How do Plants Absorb Nutrients from the Soil?

Study of Nutrient Uptake

G Sivakumar Swamy

The study of nutrient uptake by plant roots has been a fascinating subject both from the academic viewpoint and also its application in crop productivity. This article provides a very brief account of our current understanding of this phenomenon which requires an interdisciplinary approach. Plant cell simulates an electrochemical battery cell, and also represents a complex electronic circuit.

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Introduction

Animals, including man, require food in the form of carbohydrates, proteins, vitamins, etc., which in turn are provided either directly or indirectly by plants. Then, how do plants obtain their food? Plants have the unique ability to synthesise their own food utilising solar energy and the inorganic elements available in their surroundings. They obtain their carbon, hydrogen, and oxygen from water and from the atmospheric CO_2 and O_2. The soil is the source of other inorganic nutrient elements which are normally available as ions such as NO_3^-, $H_2PO_4^-$, SO_4^{--}, K^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, etc. Like all other living organisms, plants have the ability to maintain an internal environment with a composition different from that of their surroundings. The internal environment (chemical contents) of the plant body remains more or less constant whereas the outside environment is highly variable. It is a fact that the concentration of the nutrient elements and other molecules are far greater inside the plant body compared to the outside environment. Still the plant roots are able to absorb nutrients against a high concentration gradient which would have resulted otherwise due to the natural physical forces operating in the system. This property of holding a solution of higher concentration inside the cell against a dilute solution

The widely accepted model of membrane structure is the 'fluid mosaic' model. This model includes the 'fluid' lipid bilayer interspersed with both intrinsic and extrinsic proteins, some of which are structural proteins and others are involved in various membrane functions (*Figure 1*). The interior of the membrane consists of the hydrocarbon chains of fatty acid moieties of the lipids arranged in the form of a bilayer, and as a result it is strongly non-polar. The nonpolar membrane interior acts as a strong barrier for the passage of ions and polar molecules including water. The membrane surface includes the polar groups of the phospholipids and glycolipids, and it is also associated with the polar domains of the membrane proteins. The non-polar domains of the proteins establish hydrophobic interactions with the fatty acid moieties of the lipid bilayer.

outside is the unique characteristic feature of the cell membrane that surrounds the cytoplasm of the cell. It is evident that the plant cells have to overcome the physical forces such as diffusion in order to maintain a higher solute concentration and also to absorb nutrient elements which demands a massive investment of energy by the plant cells.

The Cell Membrane is the Site of Nutrient Transport

An appreciation of the structure of the cell membrane is essential for understanding the mechanism of nutrient uptake by plant roots (see *Box 1*).

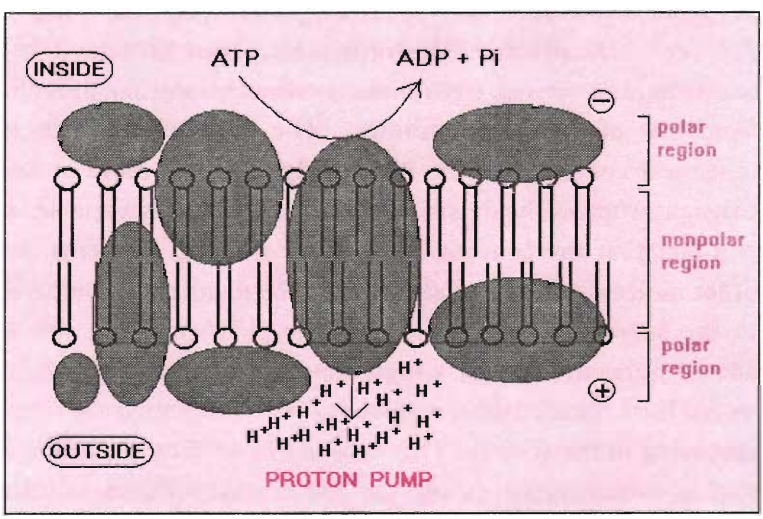

Figure 1. Cross sectional view of the cell membrane: The fluid mosaic structure. Lipid bilayer and proteins form the central non-polar and the peripheral polar regions. H^+-ATPase (proton pump) pumping protons is also shown in the figure. The + and - signs inside the small circles represent the positive and negative transmembrane potentials outside and inside the cell, respectively.

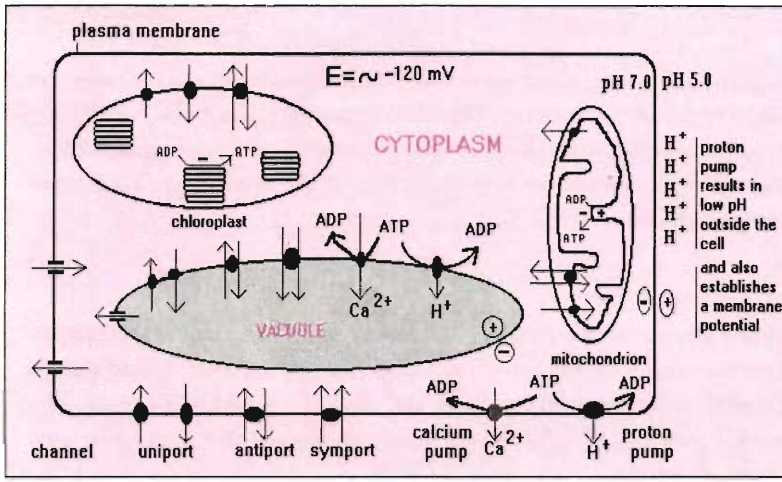

Figure 2. Diagram of a typical plant cell (cell wall not shown) showing the transport of ions and molecules across the membranes. ATP synthesis is driven by the proton motive force generated by electron transport in mitochondrion and chloroplast. On the other hand, hydrolysis of ATP mediated by H^+-ATPase generates proton motive forces across plasma and vacuolar membranes resulting in the generation of transmembrane potential (shown as + and - signs within small circles). Ca^{2+} pumping by Ca^{2+}-ATPase is also shown. Transport processes by uniport, symport, and antiport (see text for details) are also represented. (Note: Pi released/utilised not shown).

This structural design of the membrane offers scope for the transport of nutrient elements only through integral proteins of the membranes. These transport proteins are very similar to enzymes in their specificity in recognising the molecules and ions for transport. The driving force for the transport process is provided by the membrane-bound ATP hydrolysing enzyme, H^+-ATPase (*Figures 1, 2*).

H^+-ATPase Transports Protons and Generates Transmembrane Potential

Whenever an ion moves into or out of a cell unbalanced by a counter ion of opposite charge, it creates a voltage difference across the membrane called electrogenic pump. The functioning of the electrogenic pump is an energy consuming process and it provides the driving force for the ion transport across membranes by generating a transmembrane potential. In animals, the $(Na^+ + K^+)$-ATPase serves as the electrogenic pump while in plants H^+-ATPase serves this purpose. This plant enzyme, being located in the plasma membrane, generates a pH gradient across the membrane and also establishes a transmembrane potential by the vectorial pumping of protons from the cytoplasm to the exterior (*Figures 1, 2*). Under ideal conditions (when no other ions are interfering), a transmembrane potential of about –120 mV is generated inside the cell as the result of a pH difference of about

Box 2

A relationship between voltage difference across the membranes and the distribution of a given ion under equilibrium conditions is described by the Nernst equation. The Nernst equation states that at equilibrium the differences in concentration of an ion between two compartments is balanced by the voltage difference between the compartments. A simple calculation of the Nernst potential E can be made as a function of H^+ concentration in two compartments as follows:

$$E = (RT/zF) \ln (C^0/C^i),$$

where R is the gas constant, T is the absolute temperature, z is the charge of the ion, and F is the Faraday constant. C^0 and C^i represent the ion concentrations outside and inside the cell respectively (considered here as two compartments). Since H^+ is the monovalent cation, the value of z would be 1 ($z = 1$). The numerical values of the constants R, F, and T at 30°C (303 K) can be substituted, and after converting from natural logarithm to $\log_{10}$ (x2.303), we obtain:

$$E = 60 \log (C^0/C^i).$$

Suppose the H^+ concentration across the membrane is 10^{-5} M (pH 5.0) and 10^{-7} M (pH 7.0) as shown in *Figure 2*, then, $E = 60 \log 100$, and therefore, the membrane potential inside the cell is –120 mV.

2.0 units across the membrane (see *Box 2*). However, under natural conditions, membrane potentials of more than –120 mV are commonly observed in plant cells due to interaction with other ions in the outside medium as well as in the cytoplasm.

The membrane proteins that are involved in the transport process may be classified as: (i) pumps, (ii) carriers and (iii) channels (*Figure 2*). The proton-pump ATPase (H^+-ATPase) acts as a primary transporter by pumping protons out of the cell. This process creates a pH and electrical potential difference across the membrane. It is estimated that the H^+-ATPase alone utilises 25–50% of cellular ATP which indicates the amount of energy invested by the plants to absorb nutrients, and to maintain a higher solute concentration inside the cell. The plasma membrane H^+-ATPase is composed of a single polypeptide of a molecular mass of about 100 kDa. It transports one proton per molecule of ATP hydrolysed, and has a pH optimum of about 6.5 for catalysis. The plasma membrane H^+-ATPase protein stretches across the thickness of the membrane (transmembrane protein) and has ten segments of the polypeptide extending across the membrane

(membrane-spanning regions). H^+-ATPase is also present in the vacuolar and endoplasmic reticulum membranes. However, the amino acid sequences of those enzymes are different from that of plasma membrane-bound H^+-ATPase. As far as the reaction mechanism is concerned, plasma membrane-bound enzyme forms a phospho-enzyme intermediate during ATP hydrolysis whereas such an intermediate is not formed in enzymes located in other membranes. Further, there are also tissue-specific and developmental stage-specific H^+-ATPases in plants. It has been shown that there are ten different genes in *Arabidopsis thaliana* expressing just the plasma membrane H^+-ATPases, producing as many enzymes. In addition to the H^+-ATPase, a Ca^{2+} translocating ATPase has also been identified in the plant cell membranes. The Ca^{2+}-ATPase is involved in pumping out Ca^{2+} from the cytoplasm into the cell compartments or to the apoplast (outside the cell). This pump is involved in the control of cytoplasmic Ca^{2+} level which in turn regulates the process of signal transduction involving the calcium binding protein, calmodulin.

Carrier Proteins and Ionic Channels Serve as Conduit for Nutrient Transport

The transport of nutrient ions across the membrane can be explained by invoking the chemiosmotic principle which was originally put forth by the Nobel Laureate, Peter Mitchell. When protons are extruded from the cell electrogenically, both a transmembrane potential and a pH gradient are created at the expense of cellular energy (released by ATP hydrolysis). This electrochemical H^+ gradient which is also termed as proton motive force represents stored free energy in the form of H^+ gradient. The lipid bilayer is impermeable to H^+, and transport proteins (carriers) alone would allow H^+ to diffuse back into the cell if it moves with another ion or solute. The movement of the positively charged ions such as H^+ into the cell is a downhill process from the point of view of energetics as the cell interior has a negative potential compared to the positive potential of the exterior. In addition, there is also a concentration gradient of protons across the membrane favouring inward movement of

protons. If this downhill process of H^+ transport is linked to the cotransport (symport) of an oppositely charged ion into the cell, the cell can acquire a negatively charged ion against its concentration gradient (even though that particular anion is in excess amount inside the cell compared to the external soil solution). Since the free energy change (ΔG) of H^+ transfer from the exterior into the cell interior is strongly negative this process can also drive the transport of an anion or a neutral molecule against their concentration gradient.

An example of symport mechanism of transport can be cited in the uptake of NO_3^- by the plant cells. The membrane potential of the intact cell can be determined by introducing a microelectrode into the cell as shown in *Figure 3a*. As represented in *Figure 3b* there occurs an initial depolarisation of the membrane if NO_3^- is added to the bathing medium of the cell. These results would imply that NO_3^- is transported into the cell as H^+/NO_3^- symport mediated by an NO_3^- carrier protein. The symport of these two ions into the cell would decrease the H^+ concentration outside the cell, and this is expressed in the reduction of transmembrane potential (depolarisation) as shown in the figure. The movement of H^+ into the cell along with NO_3^- would result in the net transport of NO_3^- into the cell (*Figure 3b*). The depolarisation of the membrane would stimulate H^+-ATPase and this phenomenon results in the repolarisation (increase in the transmembrane potential) followed by the earlier transient depolarisation. Since the cell interior exhibits negative potential (which occurs at the expense of cellular energy) the uptake of

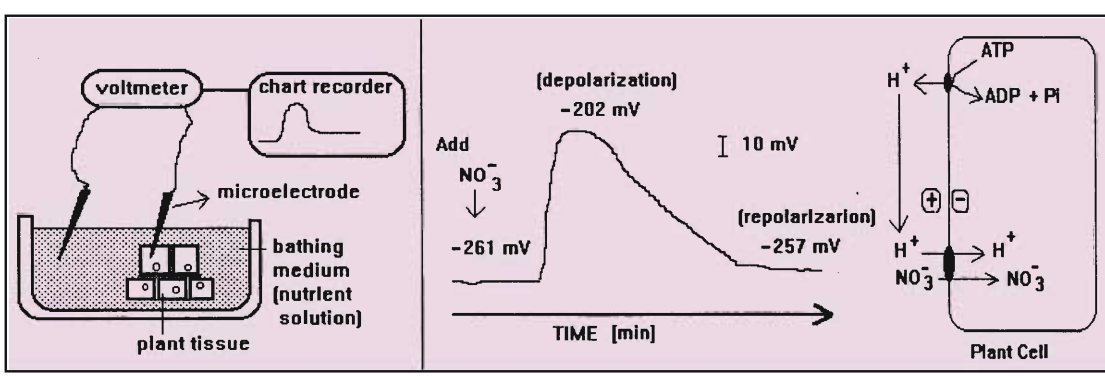

Figure 3. (a): Diagrammatic representation of the experimental set-up for measuring membrane potential. A glass microelectrode is introduced into the cell bathed in the nutrient medium. Change in the potential by the addition of a nutrient into medium can be continuously monitored by the voltmeter attached to a recorder. (b): Diagram showing the recording of the membrane potential in the experimental set-up shown in (a). Addition of NO_3^- into the nutrient medium bathing the plant tissue results in transient depolarisation followed by repolarisation. The diagram of the plant cell on the right side explains the symport mechanism of NO_3^- transport across the plasma membrane.

positively charged nutrient ions (cations) into the cell can take place by an uniport mechanism without the cotransport of protons. Similarly, anions can be extruded through specific carriers from the cell interior to the exterior since the latter has a positive potential. Two different anions can be exchanged across the membrane by an antiport mechanism resulting in the net uptake of one of the anions at the expense of the efflux of the other.

In addition to the carrier proteins, there are channels for ion transport which are also highly specific in recognising and transporting the designated ions across the membrane (*Figure 2*). The channels are the openings in the membrane, and are surrounded by proteins which would specifically allow a particular ion to pass through. The channels would invariably carry out uniport movement of ions driven by the electrochemical gradient. A number of channels have been identified in plant cells for the transport of ions such as K^+, Ca^{2+}, Cl^-, etc., and also for the transport of water. The water transporting channels are known as aquaporins. These channels possess the unique characteristic feature of opening and closing at definite time intervals, and this phenomenon is governed by the thermal motion. In addition, the opening of channels is also governed by the transmembrane potential (voltage-gated channels), and also influenced by regulatory molecules (ligand-gated channels). There are also 'stretch-activated' channels in plants which are regulated by the cell turgor. From the functional point of view, both uniport carriers and channels are similar except that the rate of transport through the channels is several fold higher compared to that of carriers.

Transfer of Genes Encoding Transport Proteins has an Enormous Economic Potential

So far, the transporter (carrier) genes for NO_3^-, H_2PO_4 SO_4^{--}, and K^+ transport have been cloned from the plants. This appears to have paved the way for transferring the genes encoding transporter proteins into crop plants. It is important to point out that high yielding crop plants require higher levels of fertilisers.

Suggested Reading

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[2] H Logan, M Basset, A-A Very and H Sentenac. Plasma membrane transport systems in higher plants: From black boxes to molecular physiology. *Physiol. Plant.* 100. 1–15, 1997.

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Box 3. Genes Encoding Transport Proteins have been Cloned.

A few genes coding for transport proteins have been cloned and made to express *in vivo*. Different strategies have been adopted to clone these genes, and the cloning of NO_3^- transporter can be considered here as an example for study. The commonly used herbicide, chlorate causes yellowing of leaves culminating in defoliation and death of the weeds and other herbs. A mutant (CHL1) of *Arabidopsis thaliana* was obtained which lacked the sensitivity to the chlorate herbicide. This mutant was unable to take up chlorate into the cell, and therefore, the plants became resistant to the herbicide. Incidentally, chlorate (ClO_3^-) is a structural analog of NO_3^-, and therefore, is transported into the cell mediated by the NO_3^- carrier. The CHL1 mutant is capable of growing in the presence of chlorate because it is defective in chlorate uptake, and consequently, NO_3^- uptake is also inhibited.

However, the CHL1 mutants could be grown in the presence of nitrogen in some form other than NO_3^- (such as ammonium salt). An insertional mutant of chlorate uptake (invariably defective in NO_3^- transport) was also obtained by using T-DNA of the bacterium, *Agrobacterium tumefaciens*. This bacterium consists of a plasmid known as Ti plasmid which has a segment in it referred to as T-DNA. When the bacterium infects the plant the T-DNA segment of Ti plasmid is transferred to the host chromosome. The T-DNA integrates into the plant DNA and expresses itself along with plant genes. The insertion of T-DNA into the plant chromosome is random, and therefore, there is a chance that it inserts itself into the NO_3^- transporter gene and causes an insertional mutation. When a large number of transgenic plants with T-DNA insertions were screened in the presence of chlorate only a few plants were found to be green and healthy. These plants were later found to be insertional mutants of chlorate (also NO_3^-) transport. A genetic cross between the insertional mutant and the original CHL1 mutant indicated that both the genes cosegregated which indicates that both mutations occurred in the same locus. The DNA from the insertional mutant was isolated and the CHL1 gene was identified by hybridising with labelled T-DNA. This had enabled cloning the flanking regions of the T-DNA which was in fact the NO_3^- carrier gene. This gene was cloned and transcribed *in vitro* to obtain the mRNA. When the mRNA of this gene was microinjected into the oocytes of the toad *Xenopus laevis*, the NO_3^- carrier protein was synthesised in the toad oocytes and exhibited all the characteristics of NO_3^- transporter in the *Xenopus* oocyte including the ability to transport NO_3^- into the cell. Similarly, the gene encoding K^+ transporter was isolated by using a different strategy from wheat roots and made to express in the yeast mutant which lacked the ability to take up K^+.

This may have resulted because efficiency in nutrient uptake was not addressed while breeding the crop plants for higher yield. On the other hand, there are a large number of plants which grow luxuriantly in wild in the soils which are poor in nutrient content. These plants may manage to grow well in such soils possibly because they have efficient nutrient uptake systems preserved in them by natural selection. Therefore, there is a bright prospect for cloning the transporter genes from those plants and transferring them to crop plants so that crop plants would become more efficient in nutrient uptake thereby bringing down their fertiliser requirement.

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