, some of the solutes can be supplied to the root sufficiently rapidly by bulk flow associated with transpiration but even for these solutes depletion or accumulation zones may be set up as transpiration and ion uptake vary. A related question is whether transpiration also affects ion transport inside the plant itself. The experiments have been reviewed by Russell and Barber (1960), Viets (1972), and Hsiao (1973) who concluded, essentially, that over long times transpiration had little effect on uptake by roots in low external solution concentrations, but had a significant effect when the external concentrations were high. Ion uptake tended to proceed independently of transpiration, probably because it depended on energy requiring processes quite different from the physical factors driving transpiration.

The action of transpiration on ion transport can be seen by varying the transpiration rate when the plant roots are surrounded by a well-stirred inorganic nutrient solution. When transpiration is rapid, xylem concentrations of the nutrient ions are low because the incoming water dilutes the xylem solution. Root ion uptake is rapid because opposing concentration gradients are small inside the root. When transpiration slows, the concentrations build up to high levels in the xylem because metabolically driven ion uptake proceeds even though water flow is slow. However, as the xylem concentrations build up, root uptake can be inhibited. Shaner and Boyer (1976a) found that NO_3^- uptake was generally independent of transpiration when transpiration was rapid (see also Schulze and Bloom, 1984) and that the concentration in the xylem varied inversely with transpiration, as expected. However, when transpiration became slow, NO_3^- concentrations climbed to such high levels (40 m*M*) in the xylem that root uptake of NO_3^- also became slow. The effect was reversible when transpiration increased sufficiently to dilute the xylem concentration. Thus, NO_3^- uptake changes from independent to dependent on transpiration in the same plant as the rate of transpiration changes.

High soil concentrations of mineral nutrients promote high xylem concentrations and increase the tendency for transpiration to affect ion uptake. Figure 9.3 shows an example in barley (Hooymans, 1969) where high potassium concentrations outside the roots caused higher potassium concentrations inside the roots. Suppressing transpiration caused root potassium concentrations to be-

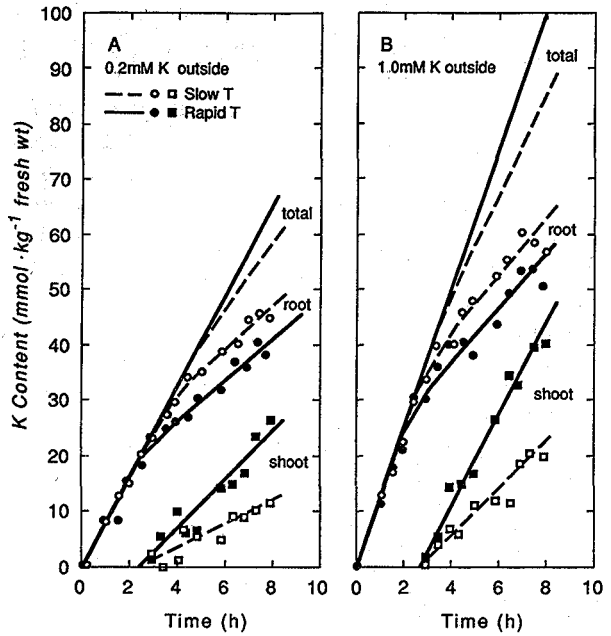

Figure 9.3 Potassium content of roots and shoots of barley plants at various times after exposing the plants to high or low rates of transpiration (T). (A) Roots in nutrient solution with 0.2 m*M* K. (B) Roots in nutrient solution with 10 m*M* K. Note that for the total plant, rapid transpiration suppressed uptake more when the external K concentration was high than when it was low. After Hooymans (1969).

come even higher. For the plant as a whole, the suppressive effect of reduced transpiration became greater when the root concentration became higher.

Effects of Dehydrating Conditions

When soil dehydration occurs, mineral nutrient uptake tends to decrease because transpiration is reduced, decreasing the bulk flow of soil solution to the root, but photosynthesis and plant growth also are curtailed. With less increase in dry matter, the decreased nutrient uptake has only a modest effect on the elemental concentration in the tissues. Several long-term field studies (Greaves and Carter, 1923; Janes, 1948; Jenne *et al.*, 1958) showed that drought had only small effects on the elemental composition of grain, in agreement with this idea. Vegetative tissues show a similar response and a typical result with apple plants (Table 9.4) showed only a moderately higher tissue concentration of N and slightly lower concentration of P, K, Ca, and Mg than in plants grown with adequate water (Mason, 1958).

Table 9.4 Effect of Soil Moisture on Percentage of Elements in Dry Matter of Apple Plants[a]

	Shoot		Root	
Element	Dry	Wet	Dry	Wet
N	0.97	0.53	1.40	1.21
P	0.09	0.10	0.10	0.21
K	0.46	0.47	0.64	0.86
Ca	0.41	0.39	0.50	0.57
Mg	0.06	0.06	0.08	0.11

[a]From Mason (1958).

For this mechanism to hold, mineral nutrient uptake should decrease as soon as the accumulation of dry mass is reduced. Eck and Musick (1979) showed that in sorghum, nutrient uptake was suppressed as soon as dry matter accumulation was inhibited. Thus, the final concentration of nutrient elements in the tissue depended on the whether the decrease in uptake was greater or less than the decrease in dry matter accumulation. O'Toole and Baldia (1982) also found that both dry matter and nutrient uptake were reduced when rice was subjected to a water deficit. Figure 9.4 shows that the decreased nutrient uptake was apparent as soon as dry matter accumulation decreased. Using the data from Fig. 9.4, calculations in Table 9.5 show that the nutrient concentrations (as a percentage of dry matter gained) were slightly less for N, P, and K in the water-deficient plants than in the controls. Thus, over the long term, the water deficit caused only moderate effects on nutrient concentrations in the tissue. The lack of a wide variation indicates that nutrient delivery remains reasonably well coupled to the demand resulting from dry mass accumulation, regardless of the availability of water. Imsande and Touraine (1994) similarly conclude that NO_3^- uptake is coordinated with biomass production.

In respect to nutrient delivery to the root, O'Toole and Baldia (1982) showed that the mechanism did not change during a water deficit. The bulk flow of water to the roots continued to be capable of supplying a large part of the N and K, and diffusion continued to supply P (Table 9.5).

NITROGEN METABOLISM

Nitrogen is the most common element in plant tissues after carbon, hydrogen, and oxygen. Like the other inorganic elements, its uptake is diminished when the supply of water is curtailed, but the tissue *concentration* shows slight changes depending on the extent of growth inhibition (cf. Tables 9.4 and 9.5). Table 9.4 shows that the direction of N concentration change sometimes can be

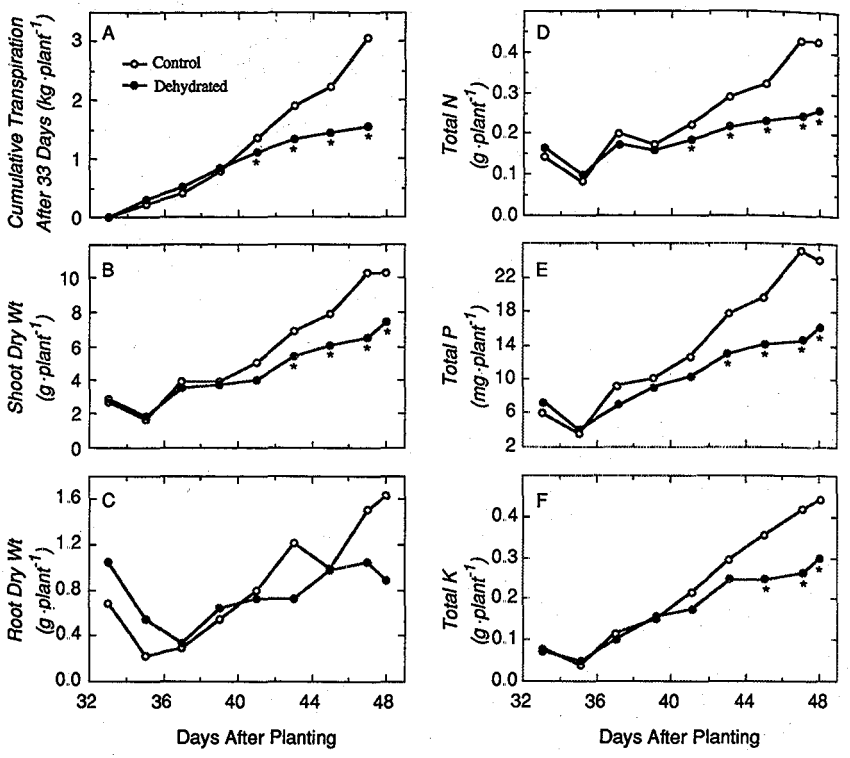

Figure 9.4 (A) Transpiration, (B) shoot dry mass, (C) root dry mass, (D) total N, (E) total P, and (F) total K contents of rice plants from which water was withheld after Day 30. The asterisk shows significant differences at the 5% confidence level. After O'Toole and Baldia (1982). The data in (E) are 10× greater than reported in the original paper because of a decimal error in the original (J. C. O'Toole, personal communication).

counter to that of the other ions during dehydration. Nitrogen acquisition differs from most of the other elements because it undergoes extensive metabolism after uptake by the cells. Nitrogen usually is oxidized in the soil and is taken up as NO_3^-. The NO_3^- must be reduced before it can be incorporated into amino acids and thence to proteins and other cell constituents. The unique changes often observed in tissue concentration of N imply that dehydration affects N metabolism.

Soil NO_3^- is present in the soil solution and, although NH_4^+ sometimes is present in small amounts, it is oxidized rapidly to NO_3^- by soil bacteria. Naturally occurring NO_3^- and NH_4^+ come mostly from the breakdown of preexisting organic matter, a process that also loses a substantial amount of N by volatilization of NH_3 or production of N_2 from NH_4^+ by some soil bacteria.

Table 9.5 Supply of Elements to Rice Roots Calculated from Bulk Flow Caused by Transpiration[a]

Treatment	Gain (% of DM gain)[b]			Transpiration ratio (g H_2O · g DM $gain^{-1}$)[b]	Concentration needed if transpiration provided nutrients (m*M*)[d]			Concentration typical in soil solution (m*M*)[e]		
	N	P[c]	K		N	P[c]	K	N	P	K
Control	2.8	0.18	3.3	256	7.8	0.23	3.3	0.1–4	0.0005–0.002	0.1–4
H_2O deficient	1.9	0.14	2.9	190	7.1	0.24	3.9			

Note. The effect of a water deficit is also shown.

[a]From O'Toole and Baldia (1982).

[b]From Fig. 9.4

[c]Data for phosphate are reported here at 10X the concentration in the original paper because of a misplaced decimal point in the original (J.C. O'Toole, personal communication).

[d]Calculated as:

$$\frac{(\text{Gain in Element})(10^4)}{(\text{Transpiration Ratio})(\text{Atomic Wt})} = \frac{(\%\text{ of DM gain} \cdot 10^{-2})(10^3\text{ g } H_2O \cdot \text{kg}^{-1})(10^3\text{ mmol} \cdot \text{g} - \text{atom}^{-1})}{(\text{g } H_2O \cdot \text{g DM gain}^{-1})(\text{g element} \cdot \text{g} - \text{atom}^{-1})}$$

$$= \text{Concentration Needed (mM)}$$

[e]From Fig. 9.1.

Also, plants volatize small amounts of N (Farquhar *et al.*, 1979). As a consequence, N needs to be continually added to the soil–plant system for plant productivity to remain stable. The input of new N comes from the fixation of N_2 gas from the atmosphere, mostly by microorganisms that can reduce N_2 to NH_3. If the microorganisms are free-living, the NH_3 is released to the soil as organic matter after the microorganisms complete their life cycle and begin to break down. The NH_3 is generally converted quickly to NO_3^- by other microorganisms and is absorbed by plants. If the microorganisms are not free-living but instead grow in symbiosis with the plant, the NH_3 is released directly to the plant without being converted first to NO_3^-. In addition to N_2 fixation by microorganisms, rainfall supplies a small amount of NH_3 because of electrical activity in the atmosphere. In agriculture, N depletion is prevented by adding nitrogen-containing fertilizers, some of which are manufactured by the Haber process that reduces N_2 to NH_3 nonenzymatically, using high temperatures and pressures.

Nitrogen Fixation

Nitrogen is fixed chiefly by bacteria growing symbiotically with other plants. The most common form is the rhizobium–legume symbiosis but others occur such as the actinomycetes found in species of alder and casuarina (Tjepkema *et al.*, 1986), and cyanobacteria in cycads (Lindblad *et al.*, 1987). Less important are some free-living bacteria and a few that form loose associations with plant roots (Bothe *et al.*, 1983; Döbereiner, 1983; Quispel, 1983) or leaves (Döbereiner, 1983). Also, it has been reported that N_2-fixing bacteria can be found in the intercellular spaces of sugarcane (Dong *et al.*, 1994). In some instances, significant N_2 fixation occurs in specialized cells of cyanobacteria (Bothe *et al.*, 1983). This is an intriguing situation because no symbiosis is involved and instead photosynthesis and N_2 fixation occur in the same plant, although in different cells.

In these microorganisms, the fixation is carried out by the enzyme nitrogenase that binds N_2 and uses ATP and reductant to form NH_3 and H_2 without releasing any intermediates:

$$N_2 + 8[H] \rightarrow 2NH_3 + H_2, \tag{9.1}$$

where [H] indicates reduced ferredoxin and the stoichiometry is only approximate. The requirement for ATP is not shown in the equation. The nitrogenase is inhibited by O_2 and thus there must be a discrimination between N_2 and O_2, which are both gases. In legumes, this is accomplished by the formation of layers of host tissue around the nitrogen-fixing bacteroids. The resulting root nodules are conspicuous and contain a modified hemoglobin (leghemoglobin) that binds O_2 making its way into the nodule. The leghemoglobin holds internal O_2 at a

low level (King *et al.*, 1988). As N_2 and O_2 diffuse from the soil pores into the nodule, the O_2 also is consumed to produce ATP and reductant and this further decreases the O_2 reaching nitrogenase (Criswell *et al.*, 1976; Dakora and Atkins, 1989; Denison *et al.*, 1992; Hunt *et al.*, 1987; Layzell *et al.*, 1989; MacFall *et al.*, 1992; Sheehy *et al.*, 1985; Weisz and Sinclair, 1987).

The nitrogenase releases NH_3 which serves as the substrate for the production of glutamate and related amino acids. The carbon skeletons for amino acid formation are provided by the host plant. Thus, the symbiosis results in an energy and carbon supply for the bacteroids but an amino acid supply for the host plant. The nodules contain xylem elements that connect to the xylem of the host root, and the amino acids are transported to the shoot in the transpiration stream.

This process requires a supply of photosynthetic products from the host plant and N_2 gas from the atmosphere as well as enough O_2 so that respiration can occur in the host and bacteroid tissues but not so much that nitrogenase becomes inhibited. The delicate balance between the supply of each substrate is dynamically controlled. Removing the source of photosynthetic products by detopping the plant or girdling the stem rapidly inhibits N_2 fixation (Denison *et al.*, 1992; Hartwig *et al.*, 1990; Huang *et al.*, 1975a,b; Vance *et al.*, 1979; Walsh *et al.*, 1987), even though substantial photosynthates (sugars and starches) are stored in the nodule tissues (Hartwig *et al.*, 1990; Vance *et al.*, 1979; Walsh *et al.*, 1987). Thus, a continued flux of recently formed photosynthate seems necessary for N_2 fixation and there is evidence that it also may play a role in the control of the O_2 concentration inside the nodule (Denison *et al.*, 1991, 1992).

The balance also is affected by the availability of soil water. Decreasing soil water at first increases the availability of N_2 and O_2 because the soil pores drain and become filled with air. The effect becomes apparent when the N_2-fixing activity is assayed with the alternate substrate acetylene, which nitrogenase can reduce to ethylene. The substrate and product are both gases and measuring the ethylene production gives a convenient assay of nitrogenase activity *in situ*. The gases can be used in intact soil–plant systems to mimic the effects of soil water on gas diffusion during fixation. Figure 9.5A shows that nitrogenase activity measured as acetylene reduction initially increased when the soil dehydrated after water was withheld, confirming that the gaseous substrates for N_2 fixation became more available. However, as dehydration became more severe, nitrogenase activity was markedly inhibited. The inhibition was accompanied by a similar inhibition in photosynthesis.

Huang *et al.* (1975b) showed that decreasing photosynthesis by decreasing the CO_2 concentration around the shoot of hydrated plants had the same effect on N_2 fixation as decreasing photosynthesis by dehydration (Fig. 9.5B). This similarity indicates that decreased photosynthesis was limiting or near limiting for N_2 fixation in dehydrated soil. Increasing photosynthesis with high CO_2

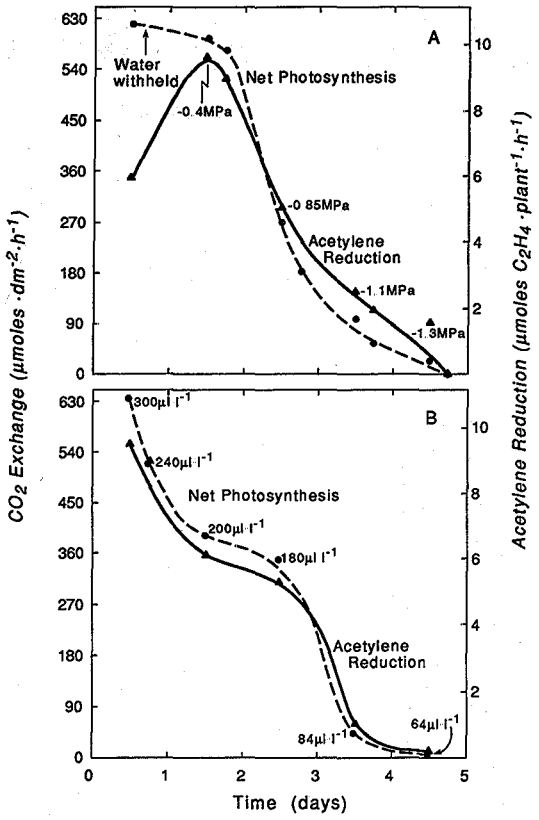

Figure 9.5 Net photosynthesis in shoots and nitrogen fixation (acetylene reduction) in roots of soybean plants at various times after (A) withholding water from the soil or (B) decreasing CO_2 around the shoot without withholding water. The nodule water potentials are shown in (A). The CO_2 concentrations around the shoot are shown in (B). In (B), the low CO_2 was selected to mimic the effects of low water potentials on photosynthesis as in (A). Note that because (B) involves only decreased CO_2 around the shoot, acetylene reduction was inhibited by the lack of products of photosynthesis. Therefore, in (A) the effect on photosynthesis indicates that acetylene reduction must have been similarly inhibited by a lack of photosynthetic products. In (A) and (B), photosynthesis and acetylene reducing activity were measured simultaneously in the same plants *in situ*. After Huang *et al.* (1975a,b).

around the shoot reversed some of the inhibition of N_2 fixation in the dehydrated plants (Huang *et al.*, 1975b). These experiments established that the loss in photosynthetic activity caused the decrease in nitrogen-fixing activity at least in part. Huang *et al.* (1975b) further showed that decreasing the availability of recently formed photosynthate to the nodules inhibited nitrogenase activity perhaps because the production of ATP, reductant, and carbon compounds for the

fixation process would have decreased [Eq. 9.1]. In effect, the lack of photosynthate was a signal from the shoot to the root that caused the decreased N_2 fixation.

Others (Davey and Simpson, 1990; Fellows *et al.*, 1987; Irigoyen *et al.*, 1992b; Pararajasingham and Knievel, 1990) showed that there is a significant amount of sugars and starches in nodules of dehydrating plants and argued against a limitation of N_2 fixation by the limited availability of photosynthate. However, in view of the dependency of N_2 fixation on recently formed carbohydrate (Hartwig *et al.*, 1990; Huang *et al.*, 1975b; Vance *et al.*, 1979; Walsh *et al.*, 1987), the flux of carbohydrate appears more important than the amount that is stored (Hunt and Layzell, 1993). Thus, the nitrogenase appears to depend on a small pool of photosynthetic products that is rapidly diminished when the delivery of new products decreases.

Pankhurst and Sprent (1975, 1976) observed that increasing the O_2 concentration around the nodules also could reverse some of the inhibition of nitrogenase activity caused by dehydration. Figure 9.6 shows that the recovery was complete in nodules that had been slightly dehydrated but was not complete when dehydration was more severe. Nodule respiration showed a similar response to high O_2 (Guerin *et al.*, 1990; Pankhurst and Sprent, 1975). The enhancement by O_2 could be overcome by slicing the nodules, indicating that there was an O_2 barrier in intact nodules that was broken by slicing and that the barrier had become more effective when the nodules were dehydrated. Weisz *et al.* (1985) directly demonstrated the barrier by showing that dehydration decreased the gas permeability of nodules. Irigoyen *et al.* (1992a,c) showed that nodule enzyme activity shifted toward hypoxic metabolism. Thus, in addition

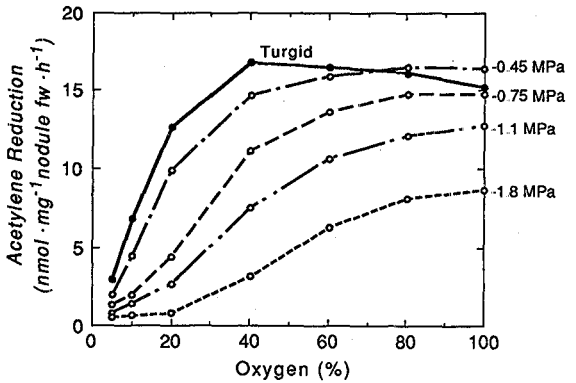

Figure 9.6 Nitrogen fixation (acetylene reduction) at various oxygen partial pressures in detached soybean nodules dehydrated to varying degrees. Dehydration is indicated by nodule water potentials beside each curve. After Pankhurst and Sprent (1975).

to an inhibition by a lack of photosynthate (Huang *et al.*, 1975b), a lack of O_2 also can be limiting in some conditions, presumably because there is an inhibition of conversion of photosynthate to the substrates needed by nitrogenase (Pankhurst and Sprent, 1975, 1976).

These results indicate that water availability has two main effects on N_2 fixation. In one, gas diffusion is increased as water drains from the soil pores and N_2 fixation tends to increase. In the other, photosynthesis and nodule gas diffusion are decreased as dehydration becomes more severe and N_2 fixation decreases. The exact conditions where the shift occurs probably depend on soil conditions, the amount of photosynthate being produced by the plant, and the structure and condition of the nodules. Sanchez-Diaz *et al.* (1990) indicate that mycorrhizal symbiosis also can alter the nodule response.

Nitrate Metabolism

The ability of plants to use NO_3^- depends on the amount of NO_3^- surrounding the roots (Beevers and Hageman, 1969, 1983). When the ion is absent, the uptake capability is negligible (Beevers *et al.*, 1965; Ingle *et al.*, 1966) but, when the ion is supplied, uptake begins rapidly and after about 30 min, the metabolism of NO_3^- increases (Beevers *et al.*, 1965). The NO_3^- is metabolized first by being reduced in the cytosol where the enzyme nitrate reductase catalyzes the reaction. The NO_3^- is reduced to NO_2^- as

$$NO_3^- + 2[H] \rightarrow NO_2^- + H_2O \qquad (9.2)$$

and metabolic energy is consumed to produce the reductant [H] which is $NADH+H^+$ or $NADPH+H^+$, depending on the tissue. The stoichiometry of the reaction has not been fully worked out.

The signal for the induction of reductive activity is the NO_3^- itself that is entering the cell (Beevers and Hageman, 1969, 1983). The activity results from the synthesis of new nitrate reductase in the cytosol and, after more enzyme has been synthesized, the NO_3^- is rapidly reduced so that NO_3^- does not accumulate in the cytosol. The NO_3^- that escapes reduction is transported to the vacuole, further keeping the concentration low in the cytosol.

The NO_2^- produced is kept from accumulating by a second reduction that converts the NO_2^- to NH_3 in the approximate reaction

$$NO_2^- + [H] \rightarrow NH_3, \qquad (9.3)$$

where the reductant is reduced ferredoxin. The enzyme is nitrite reductase which carries out the reductive steps requiring ATP and reductant and, as with nitrogenase, no intermediates are released. The NH_3 is combined with carbon compounds to form amino acids that are utilized in protein synthesis. NO_2^-

is toxic so that the continued presence of nitrite reductase is essential. Nitrite reductase is a constitutive enzyme thought to be in various kinds of plastids (Beevers and Hageman, 1983).

Thus, the uptake of NO_3^- sets in motion a series of reactions that are essential for the growth of the cell. Growth depends on NO_3^- metabolism to supply new protein and nucleic acids but the same metabolism is necessary to synthesize nitrate reductase for the process. As a consequence, there is always a small NO_3^- reduction activity present in plant cells. Depriving the plant of NO_3^- disrupts the flow of nitrogen to protein synthesis and limits the synthesis of nitrate reductase but does not eliminate reductase activity. Because of this, recovery from a nitrogen deficiency occurs slowly at first and more rapidly after new nitrate reductase is synthesized.

The availability of NO_3^- to the cells is thus an important signal, and Shaner and Boyer (1976a) found that the NO_3^- flux to the cytosol was the critical feature of the signal. They used several methods to change the NO_3^- flux to maize shoots and found that the nitrate reductase activity corresponded with the flux even though the NO_3^- content of the shoots (mostly in the vacuoles) did not change. Whenever the activity increased, it was because the synthesis of new enzyme protein increased (Morilla *et al.*, 1973; Shaner and Boyer, 1976b). Therefore, the flux controlled the expression of the nitrate reductase genes (Crawford and Campbell, 1990).

The fact that the enzyme responded to changing fluxes in the transpiration stream rather than NO_3^- in the vacuole indicated that a small cytoplasmic pool of NO_3^- must be involved and, because of its small size, the concentration could change rapidly. In effect, changes in flux were read from changes in concentration of this small pool.

With this in mind, Shaner and Boyer (1976b) decreased the water supply to maize roots and found that the NO_3^- flux to the shoot was decreased together with a lowered activity of nitrate reductase (Fig. 9.7). The NO_3^- flux decreased because of a combination of lower transpiration and a lower NO_3^- concentration in the transpiration stream due to the decreased root uptake (Fig. 9.7A, insets). If the plants were supplied with additional NO_3^- before the dehydration, the NO_3^- flux was elevated and nitrate reductase activity was similarly elevated during the dehydration (Fig. 9.7). This indicated that the NO_3^- flux regulated the activity of nitrate reductase during dehydration.

If water was resupplied, the tissue rehydrated and nitrate reductase activity recovered (Fig. 9.8), but the recovery depended on a high NO_3^- flux to the shoot (Fig. 9.8) and the synthesis of a new enzyme (Shaner and Boyer, 1976b). This indicates that the loss in enzyme activity during dehydration was not caused by a direct inhibition of the enzyme but rather by a decreased synthesis of the enzyme. The synthetic activity was determined simply by the flux of a

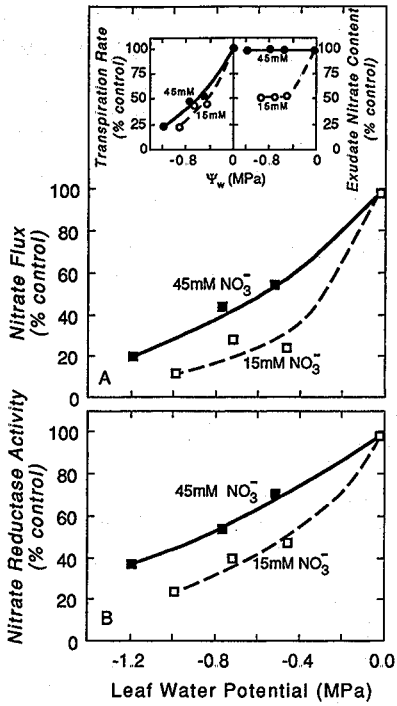

Figure 9.7 (A) Nitrate flux to the shoot and (B) leaf nitrate reductase activity in the shoot at various leaf water potentials in intact maize seedlings. (Insets) Transpiration and nitrate concentration in the xylem solution of the intact seedlings. The nitrate flux in (A) was calculated as transpiration rate × nitrate concentration shown in the insets. Immediately before dehydration, half the plants were provided with supplemental nitrate (45 m*M*) around the roots. After Shaner and Boyer (1976b).

regulatory molecule. The NO_3^- flux thus represented a dehydration signal from the roots that altered the activity of the shoot enzyme.

The basis for this response is the short half-life of the enzyme in the cell. Morilla *et al.* (1973) found that nitrate reductase has a half-life of only 4 hr in maize and that it would need to be continually synthesized to maintain steady activity. When the NO_3^- flux was diminished during dehydration, the synthesis signal was diminished and, in the absence of synthesis, the activity rapidly died away. Bardzik *et al.* (1971) suggested that similar effects would be expected if a general decrease in protein synthesis occurred in plant tissue; enzymes having short half-lives would show losses in activity before longer-lived enzymes. Therefore, decreases in enzyme activity could be caused by regulators specific for each enzyme (such as NO_3^-) or by regulators of protein synthesis itself.

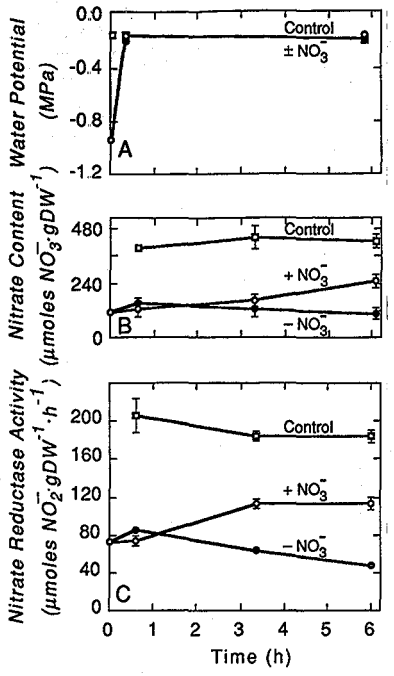

Figure 9.8 (A) Leaf water potential, (B) leaf nitrate content, and (C) leaf nitrate reductase activity in maize shoots recovering from dehydration after cutting under solution with or without nitrate. There was a substantial nitrate flux to the shoot in the $+NO_3^-$ treatment but little if any in the $-NO_3^-$ treatment. Note that the recovery of leaf nitrate reductase activity depended on a NO_3^- flux during rehydration. The recovery could be blocked by inhibitors of enzyme synthesis, indicating that recovery required new enzyme molecules to be synthesized. After Shaner and Boyer (1976b).

Protein Synthesis

Decreased protein synthesis is a central feature of plant dehydration. Synthesis often slows during mild dehydration (Hsiao, 1970; Mason and Matsuda, 1985; Mason *et al.*, 1988a; Morilla *et al.*, 1973; Scott *et al.*, 1979) and can cease entirely in severely desiccated tissues (Bewley, 1979). In part, the decrease can be explained by the decreased NO_3^- flux to the plant cells but synthesis decreases even when the NO_3^- supply does not appear to be involved. Usually, the decreases in synthesis are detected as losses in the polyribosomal content of the tissue because most other methods of measuring protein synthesis require aqueous media that rehydrate the tissue. Because polyribosomes are complexes of messenger RNA and ribosomal RNA actually synthesizing the protein, their disappearance is evidence for a general slowdown in protein synthesis.

Figure 9.9 shows that even 1 hr of dehydration causes substantial losses of

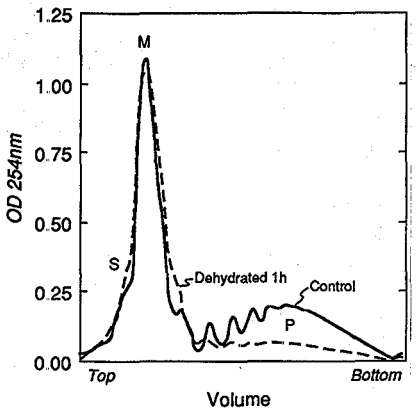

Figure 9.9 Polyribosomes (P), monoribosomes (M), and ribosome subunits (S) isolated from young maize leaves dehydrated for 1 hr to a water potential of −1.27 MPa. The ribosomes were separated from each other on a density gradient so that the largest ones (P) are on the right and the smallest ones (S) are on the left. The ribosomes are detected by their absorbance of ultraviolet radiation (OD 254 nm). Note the loss in P after the dehydration. After Morilla *et al.* (1973).

polyribosomes in maize (Morilla *et al.*, 1973). Growing cells typically have large numbers of polyribosomes and undergo large losses during dehydration whereas mature tissues contain fewer polyribosomes and lose fewer (Bewley and Larsen, 1982; Morilla *et al.*, 1973). The losses in growing tissues take place soon after growth is inhibited by the water deficit (Mason and Matsuda, 1985; Mason *et al.*, 1988a). Figure 9.10 shows that the losses in polyribosomes were apparent about 4 hr after stem growth was inhibited by transplanting soybean seedlings to vermiculite of low water content. The general loss in protein synthesis did not cause the growth inhibition because growth already had slowed, which was also noted by Scott *et al.* (1979) in wheat leaves. After a time, growth resumed in soybean and the polyribosomal content recovered. The loss also did not appear to be caused by a decrease in NO_3^- flux because amino acids were supplied by reserves in these seedlings, which were dependent on stores in the cotyledons, and NO_3^- reduction was not necessary. Importantly, the polyribosomes in the roots were not affected (Creelman *et al.*, 1990). The roots continued to grow after the transplanting and thus there was a relationship between the factors causing growth inhibition and those causing less protein synthesis. The polyribosome loss was not a whole plant response but rather a tissue-specific response.

The possibility that ABA could be a signal regulating growth began to attract attention when it was observed that treating plants with ABA inhibited shoot growth but not root growth much as occurred during dehydration (Davies *et al.*, 1986). Saab *et al.* (1990) found that a maize mutant having less ABA

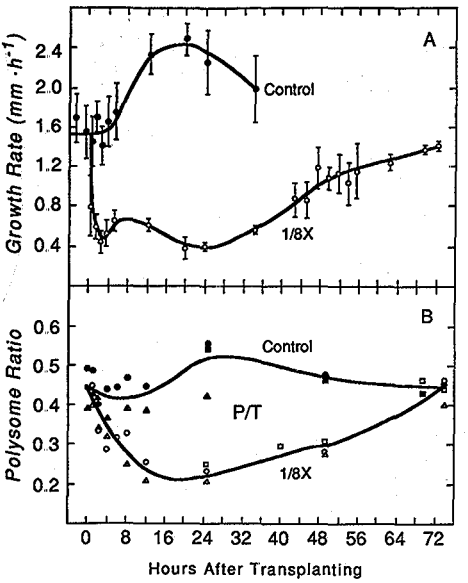

Figure 9.10 (A) Growth and (B) polyribosomal content in the elongating region of stems of intact soybean plants after transplanting to vermiculite of low water content. The dehydrated vermiculite had a water content one-eighth that of the control vermiculite. The response in control vermiculite is shown by dark points and in dehydrated vermiculite by open points. The polyribosome (P) content is shown relative to the total ribosomes (T) in the tissue. After Mason *et al.* (1988a).

production during dehydration also showed less growth inhibition of the stem and less growth enhancement of the roots. They concluded that elevated ABA might be necessary to inhibit stem growth but stimulate root growth during dehydration. Bray (1988) had shown that treating tomato leaves with ABA or dehydration affected protein synthesis in a like manner, supporting the notion that growth changes might be caused by ABA. However, Creelman *et al.* (1990) specifically studied protein synthesis in the growing regions of roots and stems and found that the patterns were different for the two treatments. Moreover, the inhibition of stem growth was followed by a loss in polyribosomes during dehydration but not after exposure to high ABA. They concluded that high ABA levels could simulate the growth responses but not the molecular responses that occur during dehydration, and thus high ABA appeared not to cause the growth responses.

Bewley and Larsen (1982) and Dasgupta and Bewley (1984) further found that the loss in polyribosomes was not due to the loss in synthesis of a particular protein, although some synthetic differences were found. Dhindsa and Bewley (1976) were unable to attribute the polyribosome loss to increases in ribonucle-

ase, and mRNA was conserved (Dhindsa and Bewley, 1978). This indicated that the losses in polyribosomes were not caused by a breakdown of mRNA. There is evidence that the polyribosomes translate some different mRNAs during dehydration (Creelman *et al.*, 1990; Oliver, 1991) and the changes in nitrate reductase activities support this concept. Therefore, two phenomena appear to be occurring. First, synthesis for certain proteins is more affected than for others and, second, the overall rate of protein synthesis tends to decrease. However, while there is some understanding of the molecular regulation of individual protein synthesis such as nitrate reductase, the molecular control of the overall rate remains obscure.

DEHYDRATION AND ROOT/SHOOT SIGNALS

The altered nitrogen metabolism and protein synthesis occurring during dehydration reflect many enzymatic changes that take place under these conditions, raising certain questions. What are the molecular signals that cause the changes? How do plants "sense" that the soil is dehydrating?

The enzymes of nitrogen metabolism can be used to gain molecular insight to these questions. It is apparent that water deficiency inhibits nitrogen acquisition at about the time growth is inhibited. Variations occur depending on the species and environmental conditions, but overall the effects are the same (Eck and Musick, 1979; Janes, 1948; Jenne *et al.*, 1958; Greaves and Carter, 1923; Mason, 1958; O'Toole and Baldia, 1982). As a consequence, the nitrogen concentration of the plant tissues is not changed by large amounts which indicates that the decreased nitrogen acquisition did not cause the decreased growth but somehow was coordinated with it.

The work described in the previous sections of this chapter indicates that the nitrogen acquisition enzymes nitrogenase and nitrate reductase are regulated by root/shoot signals that vary with changes in the root water supply and the growth of the plant. Both signals originate in another part of the plant. For nitrogenase, shoot photosynthesis is inhibited and less photosynthate travels to the root. For nitrate reductase, root NO_3^- uptake is inhibited and less NO_3^- travels to the shoot. Nitrogenase responds to the availability of photosynthate and nitrate reductase to the availability of NO_3^- and both signals are fundamental for the overall growth of the plant, which is inhibited simultaneously.

Therefore, the regulation is specific for each enzyme and represents the way in which the enzyme "senses" that the water supply to the roots is reduced. A key feature is that a pool of regulatory molecules exists that is small in the target cells. For nitrogenase, there is a small pool of useable, recently formed photosynthate (Huang *et al.*, 1975a,b). For the reductase, there is a small pool of cytoplasmic NO_3^- (Shaner and Boyer, 1976a,b). As a consequence, changes in supply are quickly detected and the molecules in the pool function as regulatory "sensors."

Another key feature is that the activities of the enzymes consume the regulator. As the pool size for the regulator increases, the enzyme activity increases, using the regulator at a faster rate. Thus, there is a feedback that stabilizes the pool size. For example, as soon as the photosynthate or NO_3^- supply increases, nitrogenase and nitrate reductase activity increase and consume the extra flux into the pool, thus stabilizing the regulator signal at the new level. Without consumption of the regulator, this stabilization would not occur and the pool would grow indefinitely, preventing its use as a sensor.

These simple elements of regulation are found in most control systems and are shown schematically in Fig. 9.11A. Note that the supply flux for the regulator acts as a signal because the pool size is small and the concentration in the pool quickly responds to changes in supply (Fig. 9.11B). When the flux to the

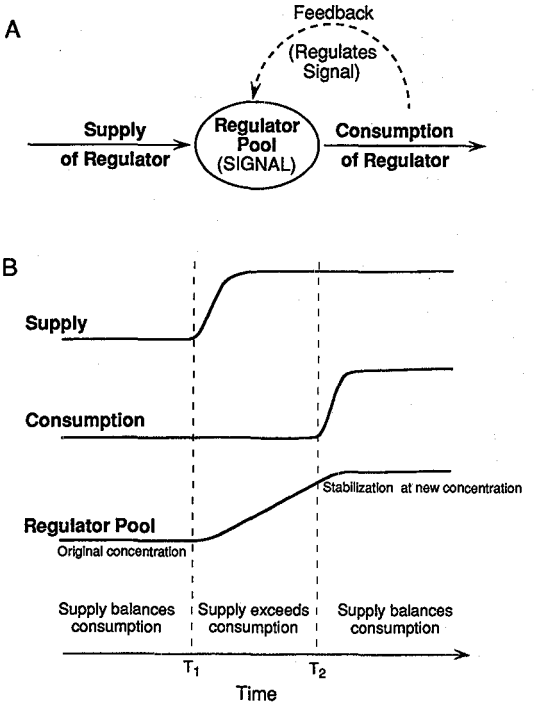

Figure 9.11 (A) Basic elements of a regulating system applicable to the control of enzyme activity in dehydrated plants. (B) Operation of the regulating system. There is a small pool of an enzyme regulator that acts as a signal. The pool is fed by a supply of the regulatory molecule (supply) and is depleted by reactions associated with the enzyme (consumption). Before time T_1, the supply is balanced by consumption. At T_1, the supply increases and the concentration of the regulator pool increases. This causes an increase in the enzyme activity at T_2, which feeds back to stabilize the regulator concentration at the new level. Because the pool size is small, it rapidly reflects changes in supply and thus responds to the flux of the regulator.

pool increases, the signal increases because the supply exceeds use and the pool concentration increases. In response, enzyme use of the pool increases until use is brought back into balance with the supply flux. This stabilizes the concentration of the pool at a new, higher level and thus maintains the enzyme activity at a new higher level.

It seems that all regulatory systems contain at least these simple control elements. For example, ABA shows certain features related to this concept. Most tissues increase in ABA content when they dehydrate because ABA is synthesized in the cells or is transported to them from other plant parts (increased supply). High ABA causes the stomata to close (Beardsell and Cohen, 1975; Kriedemann *et al.*, 1972; Mittelheuser and Van Steveninck, 1969) and can be inhibitory to shoot growth but stimulatory to root growth (Creelman *et al.*, 1990; Saab *et al.*, 1990). It has been proposed that the roots detect soil dehydration (Bates and Hall, 1981; Davies *et al.*, 1990; Saab and Sharp, 1989; Turner *et al.*, 1985) and that an increase in root ABA might be sent to the shoot in the transpiration stream and act as a signal for the shoot to close its stomata and slow its growth (Davies and Zhang, 1991; Gowing *et al.*, 1990; Zhang and Davies, 1989a,b, 1990) but Munns and King (1988) found little evidence for ABA in this role.

The issue is complicated because ABA is produced both in the root and in the shoot (Creelman *et al.*, 1990; Walton, 1980; Zeevaart and Creelman, 1988). It is more difficult to establish a signaling role than for other molecules that are produced or absorbed in one tissue and transported to another. Also, according to the root/shoot signaling described earlier, there needs to be consumption of the molecule in order for it to act as a signal, and the consumption of ABA will need to be better understood. This will require the signaling pool to be located. ABA in the xylem is not likely to be the pool because its concentration changes in passive response to changes in transpiration, and Davies and Zhang (1991) and Trejo *et al.* (1993b) point out that more attention will need to be paid to fluxes instead of concentrations of ABA in the xylem.

Root/shoot signals are important in plants and have large effects on biochemical responses to environmental conditions. Because many biochemical responses can be demonstrated in the absence of roots or shoots, root/shoot communication is not the only way that environmental signals are conveyed to metabolism. However, their presence indicates that many signals will not be understood unless enzyme studies are extended beyond the test tube to the whole plant.

DEHYDRATION AND ENZYME ACTIVITY

Direct Enzyme Effects

For many years, the causes have been sought for the metabolic changes occurring in plants exposed to dehydration. It has been proposed that low water

availability may directly cause enzyme activity to change because of changes in the free energy of water within the cells (Kramer, 1969; Slatyer, 1967), changes in spatial relationships of membrane systems, volume changes, and concentrating of enzymes resulting from losses of water, or decreases in the water of hydration surrounding macromolecules (Hsiao, 1973; Walter, 1931). These are direct effects because they involve the water molecules acting directly on the enzyme or cell structure, and some of them are undoubtedly important in some systems. Figure 9.12 shows that dehydrating an isolated enzyme, in this case urease, decreases enzyme activity. The enzyme reaction produces ammonia and CO_2, both of which escape into the gas phase where they are easily detected without disturbing the enzyme mixture. Activity is completely lost when the enzyme is dehydrated at a relative humidity below 60%. Rupley *et al.* (1983) point out that enzyme activities depend on the motion of portions of the peptide molecule and that dehydration of an isolated enzyme acts mostly by restricting this motion. The activity begins to decrease when most of the water has been removed around the peptide and only a monolayer of surface water remains. As further water is removed, the internal motion of the peptide becomes limited

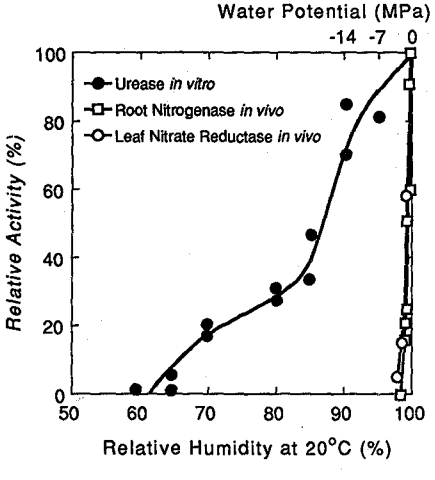

Figure 9.12 Activity of urease in equilibrium with various relative humidities, nitrate reductase exposed to various leaf water potentials, and nitrogenase exposed to various nodule water potentials. Water potentials equivalent to humidities are shown at the top of the graph (calculated according to Chapter 2). The urease catalyzes the reaction converting urea and water to NH_3 and CO_2. The enzyme was isolated, frozen, dehydrated, mixed with dehydrated substrate, and exposed to the humidity shown (*in vitro* dehydration). The rate of reaction was determined as the enzyme mix equilibrated at each humidity. The nitrate reductase was dehydrated in the intact maize leaf (*in vivo* dehydration), then isolated, and assayed in aqueous medium. Nitrogenase was assayed *in vivo* in roots of intact soybean plants. Note the large difference in the enzyme response to *in vitro* and *in vivo* dehydration. After Skujins and McLaren (1967), Morilla *et al.* (1973), and Huang *et al.* (1975a).

and the activity decreases. Eventually, activity disappears when only enough water remains to bind to a few exposed polar groups of the peptide, and the internal motion of the peptide becomes minimal.

As is readily apparent, dehydration directly affects enzyme activity only when too little water remains to cover the peptide surface with a single layer of molecules. This is much less water than is necessary for an effect on many enzymes. As pointed out earlier, nitrate reductase activity is completely lost at a water potential of about −2.5 MPa equivalent to a relative humidity of about 98% (Fig. 9.12). Similarly, nitrogenase activity disappears at a nodule water potential around −2.1 MPa (Fig. 9.12). About half of the cell water remains under these conditions, which is far more than monolayer coverage. The difference seems to be that these enzymes are functioning inside cells and depend on certain regulators to continue their function. The regulators affect the enzyme before dehydration has reduced the water content to only a monolayer.

Regulator Hypothesis of Enzyme Control

The response of nitrate reductase and nitrogenase is controlled by specific molecules different from water. For nitrate reductase the molecule is NO_3^-, and for nitrogenase it is the photosynthetic product or O_2. Other enzyme systems often follow a similar pattern. As described in Chapter 10, enzymes of photosynthesis in spinach or sunflower lose activity during dehydration, and for thylakoid ATP synthetase (coupling factor) there appear to be inhibitory interactions with Mg^{2+} and perhaps other solutes that are concentrated by water loss in the cells. This mechanism is different from that for nitrogenase or nitrate reductase and illustrates that each enzyme has its own response to changed concentrations of regulators in the cell.

To account for these effects, we suggest that enzyme control is based primarily on molecules other than water that act as regulators during dehydration. The effect is shown in Fig. 9.13B as an additional complexity of the molecular environment in the cell (*in vivo*) compared to that of an enzyme without regulators, demonstrated in the isolated enzyme urease (*in vitro*) where only water, substrates, and products are present with the enzyme. For tissues that do not withstand complete dehydration, all the enzymes studied so far show the more sensitive response of *in vivo* dehydration like that of nitrate reductase and nitrogenase (Fig. 9.12) and thus molecular regulators in the cell probably control most of these responses to dehydration. Because the effects are specific for each enzyme system, a complete picture is still being assembled to show how cell metabolism is affected. However, even with a limited picture, it seems clear that the regulator supply may be the key for these tissues and that the supply can be affected by dehydration.

For tissues that can be dehydrated to the air-dry state where water contents

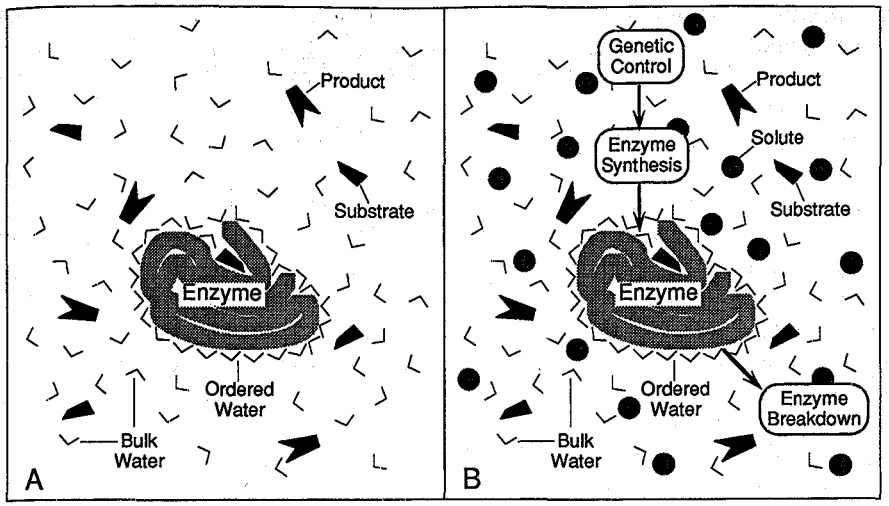

Figure 9.13 Schematic diagram of the difference between dehydration effects on isolated enzymes (A) and the same enzyme inside a cell (B). In (A), dehydration can act only on the water surrounding the enzyme and on the enzyme, substrate, or product as for urease in Fig. 9.12. In (B), dehydration has the additional possibility of altering genetic control, enzyme synthesis, enzyme breakdown, and the solute environment, as for nitrate reductase and nitrogenase in Fig. 9.12. These additional factors act as regulators of the enzyme response at dehydration levels that are less extreme than those needed for the changes in (A).

become very low, the response is more like that of urease (Fig. 9.12). As discussed in Chapter 10, photosynthesis in the marine alga *Fucus vesiculosus* does not decrease until dehydration is more severe than in sunflower and is completely lost only when water contents are less than 20% of the hydrated state (Fig. 10.2). Many seeds withstand similar dehydration and their metabolic activity becomes negligible only when water contents are extremely low (Vertucci, 1989; Vertucci and Leopold, 1987b). Desiccation tolerant *Selaginella* preserves membrane intactness after exposure to the air-dry state (Fig. 3.3). Therefore, it seems that some enzymes and cell structures can withstand very severe dehydration *in vivo* where they likely encounter water binding approaching monolayer thickness or less (Bruni and Leopold, 1991; Vertucci and Leopold, 1987a). However, regulatory molecules probably play a role in these cases as well. Chapter 12 describes some of the possibilities for protection of enzymes and membranes involving a role for sugars and specialized proteins as protective agents and it points out that gene regulation may be involved. Taken together, it seems that the regulatory control of enzymes and membranes may account for most of the behavior of metabolism during an episode of dehydration. Dehydration severe enough to remove all but monolayers of water probably in-

cludes these regulatory effects in addition to the direct effects of water on the internal motions of the peptides.

From these regulatory systems, the plant gains the possibility of modifying its response to dehydration. Thus, regulator levels can be varied before dehydration becomes severe, leading to acclimation. The ability of plants to acclimate to dehydration is a well-known phenomenon (Ackerson and Hebert, 1981; Ashton, 1956; Brown *et al.*, 1976; Matthews and Boyer, 1984; McCree, 1974; Shaner and Boyer, 1976b; Todd and Webster, 1965), and moderate episodes of dehydration appear to call into play systems that change some feature of the cell environment. This change at the molecular level may involve a diversity of regulators and suggests that acclimation might affect certain enzymes but not others and thus direct the metabolic response to dehydration.

SUMMARY

Salts of various inorganic ions are essential for plant growth and must be accumulated from the surrounding solution. They generally dissociate to ions that are freely mobile in solution, and in aquatic and marine environments the solutions generally are stirred and the nutrient ions reach the plant readily. In soil, the stirring does not occur and ions move to the root by a combination of bulk flow and diffusion. Bulk flow occurs as ions are carried along in water traveling to the root surface of transpiring plants. Diffusion occurs as gradients in ion concentrations form when the ions accumulate or are depleted next to the root surface according to their rate of uptake compared to water. Inside the plant, the rate of ion uptake is usually independent of the rate of water uptake, but very slow water entry can decrease ion entry apparently because the ion concentration in the root xylem becomes too high. Dehydration of the soil also decreases the rate of ion uptake but the rate more or less remains in balance with the decrease in dry mass occurring at the same time, hence elemental concentrations do not change much in the tissue with change in water supply.

Nitrogen is a special case because it undergoes extensive metabolism after being acquired by the cells. Nitrogen uptake by N_2 fixation involves the diffusion of N_2 and O_2 through the soil to nitrogen-fixing bacterial cells that are free-living or associated with host plants in symbiotic relationships such as with legumes. The N_2 is reduced to NH_3 by nitrogenase using energy from O_2-supported metabolism. Water-filled soil pores can restrict the diffusion of N_2 and O_2, and fixation often improves in the early phases of soil dehydration, but with more severe dehydration, fixation decreases. In legumes, the decrease results from a decreased flux of photosynthate to the nodules as photosynthesis decreases in the shoot. The decreased supply of photosynthate represents a molecular signal from the shoots to the roots that affects nitrogen fixation. There

also is decreased oxygen diffusion into the nodules, which shrink and develop a strong barrier to O_2 entry, and their ability to use existing photosynthate becomes restricted.

In nonlegumes, nitrogen is acquired mostly by uptake of NO_3^- which is reduced first to NO_2^- and then to NH_3. The first reduction is catalyzed by nitrate reductase, which is synthesized in the presence of NO_3^-, and the second reduction is catalyzed by nitrite reductase, which is a constitutive enzyme in plastids. Nitrate reduction decreases during dehydration mostly because NO_3^- is transported to the sites of nitrate reductase synthesis more slowly in the transpiration stream because of the decreased uptake of NO_3^- by the roots. The decreased flux of NO_3^- decreases the synthesis of the enzyme, and the natural degradation of the enzyme in the cell depletes the cell of reductase activity. Thus, the decreased NO_3^- flux is a signal from the roots to the shoots that controls this aspect of shoot metabolism.

Protein synthesis also is generally inhibited by dehydration. The inhibition is not the same for each protein. The inhibition is not caused by general losses in messenger RNA or increases in the plant growth regulator abscisic acid. Decreased enzyme activities in the cell appear to result in part from this inhibition of protein synthesis followed by a decline in activity determined by the half-life of the enzyme in the cell.

The regulation of nitrogenase and nitrate reductase activities during dehydration indicates that the activities respond to regulator pools that often are supplied by other parts of the plant (or even the soil). The supply of regulatory molecules (photosynthate for nitrogenase, NO_3^- for nitrate reductase) represents a signal for the level of dehydration that changes the enzyme activity. The changes thus depend on root/shoot signals of a specific molecular nature for each enzyme system. The control of these enzymes contains the basic components of any feedback control system and indicates that dehydration effects on biochemistry will be understood only in the whole plant context.

A hypothesis is suggested that accounts for the effects of dehydration on enzymes and involves molecules other than water that act as regulators of enzyme action. The regulators change in concentration either because of changes in cell water content or altered transport or biosynthesis of the regulatory molecule. Enzymes subjected to dehydration outside of cells lack these regulatory systems but are directly affected by dehydration nonetheless. However, the dehydration must be considerably more severe than in most cells, which indicates that regulatory processes probably take precedence over direct dehydration effects inside the cells. The concept that regulatory molecules control the biochemical response to dehydration suggests that plant acclimation might be explained by alterations in the cell regulator environment that would predispose the plant for a particular biochemical response when dehydration occurs.

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