Bio-inspiration from Plants' Roots

Final Report

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Plant-inspired robots for planetary exploration (SeedBot)

Introduction

Plants are dynamic and highly sensitive organisms, actively and competitively foraging for limited resources both above and below ground, and they are also organisms which accurately compute their circumstances, use sophisticated cost–benefit analysis, and take defined actions to mitigate and control diverse environmental insults. The specialization of tissues and cells in plants needs for a well-organized sensing system, which allows plants to explore efficiently the environment and to react rapidly to potential dangerous circumstances. In order to exploit distributed environmental resources, plants develop a network of growing and branching root apices, which efficiently explore and mine mineral and water.

Planetary and asteroids' soil exploration represents a very important and challenging space application. This activity can make it possible to acquire fundamental information on the history of the celestial bodies and, in rare cases (e.g., Mars), to search traces of extraterrestrial life, holing relatively in deepness.

Plant-inspired robots or *plantoids* distributed on the planetary surface and able to explore soils could be a very demanding and appealing solution to a variety of different problems in planetary exploration.

This document aims at contributing to the discussion on the space mission concepts of planetary (e.g. Martian) subsurface analysis carrying out a feasibility study on a novel plant-inspired approach. The study covers: a definition of realistic scenario where the plantoid will operate, a review of plants that may enable new strategies for planetary exploration, an assessment of technologies envisaged for the plantoid robot, and a review on the anchorage systems.

Objectives

This document aims at contributing to the discussion on the space mission concepts of celestial bodies (Planets, Asteroids) subsurface analysis carrying out a feasibility study on a novel plant inspired approach. A deep analysis of plant beings from a "biomechatronic" viewpoint can supply innovative and advanced solutions for designing and developing high performance bio-inspired machines (at the macro and micro scales) for space applications.

The study aims at verify the feasibility of a plant robot (SeedBot) with particular attention on the root and radical apex. The apex, like explained in the "review and characterization of plant roots", is the core of the plants and his characteristics are very interesting to perform a soil exploration.

The present proposal is structured into 5 work packages, which aim to explain how the feasibility study has been carried out and its goals: **WP1** defines the scenario in which the plantoid should work, and provides design guidelines and system specifications to the workpackages implementation. **WP2** aims at presenting plant's characteristics in terms of root sensors, actuators, intelligence, control and behaviour, in order to provide concepts that should lead to the design of new engineering strategies for space exploration. **WP3, WP4** and **WP5** describe the goals of the feasibility study from a technological viewpoint. In particular, **WP3** reports the concept and preliminary design of electro-osmotic actuators, and gives an assessment of technologies envisaged for the plantoid robot, with special attention to actuation solutions, suitable materials and assessment of power consumption of a system inspired to plant behaviour; moreover, it introduces a review on anchorage systems; **WP4** focus on the main technological show stoppers, and **WP5** on the plantoid biomechatronic design.

WP1. Scenario

Plant-inspired space probes could hopefully represent a new generation of planetary robotic explorers. A plantoid, or eventually many plantoids, disseminated like seeds in large lands, extending their roots in the ground, can autonomously analyse the composition of soil and detect the presence of a variety of chemical-physical parameters. This study aims at contributing to the discussion on the space mission concepts of planetary subsurface analysis with a novel biomimetic approach. The main target of the study is a novel approach in digging systems for sub-soil planetary exploration, stressing the concept of minimal power consumption and trying to conceive devices whose penetration in the soil is possibly far less traumatic than that accomplished by the current drilling system (this last feature is also particularly important thinking to dig in search of life traces, which can be considerably damaged, if not destroyed, by the harsh friction and consequently heat generated by the drilling tip).

The investigated scenarios will not take into consideration all the issues and constraints involved in designing and planning the whole space mission (i.e., launch requirements, space navigation, etc.).

On the other hand it can be said that the first stages of the proposed scenarios could be qualitatively similar to those adopted by present planetary probes. The main difference could rely in skipping the final landing phase, i.e. the airbag-based landing, and make the plantoid withstand a free-fall by few meters in order to partially penetrate into the soil (as a plant “seed”), also assuring a first anchorage. However, it is known that a free-fall impact of the whole space probe on the planetary surface is a very risky procedure to accomplish, first of all because the exact landing site is unknown, and the probe could crash on stones, being consequently damaged or destroyed. A soft land can be also evaluated, but this requires that roots start to dig into the soil from the outside, eventually requiring a counterbalance action by the probe in order to avoid a lift of the probe itself, especially in low-gravity celestial body (as Mars and Moon can be considered).

Three primary space mission targets have been envisaged so far, in partial accordance with main current ESA programs and ongoing projects:

- Mars
- Moon
- Asteroids (e.g., Near-Earth asteroids (NEAs) or even asteroids in the belt between Mars and Jupiter).


a) b) c)

Fig. 1. Possible targets for the plantoid: Mars, Moon and asteroids (Eros pictured by NEAR Shoemaker); courtesy of NASA.

Harsh environments are expected in an increasing scale going from Mars, to the Moon and finally to the asteroids.

Mars exploration can be considered as the most mild application for such a robot, thanks to its less strict constraints (if compared to the Moon or asteroids). On Mars the temperature averages between -87°C and -5°C (http://solarsystem.jpl.nasa.gov/planets/profile.cfm?Object=Mars), and a mean surface level atmospheric pressure of 0.6 kPa can be found (less than 0.01 terrestrial atmosphere).

On the Moon the atmospheric pressure is negligible, and temperature never exceeds about −173 °C in the south polar craters, for instance, which are so far among the most interesting spots where to perform soil exploration, e.g. the Shackleton crater. Shackleton crater is one of the potential site candidate for the first manned moon base, according to NASA. These crater floors are hopefully supposed to host deposits of hydrogen in water form (following Clementine and the Lunar Prospector probes investigation, which provided key data, even if not necessarily conclusive evidence, supporting claims that water ice deposits exist at the lunar poles (Campbell and Campbell, 2006; Feldman, 1998).

On asteroids finally, there are large temperature variations and there is absence of any atmosphere. The temperature of the asteroid varies with the distance from the Sun. However, the surface temperature can vary considerably due the asteroid rotation, because the sides are alternately exposed to solar radiation and then shadowed: on EROS, for example, one of the largest NEAs, the daytime temperature is around 100 °C and during night about −150 °C (rotation period is around 5.27 hours), while, on Vesta, one the larger asteroid in the belt, typical day-time and night-time temperatures are -60°C and -130° respectively. In general for dust particles within the asteroid belt, which can always be considered exposed to the Sun, typical temperatures range from -73°C at 2.2 A.U. down to -108°C at 3.2 A.U., (Low, F. J. et al.,1984), where 1 Astronomical Unit = 149.598.000 Km).

Sub-surface characteristics are of crucial importance for the success of the penetrating principle of the roots. This is likely to be limited to a loose and fine sand and soil, while it is intuitively not able to perforate hard bedrock. Indeed, the plantoid should be able to move away from similar obstacles, looking for different paths. Main terrain characteristics for these scenarios have been collected and described in the following. However, it seems not possible to foresee what the plantoid roots would exactly face under the surface.

On Mars, for example, while the first roughly 0.5-1 m under the surface, for instance, might be mainly composed of fine sand and loose soil, going deeper the subsurface could progressively host more hard rock layers with stones whose characteristics could correspond to those of our mafurite, carbonatite, diopside and diabase (Anttila, 2005; Exomars rover/Pasteur System Requirements Document, 2003 ; Statement of Work for the Phase A Study of the Pasteur Exobiology Payload and Rover for the EXOMARS Mission, 2003). In Fig. 2 a typical model of the Martian surface for drilling test is reported. The roots need to be sufficiently versatile and flexible to avoid stones and hard bedrock. Even if the first layer on Mars consists in an iron oxide dust with the consistency of talcum powder, Mars Odyssey orbiter has proved the possibility of water ice near the Martian surface (NASA, 2002.), for example. This means that even if there would be no rocks in the exploration spot, the soil might even be tied tightly by ice. This would represent a considerable constraint at mission level, since the root would not be able to penetrate icy crust (at least, without the help of further techniques, e.g., melting by heating).

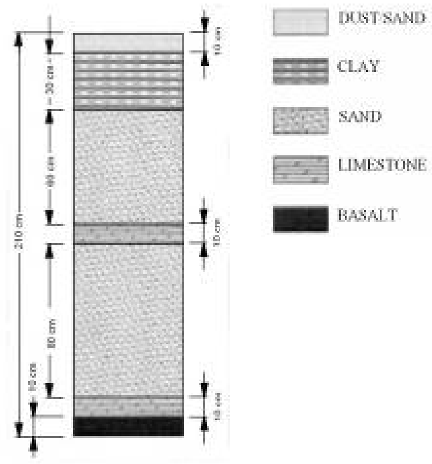
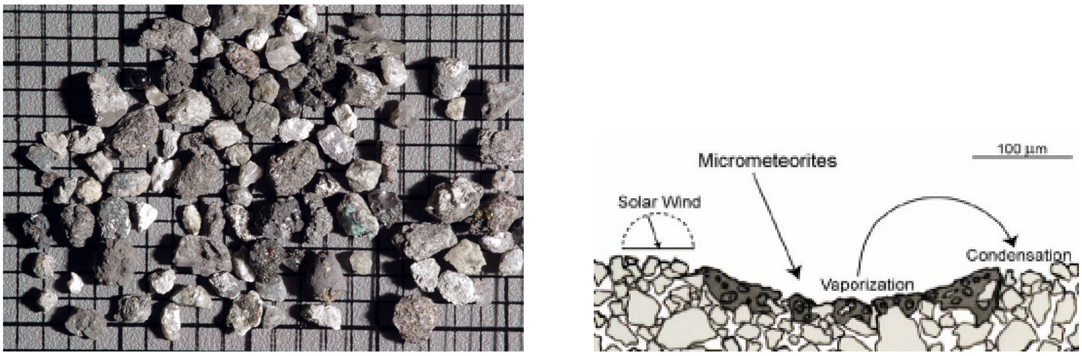


Fig. 2. Simulated Martian “sand box” developed by the Northern Centre for Advanced Technology (NORCAT), Canada.

Moon external surface is essentially regolith (Greek for stone blanket - litho + rhegos -): a sandy heterogeneous layer, whose thickness is about 4-5 meters thick in *mare* areas and 10-15 m in older highland regions, mined with some rocks. Below this, there is a region of blocky and fractured bedrock created by larger impacts, often referred to as "megaregolith".

The term lunar soil is often used as synonym of Lunar regolith and typically refers to the finer fraction of soil, that which is composed of grains 1 cm in diameter or less, see Fig. 3a. "Lunar dust" generally is used to indicate even finer grains, which are less than 100 micrometres in diameter, see Fig. 3b.



a) b)

Fig.3 a) Lunar regolith is composed of broken up rock material while with “soil” it is generally referred to as the portion of the Regolith with parts with a size < 1 cm as here shown, where all the grains are in the 2-4 mm size fraction (Photo by Randy Korotev); b) Schematic representation of some of the processes that contribute to the formation of Lunar dust (credit Larry Taylor, Univ. of Tennessee).

Finally, asteroids also have regoliths developed by meteoroid impact, even if the soil properties are generally unknown. The surface is dusty and generally the body shape is very irregular. Interesting application of the plantoid on asteroids would base on the use of the roots as a way of anchoring allowing even a partial penetration of the roots among the regolith stones. On the other hand, since the anchoring process would be quite slow, due to the low-gravity conditions on the asteroid, a continuous attitude control should be necessary in order to keep the robotic probe near the surface, necessarily exerting a counterbalance reaction to the push of the penetrating roots. This would represent a major problem.

The first soil layer that the plantoid would have to face for the proposed targets can be basically considered *powder*. Powders are probably the least predictable of all materials in relation to flowability, a fundamental characteristic in order to be able for the root to penetrate in the soil, because many factors affecting the rheological properties are present. A list of the main factors that could affect flow properties in planetary exploration are listed in the following:

Factors intrinsic to the powder or particles	External factors
▪ Particle size ▪ Size distribution ▪ Shape ▪ Surface texture ▪ Cohesivity ▪ Surface coating ▪ Particle interaction ▪ Wear or attrition characteristic ▪ Propensity to electro-static charge ▪ Hardness ▪ Stiffness ▪ Strength ▪ Fracture toughness	▪ Flow rate ▪ Compaction condition ▪ Vibration ▪ Temperature ▪ Tying substances (even frozen water) ▪ Electro-static charge ▪ Entrained gases

A powder is a blend of particles: entrained gases or vacuum space among particles drastically affect the flow properties of the powder bulk. From a preliminary viewpoint, it seems more likely that the plantoid roots could penetrate into a terrain formed by relatively larger grains, rather than fine microscopic sandy grains, which after an initial compression, and without any back expulsion strategy typically applied by drillers, would compact in a harder surface. It is known even from industrial tests on Earth, that the difference in energy needed to establish flow in a compacted powder may be 100 times that needed when the powder is aerated and possibly fluidised. For some powders, this ratio can be more than 1000 and in the most extreme case, 5000. Slight compaction, a small vibration, or the smallest amount of gas presence can significantly affect flowability.

On the contrary, relatively large and stiff grains would possibly spatially redistribute under the pushing action of the root without compacting. Too large grains, finally, could be too heavy (even in reduced gravity as on Mars and Moon) and offer too high friction due too larger surfaces, to not allow any penetration of the root of the plantoid.

Complementary penetrating strategies to simple pushing, could be considered, as blowing gases from the apex tip (in the case of Mars directly extracted from the environment), vibration of the apex, and/or even slow torsional movement of the apex itself (in order to allow grain re-distribution during penetration) as accomplished in powder characterization systems. They are all likely to ease the penetration process at least in the first stages (where dusty terrain is present).

The growing mechanisms of the root will likely to be relatively slow; therefore, the subsurface penetration will last a considerable amount of time. Considering that the current MER mission has now successfully operated on the surface for over two years, it is reasonable to consider a mission scenario with a robotic probe whose working principle is designed to operate on plant-like time scale.

The plantoid will be capable to start data transmission back to Earth right after landing, but the soil investigation will be carried out slowly, exactly as plants do, with a minimal consumption of power. This feature could allow designing relatively small solar panels and storage batteries.

WP2. Plants characteristics

Plants are photosynthetic, eukaryotic, multicellular organisms characterized by an aerial part and a root system. In an attempt to compensate for their sessile nature, they have developed growth responses to deal with the copious and rapid changes in their environment. These responses are known as tropisms and they are marked by a directional growth response that is the result of differential cellular growth and development in response to external stimulations such as light (phototropism), gravity (gravitropism), touch (thigmotropism) or water/humidity gradient (hydrotropism) (Esmon et al., 2005; Eapen et al., 2005). The combination of these mechanisms allows plants to overtake hostile or inaccessible environments and colonize the soil, leading to the generation of ramified root systems that assure their stability and survival. For that purpose, root systems often far exceed in mass and length the aboveground portions of the plant, being provided with few to many main roots and thousands of ramifications. Moreover, in spite of their slenderness and delicate structure, the spiralling forward thrust of the root tips and the pressure of their expanding cells are sufficient to split solid rock. The process by which plant tissue expands in tight places involves water uptake through osmosis and imbibition, and an overall increase in size by cell division. The amazing process starts with a seed that becomes lodged in a tiny crevice; like a living, expanding wedge driven deeper and deeper into the crack, the boulder is literally forced apart by tremendous pressures. Currently, there is a general agreement that higher plants are not only able to receive diverse signals from the environment but that they also possess mechanisms for rapid signal transmission (Baluska et al., 2004). In fact, each root tip is able to get information from the environment by their embedded sensors; the information is then transduced and processed in the whole root system and used to direct the growth towards regions of the soil with the best minerals and water availability, slowing down and stopping to grow, thus saving energy, whereas a too compact soil or a hostile environment do not allow life. So plants can effectively process information obtained from their surroundings and can show a learning behaviour which involves goal seeking, error-assessment, and memory mechanisms (Thellier et al., 1982; Knight et al., 1998; Trewavas, 2002; Trewavas, 2003). Moreover, on the surface of the epidermis of the distal portion of the root, tiny cellular projections called root hairs extend into the soil to absorb water and minerals. Root hairs are a competitive tool to increase the surface area of the roots, increasing the capacity to absorb nutrients and water. Therefore plants demonstrate to successfully reach their needs even without a conventional locomotion system. Although plants cannot physically move, active root growth allows exploration of soil niches for nutrition. This implies that root apices are not only sites of nutrient uptake but also sites of forward movement.

The root system of plants has an additional role that proves again their ability to contrive simple but effective mechanisms to overcome the varied situations occurring in their life: they are used to stock molecules important for their metabolism and energy. Reserve stored in the root system are safe and protected from many external adversities, and help plants to satisfy higher requests occurring during their annual cycle (e.g. energy for reproduction), or to survive over period of plant dormancy, or to regenerate organs or the whole aerial part after natural or deliberate destruction.

Plants are also characterized by inter-individual interactions. Plants communicate with members of the same or different species, as well as members from a different genre, including animals and insects, by means of various signals, mostly chemical (pheromones and other compounds), through which they can send and receive information over a long distance (Dicke et al., 2003; Bruine and Dicke, 2001; Chamberlain et al., 2001). As a consequence, plants have historically played a successful role in the conquest of the land. Once they had taken their first step (420 million years ago), nothing could stop them from spreading across the empty continents, giving their ability to “move” towards any region with regular rainfall and nutrient rich soil, adapting themselves each time to new landscapes and climates.

The structure of the seeds plays a key role, especially concerning their size that allows them to be dispersed ensuring a good opportunity to explore and conquer the land, and their ability to survive even for long period before reaching the optimal environment to germinate protected by special teguments and supported by the food stored inside themselves; the same food is also responsible for the sustenance of the new plant originated from the seed until it becomes completely heterotrophic.

Root system and its architecture

Plant root systems perform many essential adaptive functions including water and nutrient uptake, anchorage to the soil and the establishment of biotic interactions at the rhizosphere. Evidently, roots monitor a wide spectrum of physical and chemical parameters, and then integrate the signals obtained in order to perform appropriate and often complex growth responses to cope with the immediate environmental circumstances. The more acute sensitivity of roots to various types of signals already mentioned, when compared to shoots, is related to the **root apex**.

Changes in the architecture of the root system, therefore, can profoundly affect the capacity of plants to take up nutrients and water. Three major processes affect the overall architecture of the root system. First, cell division at the primary root meristem (i.e. of initial cells) enables indeterminate growth by adding new cells to the root. Second, lateral root formation increases the exploratory capacity of the root system; and third, root-hair formation increases the total surface of primary and lateral roots. Alterations to any of these three processes can have profound effects on root-system architecture and on the capacity of plants to grow in soils in which water and nutrient resources are limiting.

As a preliminary example of plants' ability to form an extensive, wide and deep root system, in the desert the roots of mesquite (genus *Prosopis*) may extend down more than 50 m to reach groundwater. Annual crop plants developed a root system that can usually grow until 2.0 m in depth and extend laterally to distances up to 1.0 m. As a general behaviour, the annual production of roots may easily surpass that of shoots, so the aboveground portions of a plant can be correctly defined as the tip of an iceberg. The growth of a root system can continue through the year, but this capacity mainly depends on the availability of water and minerals in the immediate microenvironment surrounding the root (*rhizosphere*). If the rhizosphere lacks in nutrients or water, root growth becomes slow. As rhizosphere conditions improve, root growth increases. Changes in root architecture can mediate the adaptation of plants to soils in which nutrient availability is limited by increasing the total absorptive surface of the root system. The development of root systems is usually highly asymmetric and reflects the ability of roots to adjust their growth and development to environmental factors (Lopez-Bucìo et al., 2006).

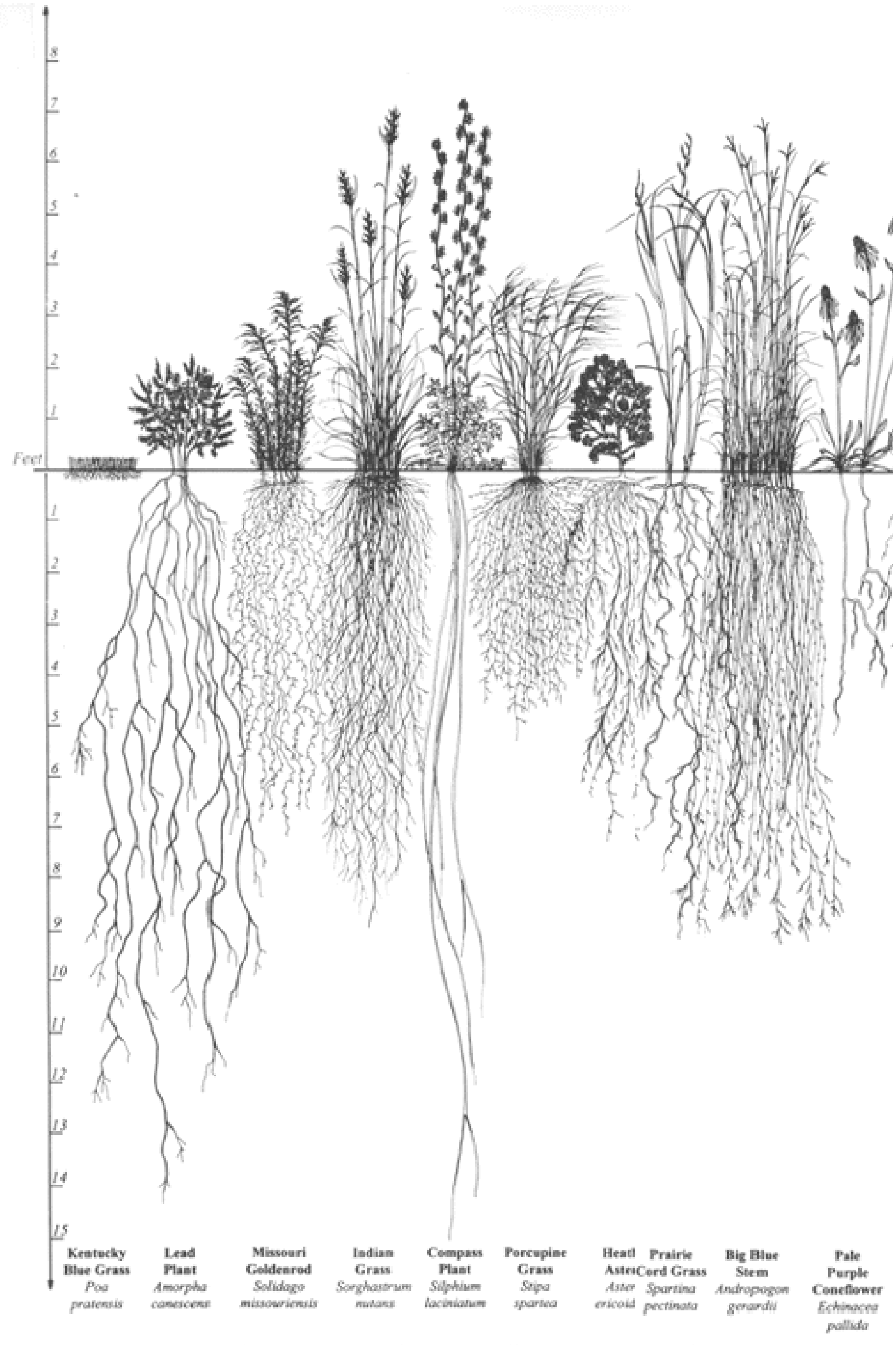


Fig. 4. Root systems of prairie plants (from http://en.wikipedia.org)

The *form* of the root system is different regarding plant species (Fig. 4). Germinating seeds in monocots initiate root development from the emergence of three to six primary root axes. Monocot plants form new adventitious roots, called nodal roots or *brace roots*, with further growth, then both the primary and nodal root axes continue their growth and branch extensively to form a complex fibrous root system. On the contrary, plants belonging to dicots develop a root system from a single root axis, called *taproot*. From this main root axis, lateral roots develop to form an extensively branched root system. The ability of dicots to activate a secondary growth from the cambium (secondary cambial activity, not present in the monocots) can thick these roots.

The development of the root system in both monocots and dicots depends on the activity of the root apical meristem and the production of lateral root meristems. As reported before, the apical region of a plant root is called **root apex** and morphologically includes three regions: meristem, transition zone (see par. 4.2), and elongation region (Fig. 5).

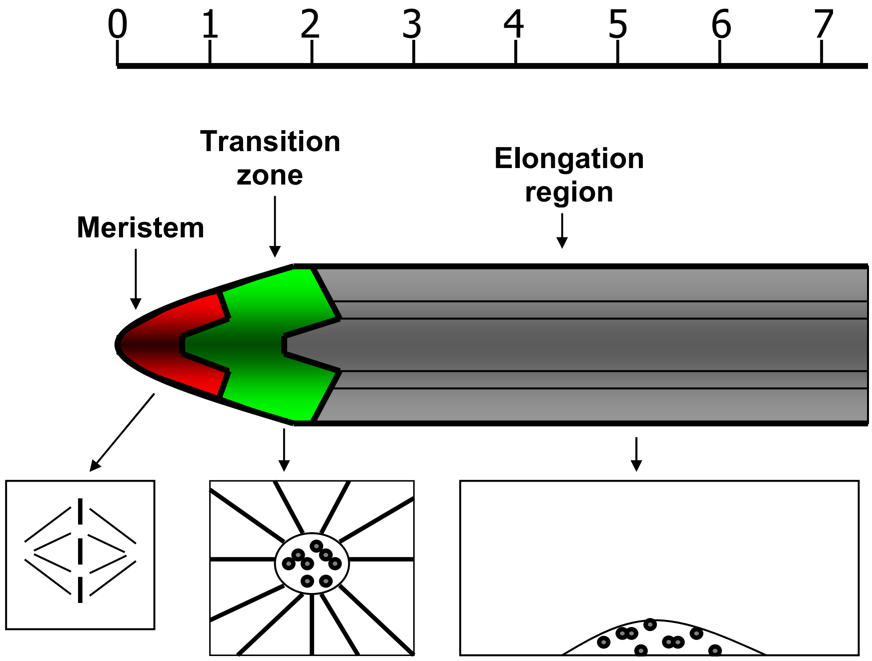


Fig. 5. On the basis of cytological observations, growing root apex is composed of three distinct zones: an apical **Meristem** or **Division Zone** (with fast dividing cells), an **Elongation Zone** (composed by elongated-shape, mature and almost fully vacuolated cells, with the nucleus close to the cell wall) and a **Transition Zone**, a unique zone intercalated between the two more obvious regions. Cells of this TZ show a unique cytoarchitecture, with centralised nucleus surrounded by perinuclear microtubules radiating towards the cell periphery (modified from Baluška et al., 2004).

The meristem is mainly delegated to cell division, which happens in both the direction of the root base in order to form new-born cells with the aim to differentiate into the tissues of the functional root and to form the root cap in the direction of the root apex.

Cell division at the real root apex proper is relatively slow; for this reason this region is known as the *quiescent center*. After a few cycles of slow cell divisions, root cells displaced from the apex by about 0.1 mm begin to divide more rapidly. Cell division again tapers off at about 0.4 mm from the apex, and the cells expand equally in all directions. After the transition zone, the root apex shows the *elongation region*, which begins 0.7 to 1.5 mm from the apex. In this zone, the elongation of the cells goes on a very rapid manner; more, they undergo a final round of divisions in order to produce a central ring of cells called the *endodermis*, whose walls become thickened due to a marked suberification to forms the *Casparian strip*, a hydrophobic structure that prevents the apoplastic movement of water or solutes across the root.

Root hairs, with their large surface area for absorption of water and solutes, first appear outside the proper root apex, in another region called the *maturation zone*. Here that the ascendant conducting pathway of the plants (xylem) develops the capacity to translocate substantial quantities of water and solutes to the shoot (Gilroy and Jones, 2000). The formation of root hairs increases the volume of soil in contact with, and therefore exploitable by, the root. It has been speculated for many decades that the primary reason for root hair existence is to increase the efficiency of nutrient ion uptake from the soil, based on the observation that the number and density of root hairs greatly increase under nutrient stress. The driving force for most nutrient uptake in plants is the electrochemical gradient across the plasma membrane, a major proportion of which is generated by the H^+-ATPase. In support of the theory that root hairs are centers of nutrient uptake, high levels of expression of H^+-ATPase genes in root hairs has been demonstrated in *Nicotiana* (Moriau et al., 1999), with the strongest expression occurring in developing root hairs and reduced expression in mature root hairs. Although the spatial localization of H^+-ATPase proteins within the root hairs themselves is unknown, evidence obtained with vibrating pH-sensitive microelectrodes indicates a strong H^+ efflux from the base of the root hair and an apparent tip-localized H^+ influx. This suggests that the proximity of H^+-ATPases to the zone of new growth is closely regulated (Jones et al., 1995).

The transition zone: a 'command centre' for the exploration of the surrounding environment

A new vision of the root system comes out from the morphological observation that root apices are composed of three distinct zones, the interplay of which allows their effective exploration of soil (Baluška et al., 2004) in searching both nutrients and water. In standard conditions, root cell elongation is much more rapid than the shoot cells one: as a consequence, any cell division in the region of rapid elongation is not allowed. On the contrary, cell cycling and cell elongation occur concomitantly in shoot apices. The clear separation of division and elongation regions in root apices permits to identify a very unique zone, the so-called transition zone, included between the two other (and well-known) regions (Baluška et al., 1994, 2001), which is a peculiar region for environmental sensing. In fact, **this region is able to detect more than 10 chemical and physical parameters from the surrounding environment.**

Why this region has a particular physiological behaviour? The main reason derives from a morphological observation (Fig. 5): in fact, the cells of the transition zone present a unique cytoarchitecture, with centralised nuclei surrounded by perinuclear microtubules radiating towards the cell periphery (Baluška et al., 2001). This configuration should be optimally suited for both the perception of signals and their transmission towards the nuclei, acting at the same time as *sensors* and *actuators*. These hypotheses come out from the fact that these cells are not occupied with the demanding tasks of either cell division or rapid cell elongation, so they can primarily focus all their resources upon perception and processing of environmental signals. Transition zone cells are not only sensory but they are also plastic in their behaviour. The distal portion of the zone includes cells which are still competent for cell division and which can, if necessary, regenerate a complete new meristem. On the other hand, cells of the proximal part of the transition zone have begun to achieve competence for rapid cell elongation, requiring only an appropriate signal to do so.

Experimental evidences show that root apices are the main sites for the first perception of low-temperature (Goulas et al., 2003) and drought (Blake and Ferrell, 1977), and are able to immediately transmit the information to the aboveground part. Root apices serve also for plant-to-plant communication, as they can emit and receive volatiles, whose production is induced by the herbivore attack of above-ground organs (Chamberlain et al., 2001; Dicke and Dijkman, 2001). Particular interest is directed to the polar auxin transport, which is accomplished along very complex pathways in root apices. There, root caps seem to act like a redistribution centre (Sabatini et al., 1999; Friml et al., 2002) as auxin transport drives root apex patterning (Friml et al., 2002; Jiang and Feldman, 2003; Bhalerao and Bennett, 2003; Barlow et al., 2004). Root apices are also equipped with high numbers of actin enriched and auxin-transporting plant (Barlow et al., 2004). Moreover, an auxin maximum (Sabatini et al., 1999; Jiang and Feldman, 2003) is localised at the quiescent centre and root cap statocytes, which responds rapidly to gravistimulation (Rashotte et al., 2001; Ottenschläger et al., 2003; Boonsirichai et al., 2003) and to exposures of extracellular auxin (Ottenschläger et al., 2003).

These observations can easily suggest that transition zone is a sort of *processor* or *command center* which, processes sensory information from the surrounding environment, stores memories and takes decisions about future exploratory and adaptive root behaviour. The *command center* needs a huge amount of ion-channel activity, endocytosis-driven vesicle trafficking, and cytokeletal re-arrangements. All these processes also require an enormous ATP consumption: consequently, it is logical that the transition zone cells should also have the highest oxygen requirements of the whole root apex, more than the division zone. The peak of oxygen uptake coincides with the peak of synaptic auxin transport (Mancuso et al., 2005) but is also extremely sensitive to gravistimulation. Repositioning a growing root apex from vertical to horizontal position induces extremely rapid changes (in the order of a few seconds) in oxygen uptake at the upper side of such a gravistimulated root (Mancuso et al., unpublished data), and almost immediate oxygen responses have been recorded during a brief period of microgravity induced by parabolic flights (Mugnai et al., unpublished data). All these results suggest that whatever process lies downstream of the oxygen uptake, it is closely linked to the gravity sensing and processing into the transition zone.

The root cap and the soil penetration

Plant roots may exert **pressures of up to 1 MPa** in order to penetrate hard soils (Pfeffer, 1893; Misra *et al.,* 1986). Consequently, virtually all plants which grow in soil have evolved root caps which protect the root meristem from physical damage or abrasion by soil particles.

Root cap consists of mucilage-secreting cells (one to two layers beneath the lateral and peripheral tissue surface); the cap cells are continually being produced by the meristem and, after a period of hours or days, sloughed off from the outer surface of the root cap where may remain alive for a period of days after being detached from the root cap (Vermeer and McCully, 1982). Despite the full knowledge of the mechanism that leads to the so called *mucigel* production, very poor certainties are given about its precise function. It has been suggested that lubricates and protects the root apex, promotes the transfer of nutrients to the root, and affects the interaction between roots and soil micro organisms (Russell, 1977). The root cap is also central to the perception of gravity, the signal that directs the growth of roots downward: this process is termed the gravitropic response (see par. 4.7.2).

The products of mucilage secretion are not the same in the various species, but the main components are carbohydrates (94% [w/w]) and proteins (Bacic *et al*., 1986) that, even when the root cap is fully hydrated, allow its integrity creating a sort of polymer network that entraps water (up to 99.9% of wet weight) (Guinel and McCully, 1986). Interestingly, the physical properties of the root cap mucilage change considerably with changing degrees of hydration: upon loss of water, the surface tension of the mucilage is strongly reduced, whereas its viscosity increases (Read and Gregory, 1997). When wet, mucilage expands to 1000 times its dry volume (Guinel and McCully, 1986).These findings support the idea that mucilage plays a major role in the maintenance of root-soil contact in drying soil: as the surface tension decreases, the ability of the mucilage to wet the surrounding soil particles becomes greater, and as the viscosity and elasticity decrease, the rhyzosphere structure is stabilized and hydraulic continuity is upheld (Read and Gregory, 1997). Moreover, the mucilage secreted by the root cap cells is exuded even under conditions of water stress (Guinel and McCully, 1986).

The root cap must, therefore, play an important role not only in protecting the root meristem from damage, but also in determining the mechanical interaction between the root and the soil, namely the mode of soil deformation and the root–soil friction (Bengough, 2006).

The mechanical resistance of the soil to root growth can be separated into two components: the ***pressure required to deform a cavity*** (in the soil) that is large enough for the root; and the ***pressure required to overcome the frictional resistance between the soil particles and the moving surface of the root***. The resistance of the soil to deformation results in a pressure on the surface of the root which, in turn, is the cause of the frictional resistance between the root surface and the soil. The frictional stress, τ, between soil and the surface of a root is given by the equation (1) (Bengough and McKenzie, 1997):

$$\tau = \sigma_N \tan\delta + c_a \qquad (1)$$

where σ_N is the stress normal to the surface of the root, δ is the angle of friction between the two surfaces, and c_a is the tangential component of adhesion between the surfaces (Stafford and Tanner, 1977). The direction of the frictional resistance is opposite to that of the velocity of the moving surface of the root. The total resistance to the elongation of a root is, therefore, the sum of the components of the cavity expansion pressure (= N), and in the axial direction. It is well proved that both mucilage exudation and root cap sloughing seem to participate in eliminating practically all frictional resistance to root growth (Greacen *et al.,* 1968). A root cap cell will only move relative to the root if the friction between the cap cell and the surface of the root is smaller than the friction between the cap cell and the surrounding soil. Thus, in order for a cap cell to move relative to the root, the coefficient of friction between the cap cell and root surface (including any adhesive forces) must be smaller than between the cap cell and the soil. Hence, by definition, a sloughed cap cell decreases the frictional resistance to soil penetration (Bengough and McKenzie, 1997).

Removal of the root cap has recently been shown to halve the elongation rate of maize roots grown in compacted sandy loam soil (penetrometer resistance 1.0 MPa), whereas root elongation rate was unaffected in loose soil (penetrometer resistance 0.06 MPa; Iijima *et al*., 2003). The reason for the slowing of root elongation was the associated increase in the root penetration resistance, equal to the force exerted per unit cross-sectional area of root tip, **from 0.31 MPa to 0.52 MPa**. This again demonstrates that the intact root cap and its associated border cells and mucilage facilitate root penetration by decreasing the coefficient of friction between the root surface and soil particles (Bengough and McKenzie, 1997). Direct measurements of the frictional forces suggest that, in wet soil, the coefficient of root cap–soil friction is approximately **0.03** (Kirby and Bengough, 2002), with the border cells forming a low-friction sleeve around the root cap (Bengough and McKenzie, 1997). In soil drier than -60 kPa matric potential, however, the coefficient of friction may well be significantly greater, depending on the lubricating action of the less-hydrated mucilage between border cells and the root cap (Guinel and McCully, 1986; Read *et al*., 1999). The relative contributions of border-cell sloughing and mucilage to decreasing root penetration resistance have been estimated as 58% and 42%, respectively, for maize roots grown in loamy sand soil (Iijima *et al*., 2004).

Values of the maximum axial growth pressure (σ_{max}) of seedling pea (*Pisum sativum* L.) roots have been reported by Clark et al. (2004), obtained using different apparatuses and cultivars. The values ranged from **0.3 MPa to 1.3 MPa**. Table 2 reports experimental values for maximum rate of root elongation R_{max}), maximum growth pressure ($\sigma_{max},$) and soil penetrometer pressure ($Q_{1/2}$), which halves the rate of elongation of plant seminal roots.

Plant species	**R_{max} (mm day^{-1})**	**σ_{max} (MPa)**	**$Q_{1/2}$ (MPa)**
Bean (*Faba vulgaris* L.)		1.08	
Cotton *(Gossypium hirsutum* L. cv. Empire)	85		0.72
Cotton *(Gossypium hirsutum* L. cv. Coker)		0.92-1.1	
Cotton *(Gossypium hirsutum* L. cv. Sicot 3)		0.29	
Maize *(Zea mays* L.)		1.45	
Maize *(Zea mays* L. cv. United)	26		1.3
Pea *(Pisum sativum* L. cv. Brunswick)		1.31	
Pea *(Pisum sativum* L. cv. Meteor)			2.03
Pea *(Pisum sativum* L.)	24		
Pea *(Pisum sativum* L. cv. Onward)	35		
Pea *(Pisum sativurn* L. cv. Brunswick)		1.2	
Pea *(Pisum sativum* L. cv. Greanfeast)		0.50	
Peanut *(Arachis hypogaea* cv. Virginia bunch)	65	1.16	1.91
Ryegrass *(Lolium multiflorum* cv. 522)			1.39
Sunflower *(Helianthus annuus* L. cv. Hysun)		0.24	
Tomato *(Lycopersicon esculentum* cv. Potentate)			1.48

Root growth

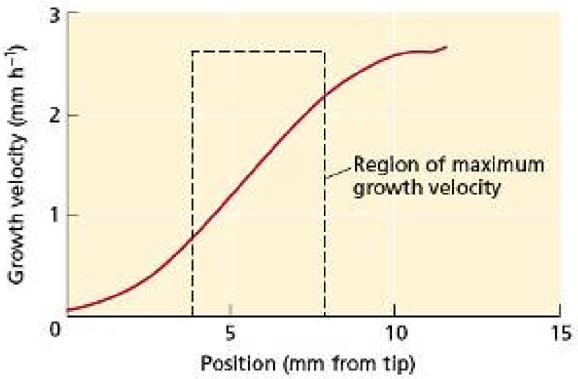
Root growth is strongly influenced by available nutrients in order to optimize acquisition. Thus, understanding root growth is of particular interest to plant physiologists, which can be clearly seen from the plentitude of publications on the subject. Models have not only been developed for the topological description of the complicated networks of root systems, but also for the growth of individual roots as their modular basis. For example, the growth of the root apex has been modeled using growth tensors and natural coordinate systems (Hejnowicz and Hejnowicz, 1991; Hejnowicz and Karczewski, 1993). Other authors have concentrated on the distribution of growth (velocity and rate) and also on cell length (Beemster and Baskin, 1998; Erikson and Sax, 1956; Goodwin and

Stepka, 1945; Silk, 1992; Walter et al., 2002). The distribution of growth is essential to understand the adaptation due to nutrients availability. Until now the approach to model such distributions has been based on conservation and kinetic equations, such as those used by Silk (1992). Empirical approaches, e.g. the use of a logistic fit, have been also used (Morris and Silk, 1992). Mechanistic understanding of root growth needs to take into account the architecture of the root growth zone. The response of growing roots to their environment is a function of their cellular compounds (Beemster et al., 2003). As a given region of the plant axis moves away from the apex, its growth velocity increases (the rate of elongation accelerates) until a constant limiting velocity is reached equal to the overall organ extension rate. The reason for this increase in growth velocity is that with time, progressively more tissue is located between the moving particle and the apex, and progressively more cells are expanding, so the particle is displaced more and more rapidly. In a rapidly growing maize root, a tissue element takes about 8 hours to move from 2 mm (the end of the meristematic zone) to 12 mm (the end of the elongation zone).

Beyond the growth zone, elements do not separate; neighboring elements have the same velocity (expressed as the change in distance from the tip per unit of time), and the rate at which particles are displaced from the tip is the same as the rate at which the tip moves through the soil. The root tip of maize is pushed through the soil at ***3 mm h^{-1}***. This is also the rate at which the nongrowing region recedes from the apex, and it is equal to the final slope of the growth trajectory.

The velocities of different tissue elements are plotted against their distance from the apex to give the spatial pattern of growth velocity, or **growth velocity profile** (Fig. 6). The velocity increases with position in the growth zone. A constant value is obtained at the base of the growth zone. The final growth velocity is the final, constant slope of the growth trajectory equal to the elongation rate of the organ, as discussed in the previous section. In the rapidly growing maize root, the growth velocity is 1 mm h^{-1} at 4 mm, and it reaches its final value of nearly ***3 mm h^{-1} at 12 mm***.

(A) Growth velocity profile



(B) Relative elemental growth rate

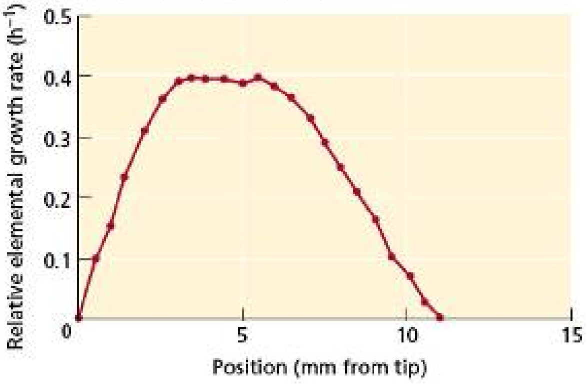


Fig 6. The growth of the primary root of *Zea mays* can be represented cinematically by two related growth curves. (A) The growth velocity profile plots the velocity of movement away from the tip of points at different distances from the tip. This tells us that growth velocity increases with distance from the tip until it reaches a uniform velocity equal to the rate of elongation of the root. (B) The relative elemental growth rate tells us the rate of expansion of any particular point on the root. It is the most useful measure for the physiologist because it tells us where the most rapidly expanding regions are located (From Silk 1994).

If the growth velocity is known, the **relative elemental growth rate**, which represents the fractional change in length per unit time, can be calculated (see below). The relative elemental growth rate shows the location and magnitude of the extension rate and can be used to quantify the effects of environmental variation on the growth pattern (see Figure 6B). In physical terms, cell growth can be defined as an irreversible ***increase in cell volume and surface area*** (Schopfer, 2006). Plant protoplasts are characterized by an univocal structure and organization, as they are surrounded and encased by a rigid, but expandable, cell wall due to elastic characteristics. The cell wall is infiltrated with water containing only a very low osmotically active amount of solutes. **This situation enables the formation of a large difference in osmotic pressure ($\Delta\pi$) between the apoplastic and the symplastic space (0.6-1.0 MPa),** compensated by a hydrostatic pressure, named *turgor* (*P*), of equal value when the cell is in a fully turgid state. **The cell can hence be described as a simple osmometer that can enlarge by water uptake** powered by a difference in water potential ($\Delta\Psi$) between protoplastic and simplastic space.

$$\Delta\Psi = P - \Delta\pi \quad (2)$$

According to eq. (2), water uptake driven by $\Delta\Psi$ can be achieved in two ways (Schopfer, 2006):
increase of π in the protoplast. In this type, cell enlargement is reversible (i.e. stomata guard cells)
decrease in P. For producing growth, irreversible increase in cell volume is needed, and this mechanism is the most suitable. The only way to achieve this is by lowering the counter pressure exerted by the cell wall on the protoplast, and this happens only if the cell wall is a tensile stress state due to the turgor, and this stress is released by changes in the mechanical properties of the wall material (stress relaxation by wall loosening). Continuing wall loosening permits to liberate a driving force for water uptake, and it will restore the turgor to its previous level. This mechano-hydraulic process produces irreversible cell enlargement, namely growth. As growing cells generally maintain a constant $\Delta\pi$ by the osmoregulation process, they can reach a steady-state growth for hours or days.

Root growth can be described by a two-level characterisation: individual root elongation, and a network expansion through branching. Recently, Chavarria-Krauser et al. (2005) proposed a novel approach to model growth distribution, finding that the root apex can be modelled by a string of dividing and growing (elongating) cells controlled by hormones. In fact, active auxin transport is essential for the control of cell division, elongation and differentiation (Teale et al., 2005), while cell extensibility, turgor and water conductivity influence the growth rate profiles of roots (Pritchard, 1994). In 1965, Lockhart formulated the following empirical equation for the description of cell growth rate in length:

$$g = \frac{dl}{dt} = l\phi(\Psi_p - Y) \qquad (3)$$

where *g* is the growth rate (μm min^{-1}), *l* is the cell length (μm), Φ is the wall extensibility (MPa^{-1} min^{-1}), Ψp is the turgor pressure (MPa) and *Y* is the yield threshold (MPa).

As growth occurs by uptake of water transported through the cell wall and cell membrane, the cell can also be described as a simple osmometer:

$$\frac{dV}{dt} = -A_s L(\Pi + P) \qquad (4)$$

where V is the volume of the cell (μm^3), A_s is the area of the cell surface (μm^2), L is the conductivity coefficient ($\mu m\ min^{-1}\ MPa^{-1}$) and Π is the osmotic potential (MPa).

The Lockhart equation has been useful for the determination of the physical growth potentials and cell wall extensibility coefficients in a variety of growing tissues. An important observation derives from such measurements: the turgor remains unchanged (or decreased) if growth is induced by auxin, while cell wall dramatically changes. Same results during growth inhibition by abscisic acid. These findings confirm the notion that changes in cell wall extensibility, produced by active cell wall loosening or stiffening, are the basic mediators controlling cell growth (acting as *actuators*).
Cell wall extensibility is determined by several factors, but the complete mechanism and control of wall extension are still unknown. Chavarria-Krauser et al. (2005) assumed that an enzyme, deposited on the cell wall, is essential for cell wall extensibility, and Φ will be proportional to the enzyme concentration c_e for small enzyme concentration. On the contrary, for high concentrations the enzyme is not a restriction parameter, and other factor plays a key role.
Information about the *in vivo* extensibility properties of cell walls during growth has been obtained by several techniques (Lockhart et al., 1967; Cosgrove et al., 1984; Kutschera and Schopfer, 1986). Cosgrove (1985) showed that auxin increases Φ from 0.08 to 0.24 $MPa^{-1}h^{-1}$. As stress relaxation proceeds, the turgor decays from 0.60MPa to the threshold Y of 0.29 MPa that is not affected by auxin.
In a first approximation, the cell wall can be regarded as a composite material composed of cellulose microfibrils embedded in an amorphous matrix of hemicelluloses and pectins (Fry, 2004). Turgor pressure exerts a homogeneous, multidirectional force on the elastically stretched cell wall, but the tensile forces generated in the wall by the turgor and the ability to yield by liberation of these forces can be quite different in the different zones of the cell, depending on the local structure of the wall. If surrounded by a structurally homogeneous wall, a cell would expand in the form of a sphere (at the lowest level of energy). From this consideration derives the fact that in the wall of a cylindrical cell, the tangential stress produced by turgor in the side walls is given by $\sigma = \mathrm{Pr}/d$, where r is the radius of the cylinder and d the thickness of the uniform wall. By contrast, the longitudinal stress is given by $\sigma = \mathrm{Pr}/2d$. These geometric constraints predict that the wall must exhibit anisotropic properties in order to maintain the cell shape of a cylinder during growth. Anisotropic mechanical properties are consequences of anisotropic molecular wall structure, determined by the spatial arrangement of the cellulose microfibrils that are generally organized in layers of parallel fibers. Microfibrils convey a relatively high strain resistance (*tensile strength*) to the wall in the direction in which they are laid down by cellulose-synthase complexes drifting in the plasma membrane. The direction of the drift is thought to be directed by cortical microtubules attached to the inner side of the membrane (microtubule–microfibril paradigm). In primary walls of elongating cells, the microfibrils are typically oriented perpendicularly to the longitudinal axis, reinforcing the wall in girth similar to the hoops of a barrel. This particular orientation of the microfibrils determines the direction of growth. The wall of a long, cylindrical cell with net orientation of microfibrils in the side walls perpendicular to the long axis will expand preferentially in length even though the wall stress in girth is twice as high as in length and therefore per se favors growth in girth. It follows from these considerations that cell growth is oriented by the specific architecture of the cell wall. This statement can be supported by a multitude of observations. For example, the outer epidermal wall of elongating plant organs shows hoop reinforcement during early growth, followed by a transition to net longitudinal microfibril orientation toward the end of the growth period (Paolillo, 2000). Extrapolated to the level of the whole plant, the biomechanical concept outlined above implies that the specific form of an organ is modeled during growth by local regulatory changes in the orientation of microfibrils newly deposited at the inner wall surface.

Hormones and growth. The role of auxin

The growth and development of root cells and tissues rely on the establishment of cellular and sub-cellular asymmetries. The formation of the real cell shape and the positioning of molecules in the intracellular space commonly involve a persistent directional orientation along an axis, named *cell polarity.* Polar transport of auxin is directly linked as a signal to the regulation of both growth and polarity in the plants. As the plant body is shaped in response to numerous environmental stimuli (i.e. light and gravity; Muday et al. (2003), Friml J (2003)), these factors are able to influence the transport of auxin in a way that this hormone is delivered to tissues induced to grow *via* the establishment of auxin gradients, transport and response. Auxin is transported across the whole plant body via effective cell-cell transport mechanisms involving both the symplast and the apoplast. Cellular auxin influx and efflux, and the mechanisms that mediate the delivery and removal of potential polar auxin transport components from the plasma membrane, remain still open and discussed. For example, it is not clear why auxin bypasses the cytoplasmatic channels of the plasmodesmata crossing through the apoplast, as their diameter could easily accommodate several auxin molecules. This suggests the presence of an active mechanism that prevents auxin entering the plasmodesmata (Šamaj et al., 2004) and implies a functional benefit for including an apoplastic step in the polar transport of auxin. Transcellular auxin transport is accomplished via a poorly understood vesicle-based process that involves the putative auxin transporters, or transport facilitators, recycling between the plasma membrane and the endosomes.

Active auxin transport mediates cellular auxin concentration and is therefore a crucial component in the coordination of plant development. However, the specific relationship between auxin signalling and auxin transport is still quite unknown.

Tropism in plants

Plants, along their evolution, have had to face and solve a variety of problems to survive: developing support structures, creating a system of transport of water and nutrients through the whole organism, protecting the delicate reproductive organs, assuring the reproduction in the most convenient period of the year, improving the mechanisms of adaptation to the variable climatic conditions etc. All these needs has led to large modifications in metabolism and has brought to the acquisition of sensorial structures to set up a precise biological clock necessary to guarantee the constant monitoring of the surrounding situation to acclimatize them.

Plants can **sense gravity, temperature, light quality and direction**, etc., and if necessary they can act consequently. Plants feed themselves, breathe, fight the infections, in some cases generate symbiosis with fungi and bacteria and communicate with them.

They live in continuous competition both with environmental agents and with other plants for the conquest of light, space and nourishing substances, and all those necessary elements to their survival. They also live in continuous competition with other predator organisms, fungi, bacteria or animals. When circumstances become unfavourable for optimal growth and development of animals, they can respond accordingly by moving to a more favourable environment. Plants are not afforded this luxury. Due to their sessile nature, plants are forced to make the most of their immediate surroundings, which means adapting to an ever-changing environment (Liscum, 2002). So they have been adapted to perceive and react to adverse situations with varies useful movements to the survival. Darwin noted that plants had a tendency to sense their environment so as to orient themselves for optimal growth and development, and he dedicated part of his studies to vegetal biology publishing with his son Francis a fascinating book "The Power of Movement in Plant" (Darwin and Darwin, 1880) in which he encloses many interesting observations on plant life with special interest in movement. The Darwins studied the two great categories of "movements in the plants": "tropisms" (directional movements in answer to external directional stimuli) and "nastic movements" (movements in answer to external stimuli, but independent to their direction). Plants are constantly being bombarded with changes in their environment. Temperature fluctuations, not enough light or water content in the soil, are just a few of the factors to which plants must be able to respond. Moreover, plants must respond to physical forces of nature such as gravity or touch stimulation. Over evolutionary time, plants have adapted to their surroundings with a high degree of plasticity, affording them the ability to respond to ever-changing conditions that provide constant stimulation. Plant tropisms are operationally defined as differential growth responses that reorient plant organs in response to direction of physical stimuli. An example of tropism regards the perception of gravity, also called “Gravitropism”. “Phototropism” is the directional response in answer to a luminous source. Tropisms can be negative, such as a stem bending away from a

gravity stimulation (Blancaflor and Masson, 2003), or they can be positive, as in a stem bending toward a light stimulation (Liscum, 2002) while example of nastic movements are the closing leaves, modified as fly-trap, of *Dionea muscipola*, the fast closing of the composed leaves of *Mimosa pudica* to a tactile stimulus, or the closing leaves of many leguminous during night (*nictinastic movements*). Darwin studied also another type of movement, oscillating and rhythmic, called "circumnutation": plants, during their growth, perform circular movements around a central axis (Mugnai et al., 2007). Nearly all the plants present this growing movement, however it turns out to be more obvious in some species rather than in others, such as climbing plants (Brown, 1993; Larson, 2000).
Therefore tropisms are that group of reaction mechanisms that the plant acts in relation to directional stimuli. The most known and studied are:

1. Phototropism – the answer to light
2. Gravitropism – the answer to gravity
3. Electrotropism – the answer to an electric field
4. Hydrotropism – the answer to a gradient of water in soil

Phototropism

Plants need light to grow. They will do everything they can to get as much of it as possible. One of the ways plants maximize their amount of light is phototropism or "moving toward light". Since most plants cannot really move their whole body, they move part of it. When light is all around, the plant grows straight, but if light is coming from only one side (for instance, from a window) plants will start to grow toward the source of the light. Stems exhibit positive phototropism (growth towards the stimulation), while roots exhibit negative phototropism (growth away from the stimulation). The way the plant does this is by making one side of the stem grow faster than the other side. This causes the bending. The part away from the light (the darker part) grows faster, and this has the effect of getting the rest of the plant closer to the light. The responsible of this asymmetric bending is a plant hormone, auxin. Auxin is produced at the tip of the plant and goes down promoting the elongation of cells. When one side is darker than the other, more auxin goes to the darker side and it grows faster, bending the stem toward the light.
In the 19th Century, Darwin postulated that there was “something” being moved from the tip of the plant to the shoot that enabled it to bend toward the light stimulation. In the early portion of the 20th Century, Cholodny (1927) and Went and Thimann (1937), working independently, first proposed that an asymmetric redistribution of a growth-promoting substance lead to this phototropic response. They named this substance “auxin” which is a Greek term meaning “to increase” – an appropriate name given to its properties to promote cell elongation. It would be many years before the substance was purified and a structure determined, but auxin would become the first plant substance to be termed as “hormone.” In addiction, chloroplasts, which are cell organelles specifically differentiated for photosynthesis, change their intracellular arrangements and/or positions in response to irradiation with light (Senn, 1908; Haupt and Scheuerlein, 1990; Wada and Kagawa, 2001). These movements are widely observed in a variety of plant species and are designated as the photo-orientation movement of chloroplasts. Basically, chloroplasts move to expose their 'faces' to incident dim light and their 'profiles' to strong light. These movements are believed to play important roles in the maximization of photosynthetic activity (Zurzycki, 1955) and the minimization of photo-damage (Zurzycki, 1957; Park et al., 1996) in plants under fluctuating light conditions.

Gravitropism

Plants answer to a series of stimuli during their development, and gravity represents the main one. In the earth gravitational field the vegetative shoots grow upwards in order to maximize the absorption of light for the photosynthesis, while the roots grow towards the bottom for better supplying water and nutrients. This directional increase of plant organs in answer to the gravity force, takes the name of "Gravitropism". In the early portion of the 20th century the mechanism of perception of the gravity already was localized in root tip, and in particular in the root cap, where gravity is perceived by amyloplasts that lay down at the bottom and give to the cell the information of the gravity vector (Haberlandt, 1900). This theory, commonly famous like "starch/statolith hypothesis ", due to the sedimentation of the amyloplasts in the cells of the cap, is proposed to be the first step in the gravitropic response (Sack, 1997; Kiss, 2000). Starch-filled amyloplasts are displaced when the gravity stimulation changes (Kiss et al., 1989). Amyloplasts are found in the columella cells of the root cap (statoliths) and in the endodermal cells of the shoot (statocytes). When laser ablation was used to remove the central root columella cells in Arabidopsis, a large inhibitory effect was seen with respect to root curvature in response to a gravity stimulation (Blancaflor et al., 1998). Genetic studies using mutants that have few or no endodermal cells, lack amyloplasts, or have a problem in sedimentation of amyloplasts have proven to be useful tools in establishing the necessary role of the organelle in a plant's ability to respond to a change in gravity stimulation (Boonsirichai et al., 2002). More recently, however, other theories funded on scientific evidences have been formulated and the gravitropic response mechanisms is still controversial.

Root gravitropic response: sensors and actuators. As gravity is a physical force which only act on masses, several organelles or particles which are denser or lighter than the cytoplasm can be involved in gravity sensing. The stages of gravitropism in plants can be divided into: perception of the signal, transduction, and response (Salisbury, 1993). In roots, gravity perception is hypothesized to occur in the root cap region, in columella cells, and the response (differential growth) occurs in the zone of elongation (Sack, 1991). The response involves the plant hormone auxin (Simmons et al., 1995; Firn et al., 2000). In higher plants, there is a temporal and spatial separation between perception and response, and the signal must be transmitted over a relatively large distance (i.e., multiple cell layers).

Gravity perception. More than one model are proposed:

1. The starch-statolith hypothesis proposes that perception is mediated by the interaction of dense organelles (statoliths) with other cytoplasmic structures. In higher plants, the statoliths appear to be amyloplasts in specialized columella cells (MacCleery and Kiss, 1999) (Fig. 7). Some of the strongest evidence that amyloplasts function as statoliths comes from research (Kiss et al., 1989) with starchless mutants of *Arabidopsis*. Detailed studies of the kinetics of gravitropic curvature demonstrated that WT roots (with a full complement of starch) are more sensitive to gravity than starchless roots.

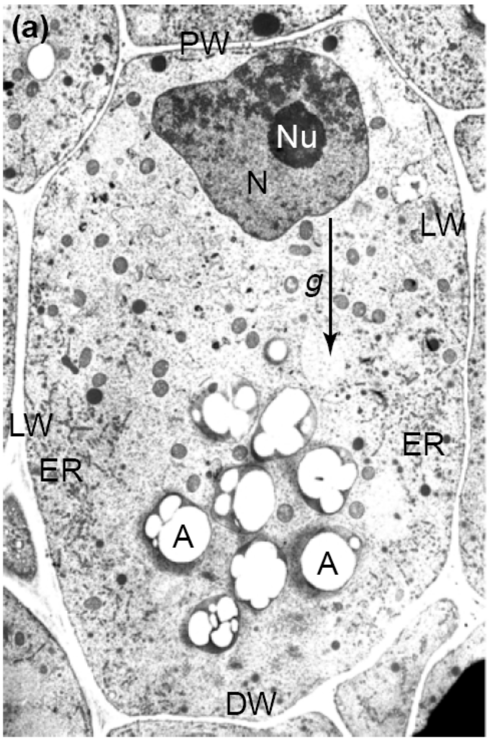


Figure 7. Gravisensing root cells. Root statocytes (*Lens culinaris*) show a structural polarity. However, the nucleus is located close to the proximal wall and the endoplasmic reticulum is situated beneath the amyloplasts and along the longitudinal wall. In shoots, the statocyte is highly vacuolated and the nucleus can sediment with the amyloplasts.
Abbreviations: A, amyloplast; DW, distal wall; ER, endoplasmic reticulum; g, gravity; LW, longitudinal wall; N, nucleus; Nu, nucleolus; PW, proximal wall; V, vacuole. Scale bar: 5 μm.

2. The protoplast pressure hypothesis, which suggests that the entire mass of the cytoplasm participates in perception (Wayne et al., 1992; Staves et al., 1997). Sedimentation of internal organelles appears to be absent from most gravitropic cells and from the internodal cells of *Chara* (Wayne et al., 1990; Machemer and Braucker, 1992). Instead, in both systems the mass of the entire cell has been implicated in gravity sensing. Wayne et al. (1990) hypothesized that the mass of the cell functions in gravity sensing. Tension at the top of the cell and compression at the bottom are thought to act via ”integrin-like" molecules in the plasma membrane to regulate calcium uptake differentially and thus influence the polarity of streaming (Staves et al. 1995).

Polar regulation of streaming seems to prevent a gravity-induced accumulation of cytoplasm in the bottom of the cell by increasing the thickness of upward-streaming cytoplasm (Staves et al., 1995). It has been argued that the mass of the starch in columella cells contributes to gravitropic sensing by increasing the weight of the cell, rather by functioning as statoliths that act upon an intracellular receptor (Wayne et al., 1990). The mechanism through which the plant transforms the indication of cell position into a biochemical message to start the bending process remains an argument of intense research (Boonsirichai et al., 2002; Chen et al., 2002). An important question in sensor physiology is the determination of the threshold dose of a stimulus in order to provoke the reaction of the plant organ. For the gravireaction of plant roots**, it has been noticed that gravitational stimuli which last 1 s (*perception time*) can be actively perceived by plant roots** (Hejnowicz et al., 1998), but one single stimulus is not sufficient to determine any organ response. So, a minimum time of continuous stimulus must occur (*presentation time*). **Experimental data (Hejnowicz et al., 1998) shows that the presentation time is probably less than 10 s in roots in ground experiment.** However, Mancuso et al. (unpublished data) found that during parabolic flights, the minimum presentation time for the evoking of a physiological response (in that case, a change in oxygen influx pattern) in maize roots was less than 2 sec after the beginning of microgravity conditions.

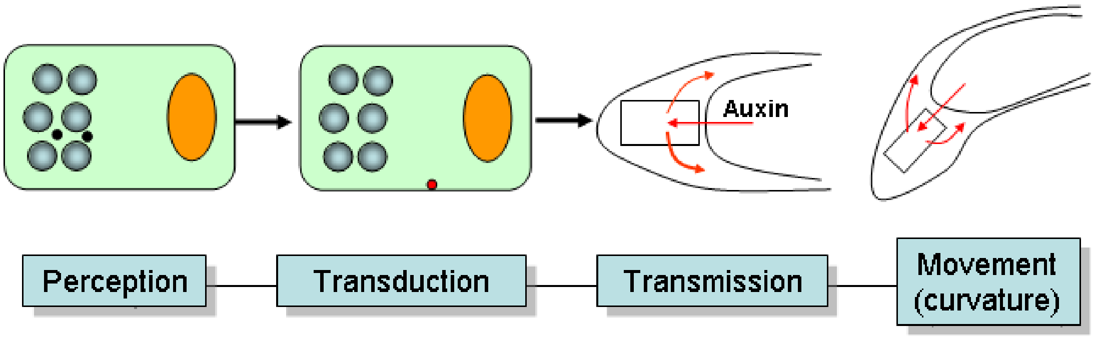


Fig. 8. The different phases of root gravitropism. When a root is subjected to a change of orientation in the gravitational field, the stimulus is perceived in less than 1 s. However, this 1 s stimulus is not sufficient to induce a gravitropic response – it must be repeated about ten times (or the stimulation must last 10 s) to initiate an asymmetrical signal within the statocytes (**transduction phase**). This leads to a downward lateral movement of auxin (**transmission phase**) that is the cause of the differential growth occurring after a latent time of 10 min. The four phases are represented in sequence but **perception**, **transduction** and **transmission** can persist for the whole period of the gravitropic reaction. The black circles represent activation of mechanosensors by amyloplasts. The red circle represents the start of the relocation of auxin efflux carriers. The green spheres represent the amyloplasts and the purple sphere represents the nucleus (modified from Perbal and Driss-Ecole, 2003)

Gravity signal transduction. It is also not yet clear which cellular structure perceives the settling statolith or the tension of the protoplast. The plasma membrane, cytoskeleton, and endoplasmic reticulum have been suggested as cellular organelles that may accept the force of the settling statolith and transmit this information via a signal transduction cascade leading to altered growth (Sack, 1991; Baluska and Hasenstein, 1997). What is quite clear about this complex process is that the ultimate response to the change in gravity vector is a root alteration in the direction of growth. Depending on the age, size, and species of plant tissues, these growth alterations can be observed within minutes of plant reorientation and completed within hours after gravity stimulation (Mancuso et al, unpublished results). Cytoskeleton is the best candidate for transmitting the force exerted by gravisensors to the mechanoreceptors. Cytoskeleton constitutes the structure of the cell. Its main functions are structural, of protection and of intercellular and intracellular movements. It seems to be involved actively in gravitropism of the organisms through various mechanisms. For some authors the cytoskeleton could be involved in the phase of perception, while others suggest its involvement in the transduction of the signal. In one or the other case, however, cytoskeleton participates to the gravitropic mechanism in the initial stages, before root bending (Baluska and Hasenstein, 1997; Braun, 1997; Perbal et al, 1997; Sievers et al., 1996; Nick et al., 1997). The actinic microfilaments of the cytoskeleton play an essential role, the greater part of the studies connected to the role of them with the perception and transduction of the signal have been carried out on the rhizoid of seaweed *Chara* (Sievers et al, 1996; Braun, 1997; Braun and Wasteneys, 1998). Thanks to experiments in microgravity condition has been put in evidence the role of actinic microfilaments for the positioning of statoliths in the rhizoid (Buchen et al., 1993). More recently, myosin has been localized on the surface of statoliths from Braun (1996), who therefore has suggested that the system of the acto-myosin has involved in the positioning and the transport of statoliths rhizoid.

The effect of the actin network on statolith sedimentation has been analysed in living columella cells of corn roots. Following a 90° rotation of the root, the statoliths move downwards along the distal wall and spread out along the bottom. The sedimentation of these organelles is completely different when statoliths traverse the complete width or length of the cell. In this case, they move horizontally towards sites of formation of channels and fall down vertically. Analysing the sedimentation of the amyloplasts within the root statocytes revealed the apparent viscosity of the cytoplasm (1 Pa s). This sedimentation implies that the actin network is loose or that it is subjected to constant modification and to the formation of channels in the cytoplasm owing to partial depolymerization in restricted areas.

Many studies have demonstrated that the cytoplasmic free Ca^{2+} concentration ($[Ca^{2+}]$cyt.) is affected by environmental stimuli such as touch, wind, cold, drought, light and oxidative stress. Efflux of Ca^{2+} from the cytosol is mediated by pumps powered by either ATP hydrolysis or a proton motive force. On the contrary, the passive entry of Ca^{2+} into the cytosol is mediated by ion channels. The root cap cells are the most sensitive to gravity stimuli and there is a specificity of a cellular response to Ca^{2+} because of the timing, duration and magnitude of the Ca^{2+} pulse elicited. To demonstrate that cytosolic Ca^{2+} transients are induced by gravitropic stimulation, measurements have been made in groups of young seedlings of Arabidopsis. After gravistimulation, there is an initial $[Ca^{2+}]$cyt. spike (20–25 s) followed by a much longer shoulder that peaks, 90 s after the change in orientation of the seedlings. The spike could be related to the early steps of gravisensing, whereas the shoulder could be related to the movement of the amyloplasts (which takes minutes).

The results obtained recently on calcium transients induced by gravistimulation agree with the hypothesis that the sedimentation of amyloplasts can induce the opening of stretch-activated Ca^{2+} channels (SACs). These organelles are in contact with actin filaments that are connected to the cytoskeleton lining the plasma membrane. SACs might be activated either by tension exerted by statoliths on actin filaments or by sedimentation on bridging filaments (Fig. 9), the nature of which remains to be determined. When SACs are activated, Ca^{2+} in the cell wall can enter the cytosol and be trapped by Ca^{2+}-sensing proteins, leading to an asymmetry in the statocytes. The biphasic Ca^{2+} signature of gravistimulation can be caused by rapid action of statoliths on the actin network and a stronger but slightly delayed action on the bridging filaments lining the plasma membrane. The sedimentation of amyloplasts can open or close SACs because of their structural asymmetry (Plieth and Trewavas, 2002). Complete sedimentation of the amyloplasts should therefore inactivate all channels along the lower wall of the statocytes. This could explain why the shoulder of the calcium signal ends after 15 min which corresponds roughly to the period necessary for the complete sedimentation of amyloplasts. When the root is in the vertical position, the amyloplasts are sedimented on the ER tubules, which keep these organelles away from the peripheral cytoskeleton and prevent any gravitropic stimulation.

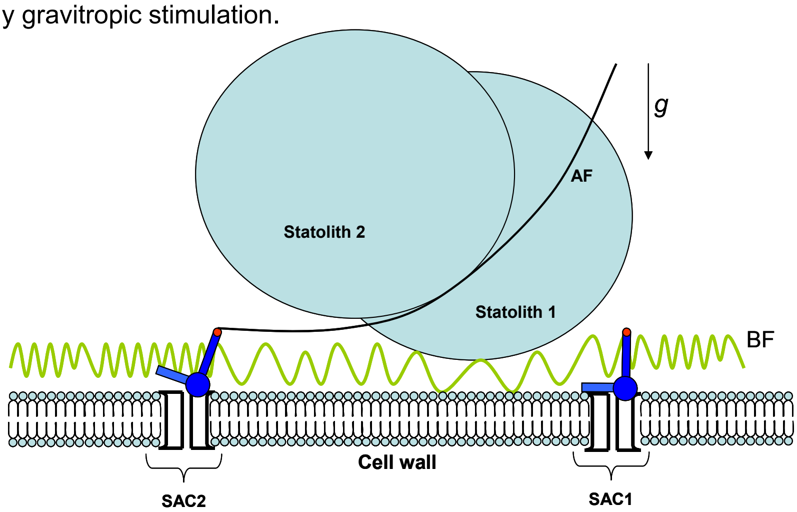


Fig. 9. Model of mechanosensing in root statocytes. The statoliths can open the stretch-activated $Ca2^+$ channels (SACs) by exerting tension on the actin filament (AF; statolith 2) or by pressing on bridging filaments (BF; statolith 1) that link SACs. Activating SACs by pressing on bridging filaments should be more efficient, because statocytes treated with latrunculin B (which perturbs the polymerization of actin filaments) are more sensitive. This treatment could reduce the cytoplasmic viscosity, increase the velocity of statolith sedimentation and hence activate SACs more rapidly. Abbreviations: PM, plasma membrane ((modified from Perbal and Driss-Ecole, 2003).

Gravity signal transmission. Although auxin was suggested to be a signal controlling this differential growth more than 100 years ago, recent evidence has provided solid support to this hypothesis (Muday, 2001). Auxins, of which indole-3-acetic acid (IAA) is the predominant naturally occurring hormone, move through plants by a unique polar transport mechanism (Goldsmith, 1977; Lomax et al., 1995). This polar movement of auxin is from the shoot meristem towards the base of stems, and is a cell-to-cell movement. Polar auxin transport results in an auxin gradient down the length of the stem or hypocotyl, with the highest auxin concentrations found in the regions of greatest elongation (Ortuno et al., 1990). In roots, auxin transport is more complex, with two distinct polarities. IAA moves acropetally (toward the root apex) through the central cylinder, and basipetally (from the apex toward the base) through the outer layers of root cells (Jones, 1998). In graviperception, there is a lateral (downward) transport of auxin from the columella toward the zone of reaction (Fig. X) and this lateral transport is required for the gravitropic reaction. The lateral transport of auxin can not explain the entire process of gravitropic reaction, but it can be considered the primary cause of differential growth. The asymmetric growth has been analysed in detail in roots and it has been shown that the peak of maximal growth is displaced towards the tip in their upper side and towards the base in the lower side during gravireaction.

Electrotropism

Like all living tissues, the cells of plant roots produce an electric field due to the activities of ion transporters. This ion movement creates a flow of current through the tissue and the formation of electrical potential differences across the membrane. As a consequence of these ion transport activities, plant roots generate long-lasting electric fields in the apoplast and rhizosphere (Weisenseel et al., 1992). These electric fields can polarize cells and tissues and can affect growth of the root. For instance, electric fields may generate a lateral asymmetry of ions and hormones in the elongating zone. Application of electric fields can modify the direction of growth of certain plant cells or organs. This phenomenon, known as electrotropism, has been reported in fungi (McGillavray and Gownar, 1986) and algae (Brower and Giddings, 1980) as well as in roots (Fondren and Moore, 1987; Schrank, 1959), and shoots (Schrank, 1959) of higher plants. The preferred direction of growth relative to the applied electric field varies with the type of cell or organ tested and, in some cases, is species dependent. Some studies demonstrate the effect of electric field on the primary root of maize (*Zea mays* L.). Using different intensity of voltage, it has been observed a root curvature towards cathode. Electrotropism was partially inhibited using different concentration of TIBA (2,3,5-triiodobenzoic acid) and NPA (naphthylphthalamic acid), two auxin efflux inhibitors. Another interesting phenomenon was observed adding different concentration of glutamate to the medium. Glutamate is one of the 20 standard amino acids but it is also the most abundant excitatory neurotransmitter in the nervous system. Numerous glutamate receptor-like (GLR) genes have been identified in plant genomes, and plant GLRs are predicted, on the basis of sequence homology, to retain ligand-binding and ion channel activity (Davenport, 2002). Electrotropic effect on root growth was inverted in presence of glutamate: roots turn towards anode and show a corkscrew kind of growth. BMAA, a glutamate antagonist, inhibited this glutamate effect on the root apex electrotropism (Pandolfi et al., 2006).

The correlation between electrical changes and gravitropic curvature suggests the possibility that the curvature of roots in an electric field results from electrical changes within the root that mimic those caused by gravistimulation. This possibility is strengthened by reports that root electrotropism is suppressed by inhibitors of auxin transport (Moore et al., 1987). The effects of electrotropism in solutions of low electrolyte concentration using primary roots of *Zea mays* was also investigated by using a vibrating probe system (Pandolfi et al., 2007). The experimental measurement of ion or gaseous molecules fluxes in roots is fundamental when discriminating normal physiological function from abnormal or stressed states. Measures were taken in three basic anatomical parts of root apex: meristematic zone, transition zone, and elongation zone (Verbelen et al., 2006). The goals of the research led to determine the rapid changes of oxygen fluxes in the different zones of the root related to the electrotropic curvature in maize roots, investigating the early phase of the electrotropic response, from the apply of the current to the visible bending.

Hydrotropism

Roots display hydrotropism in response to moisture gradients, which is thought to be important for controlling their growth orientation, obtaining water, and establishing their stand in the terrestrial environment. However, the molecular mechanism underlying hydrotropism remains unknown. Although some studies have identified the existence of hydrotropism (the directed growth of roots in a moisture gradient), it has proven difficult to differentiate between this response and gravitropism because the latter always interferes with the former here on Earth (Takahashi, 1997). However, by using an agravitropic mutant (i.e. pea mutant *ageotropum*) or clinorotation to randomize the angle of gravitropic stimulation so that plants no longer exhibit gravity-directed growth, positive hydrotropism in roots has been demonstrated (Jaffe et al., 1985; Takahashi et al., 1991; Takahashi et al., 1996). This rediscovery of a genuine hydrotropism in roots enabled us to elucidate some physiological aspects of hydrotropism and its universality in a wide range of plant species (Takahashi, 1997), but the underlying mechanisms that regulate hydrotropism remain unknown. Although the ecological role of hydrotropism in nature still needs to be clarified, the root response is thought to be important for finding water. Considering that limited water availability and/or precipitation affects agriculture and ecosystems throughout the world, understanding the molecular mechanisms mediating hydrotropism in roots could lead to methods for improving crop yield and biomass production (Kobayashi, 2007). Compared with gravitropism, hydrotropism has not been popular in genetic studies because of the difficulty of establishing a large-scale screening system that provides an appropriate stimulus–response interaction. Therefore, the implementation of a screening procedure for the isolation of mutants with aberrant responses to water potential gradients is remarkable (Eapen et al., 2005). Putative mutants are selected based on their inability to develop a positive hydrotropic curvature and their failure to sustain continuous growth in the severe water-deficit conditions of the medium – this is important for distinguishing hydrotropic mutants from drought mutants. In our screen, we found only two negative hydrotropic mutant alleles (including one that germinates poorly). This is perhaps a consequence of either the screening medium (for instance, the threshold water potential for hydrostimulus perception was not developed in the plate) or of the low number of loci involved in the signaling mechanism of water sensing.

Synopsis of plants characteristics

- Plants are photosynthetic, eukaryotic, multicellular organisms characterized by an aerial part and a root system, with a peculiar directional growth in response to external stimulations such as *light (phototropism), gravity (gravitropism), touch (thigmotropism)* or *water/humidity gradient (hydrotropism).* The combination of these mechanisms allows plants to overtake hostile or inaccessible environments and colonize the soil.

- **Root system and root tips: sensing, transducing and processing environmental stimuli**. The root system, which is the plant organ devoted to soil exploration, is composed by thousands of root apices or tips. Each root tip can get information from the environment by their embedded *sensors*, *transduces* and *processes* them to direct the growth towards regions of the soil with the best minerals and water availability.

- **The transition zone. Sensors, actuators, processors: a command centre?** Root apices are composed of three distinct zones. The separation of *division* and *elongation* regions in root apices permits to identify the *transition zone*, included between the two other regions, peculiarly able to detect more than 10 chemical and physical parameters from the surrounding environment, due to the unique cytoarchitecture of the cells, with centralised nuclei surrounded by perinuclear microtubules radiating towards the cell periphery. This configuration should be optimally suited for both the perception of signals and their transmission towards the nuclei, acting at the same time as ***sensors*** and ***actuators***. Transition zone is also a sort of *processor* or *command center* which processes sensory information from the surrounding environment, stores memories and takes decisions about future exploratory and adaptive root behaviour. The *command center* needs a huge amount of ATP (the main energy source for plant metabolism) consumption.

- **Soil penetration.** Plant roots may exert **pressures of up to 1 MPa** in order to penetrate hard soils, and have evolved root caps which protect the root meristem from physical damage or abrasion by soil particles. Root cap consists of mucilage-secreting cells. The products of mucilage secretion are mainly composed by carbohydrates (94%) and proteins that allow its integrity creating a sort of polymer network that entraps water (up to 99.9% of wet weight). Interestingly, the physical properties of the root cap mucilage change considerably with changing degrees of hydration: upon loss of water, the surface tension of the mucilage is strongly reduced, whereas its viscosity increases. When wet, mucilage expands to 1000 times its dry volume. These findings support the idea that mucilage plays a major role in the maintenance of root-soil contact in drying soil: as the surface tension decreases, the ability of the mucilage to wet the surrounding soil particles becomes greater, and as the viscosity and elasticity decrease, the rhyzosphere structure is stabilized and hydraulic continuity is upheld. The root cap must, therefore, play an important role not only in protecting the root meristem from damage, but also in determining the mechanical interaction between the root and the soil, namely the mode of soil deformation and the root–soil friction.

- **Root growth**. Mechanistic understanding of root growth needs to take into account the architecture of the root growth zone. As a given region of the plant axis moves away from the apex, its growth velocity increases (the rate of elongation accelerates) until a constant limiting velocity is reached equal to the overall organ extension rate. In a rapidly growing maize root, a tissue element takes about 8 hours to move from 2 mm (the end of the meristematic zone) to 12 mm (the end of the elongation zone), with a main velocity of *3 mm* h^{-1}. In physical terms, cell growth can be defined as an irreversible *increase in cell volume and surface area*. Plant protoplasts are characterized by an univocal structure and organization, as they are surrounded and encased by a rigid, but expandable, cell wall with elastic characteristics. The cell wall is infiltrated with water containing only a very low osmotically active amount of solutes. This situation enables the formation of a large difference in osmotic pressure ($\Delta\pi$) between the apoplastic (outer) and the symplastic (inner) space (0.6-1.0 MPa), compensated by a hydrostatic pressure, named *turgor* (*P*), of equal value when the cell is in a fully turgid state. The cell can hence be described as a simple osmometer that can enlarge by water uptake powered by a difference in water potential ($\Delta\Psi$) between protoplastic and simplastic space.

- **An example of the response chain perception > transduction > processing: the gravitropism.** In the earth gravitational field the roots grow towards the bottom for better supplying water and nutrients (gravitropism). As gravity is a physical force which only act on masses, several organelles or particles which are denser or lighter than the cytoplasm can be involved in gravity sensing. The stages of gravitropism in plants can be divided into: *perception of the signal*, *transduction*, and *response*. In roots, gravity perception occurs in the root tip and the response (differential growth) in the zone of elongation, involving the plant growth hormone *auxin*. In higher plants, there is a temporal and spatial separation between perception and response, and the signal must be transmitted over a relatively large distance. Two models are proposed for gravity perception. (1) The *starch-statolith hypothesis* proposes that perception is mediated by dense organelles (statoliths, amyloplasts located in the root tip cells). (2) The *protoplast pressure hypothesis* suggests that the entire mass of the cytoplasm participates in perception. The mechanism through which the plant transforms the indication of cell position into a biochemical message is still under discussion. An important question in sensor physiology is the determination of the threshold dose of a stimulus in order to provoke the reaction of the plant organ. For the gravireaction of plant roots, it has been noticed that gravitational stimuli which last 1 s (perception time) can be actively perceived by plant roots, but one single stimulus is not sufficient to determine any organ response. So, a minimum time of continuous stimulus must occur (*presentation time*). When a root is subjected to a change of orientation in the gravitational field, the stimulus is perceived in less than 1 s. However, this 1 s stimulus is not sufficient to induce a gravitropic response – it must be repeated about ten times (or the stimulation must last 10 s) to initiate an asymmetrical signal within the statocytes (*transduction phase*). This leads to a downward lateral movement of the growth hormone auxin (*transmission phase*) that is the cause of the differential growth occurring after a latent time of 10 min. The four phases are represented in sequence but *perception*, *transduction* and *transmission* can persist for the whole period of the gravitropic reaction.

WP3. Technological assessment

State of the art on actuators for space application

Space applications require safe and robust actuation principles, capable to withstand harsh environment. There is plenty of reports in literature about actuators for space, novel actuators (Jänker and Claeyssen, 2006) and bio-inspired actuators for space applications (Ayre, 2003; Ayre, 2004).]. Aim of this paragraph is to investigate interesting actuation systems, capable to offer similar features to the osmotic actuation, in particular regarding the low-heating penetration capability and low-power consumption and thus to be competitive with the proposed osmotic actuation principle. In the following, some of the most interesting actuation technologies are quickly surveyed.

Electroactive Polymers (EAPs)

EAPs is a general term describing polymers that respond to electrical stimulation . There are two principal classes of EAPs:

- Electronic EAPs: ferroelectric polymers, dielectric EAP or ESSP, electrostrictive polymers and Liquid Crystal Elastomers (LCE). They generally require high voltages, even if stacks of thin layers interleaved with electrodes can drastically reduce the actuation voltage. The main constraints for their application in space (e.g., planetary exploration) are limited temperature range working capability and poor chemical stability in harsh environments. Furthermore, in order to have appreciable displacement, they need very high voltages.
- Ionic EAPs, in which actuation is caused by the displacement of ions inside the polymer. Examples are: Ionic Gels (IGL), Ionic Polymer-Metal Composites (IPMCs), Conductive Polymers (CP). They require less voltage for actuation compared to dielectric EAP but the ionic flow implies higher electrical current needed for actuation. They generally features short life and low actuation force. Furthermore, some of them, e.g. IPMC requires wet environments for working. While displacement is considerably large, the actuation force is so far relatively low (bulky systems have reached a force capability comparable to that of human muscles so far). Finally, except for CP, they don't hold strain under DC voltage

Possible application of these materials and performance for planetary applications have been investigated yet (e.g., Bar-Cohen, et al., 1999).

Shape Memory Alloys (SMA)

SMA, also known as a smart alloy, memory metal, or muscle wire, is an alloy that "remembers" its shape. After a sample of SMA has been deformed from its original crystallographic configuration, it regains its original geometry by itself during heating (one-way effect) or, at higher ambient temperatures, simply during unloading (pseudo-elasticity or superelasticity). Usually the heated condition is achieved by the Joule effect, but there other techniques, including exposition to radiation or convection. SMA are commercially available in wires in several diameters, typically ranging from 50μm to 250μm, in wedges, or it can also be sputtered in order to form custom geometries especially for micro and nano actuation. One of the most diffused SMA, Nitinol (an alloy of Nickel and Titanium) has low energy conversion efficiency (around 5%), and a work output of around 1 kJ/kg. In general it can not be seen as a competitive actuator, in particular it needs relatively high power consumption for relatively low work output. Space applications have been introduced for example in (Godard, et al., 2003).

Piezoelectric Materials

They are materials capable to produce and internal stress and/or strain when an electric field is applied (and viceversa). For instance, lead zirconate titanate (PZT) crystals exhibit a maximum shape change of about 0.1% of the original dimension. Typical applications of piezo actuators in space are in telescopes, optics and fine instruments positioning. As a disadvantage they perform low displacement with relatively high voltages.

There are some other interesting actuating technologies that could be applied to the plantoid roots. In the following two classes of materials are shortly presented, which are:

- *electrorheological materials*
- *magnetorheological materials*

Electrorheological (ER) fluids consist of solid particles suspended in an insulating oil. They can be reversibly transformed from a liquid-like state to a solid-like state by applying an electric field, causing their viscosity to increase dramatically (Halsey, 1992; Chen, et al., 1992). A yield stress as high as about 27 kPa in an applied electric field of 3 kV/mm with low leakage current can be achieved (Zhang, et al., 2002) wide dynamic ranges in temperature and shear rate, and a long-term stability against sedimentation.

Magnetorheological (MR) fluids act in a similar way to ER fluid, but in presence of a magnetic field. A typical MR fluid consists of 20-40% by volume of relatively pure, 3-10 micron diameter iron particles, suspended in a carrier liquid such as mineral oil, synthetic oil, water or glycol. MR fluids made from iron particles exhibit maximum yield strengths of 50-100 kPa for applied magnetic fields of 150-250 kA/m.

Considering their working principle and performances, it is quite clear that the mentioned actuating principles are not likely to be able to directly dig the soil, especially for harder terrains. However they could be an interesting technology to be employed as a mean for making roots stiffer wherever and whenever is necessary. In particular, during root extension and push, it can be important to make the eventual mechanical joints of the root stiff to at least partially withstand the push, avoiding too large bending or back reaction of the root structure, up to the surface main module. Stiff joints could help in anchoring the root into the terrain, allowing the push of the part of the root to be extended to be completely and rigidly propagated to the apex for forward movement.

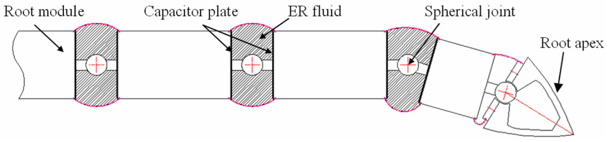


Fig. 10. A rough schematic of a plantoid root concept, with a possible application of Electrorheological (ER) fluids in order to fabricate active joints between root stages. Establishing a voltage potential between the two capacitor plates, the ER fluids become stiff blocking the spherical joint movement.

Among the advantages that these actuators can offer to the plantoid there is compactness, low power consumption (current leakages are generally low, refer e.g. to Zhang, et al., 2002), although long duration of the actuation is an issue regarding this aspect), capability to withstand low temperatures, and quick reversibility and speed of the actuation is also possible, even if probably not necessary. However, among the disadvantages it has to be highlighted the high voltage requirements in order to achieve suitable solid-like states of these actuators, and, finally the not so high yield strengths it is possible to obtain.

Electro-Osmotic Actuation principle and preliminary study results

Bio-inspiration from plant root actuation

The movement/growing process in plant's roots is essentially actuated by two mechanisms: roots grow by a mechanism of cell division in the apical meristem just behind the tip, and by a cell elongation in a zone just behind the apex (Clark et al., 2003).

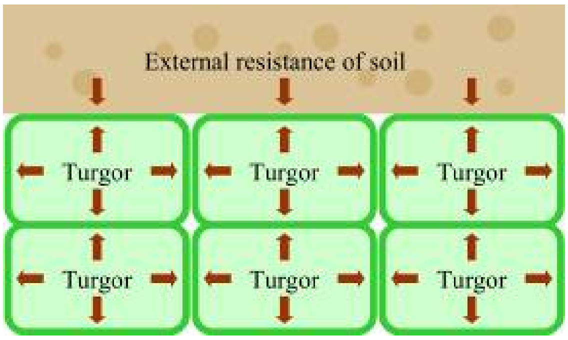


Fig. 11. Schematic diagram showing pressures acting in the epidermis of a root growing in soil

Water influx into cells generates turgor pressure (*P*), which provides the driving force for elongation and hence root growth (Fig.5). The osmotic potential within cells in the elongation zone of a root generates the water potential gradient for water uptake into the cell (Bengough et al., 1997b). Turgor pressure (*P*) is generated as the influx of water, which presses the protoplast against the confining pressure of the 'rigid' cell wall. Turgor pressure (*P*) is then given by

$P = \pi_o - \pi_I$ (1)

where π_i is the osmotic potential inside the cell, and π_o is the osmotic potential outside the cell. This equation assumes that water influx into the cell is not limiting to growth, there is no transpiration tension in the wall, and solutes have a reflection coefficient near unity. The water relations of expanding cells have been reviewed in detail (Pritchard, 1994). A typical value for the vacuolar osmotic potential (equal toπ_i) inside a cell in the growing zone of an unimpeded root grown in hydroponics is around -0.7 MPa (about 7 Atm).

Classically, following experiments on cell walls isolated from giant algal cells (Probine and Preston, 1962)., cell elongation has been regarded as plastic flow of the wall material under stress (Lockhart, 1965).

Where existing soil channels are smaller than the root diameter, roots must exert a growth pressure in order to displace soil particles, overcome friction and elongate through the soil. The growth pressure (σ) is equal in magnitude to the soil pressure that opposes root growth. In a root tip elongating through soil, cell turgor pressure (*P*) generates the growth pressure (σ), which results from the difference between *P* and the wall pressure (*W*) so that (Greacen and Oh, 1972).

$\sigma = P - W.$ (2)

In unimpeded roots, σ is by definition zero and *P* is balanced by *W*. When roots are completely impeded and cannot elongate, σ attains a maximum value (σ_{max}). Roots exert growth pressures in both radial and axial directions, but we will only deal with axial growth pressures here. When water transport into growing cells is not limiting, root elongation rate can be considered in terms of a simplified Lockhart equation (Lockhart, 1965), as modified by Greacen and Oh (Greacen and Oh, 1972) to take account of the soil impedance:

$R = m[W - Wc] = m[P - Wc - \sigma]$ (3)

where *R* is the elongation rate, *m* is a cell wall extensibility coefficient, *W* is the wall pressure, *Wc* is the cell wall yield threshold, *P* is the turgor pressure and σ is the soil impedance (or growth pressure).

As showed the mechanism of root cells expansion is driven by the osmotic pressure. This osmotic expansion mechanism could be successfully used to design a new class of electrochemical actuators able to fit the necessary requirements (low power, slow actuation, high force/pressure). The concept of this new electro-osmotic actuators inspired by plants will be presented in the next sections.

The electro-osmotic actuator concept

The idea of a new electro-osmotic actuators inspired by plants is based on the control of the osmotic pressure in a suitable "cell" containing liquid or gel matrix, by applying a small electrical current, and using the resulting osmotic pressure to induce an actuation (electro-osmotic actuation). This kind of actuator will have the following features:

- Very low power consumption (small current requirement);
- Actuators response with high modulation capability (high accuracy, resolution);
- Slow actuation;
- High force/pressure actuation.

The theoretical working principle of the plant inspired electro-osmotic actuator is now described in detail. Fig.2 shows two cells connected through a semi-permeable membrane. Each cell has a metallic electrode (M and N) immersed in a water solution of own ions (M+ and N+, respectively molal concentration c_{M^+} and c_{N^+}) in presence of a counter-ion (X-), required for the electroneutrality of the solution. If the concentration of the two cells is different, an osmotic pressure is generated across the semi-permeable membrane. In this case the osmotic pressure π is given by the Van t'Hoff's low (Atkins, 1997):

:

$$\pi = \Phi \cdot \left(c_{M^+} - c_{N^+}\right) \cdot RT \qquad (4)$$

where R is the gas constant (0.082 l atm mol^{-1}), T the temperature (in Kelvin) and Φ is the molal osmotic coefficient, a coefficient that takes into account the non-ideality of the solution.

On the base of the equation (4) it is possible to evaluate the theoretical pressure achievable by using the osmotic mechanism. Considering a difference of electrolytic concentrations between the two cells of 1M (a realistic achievable concentration) and considering the molal osmotic coefficient Φ = 1, we obtain a pressure of about 2.5MPa (24 Atm).

The force that can be exploited by this pressure is obviously essentially related to the geometry and dimensions of the transduction mechanism. Few examples of devices that use pure osmotic actuation mechanism are available in literature (Su and Lin, 2004).

Fig. 12 shows a simplified ideal mechanism that should convert the difference of pressure between cells in a displacement.

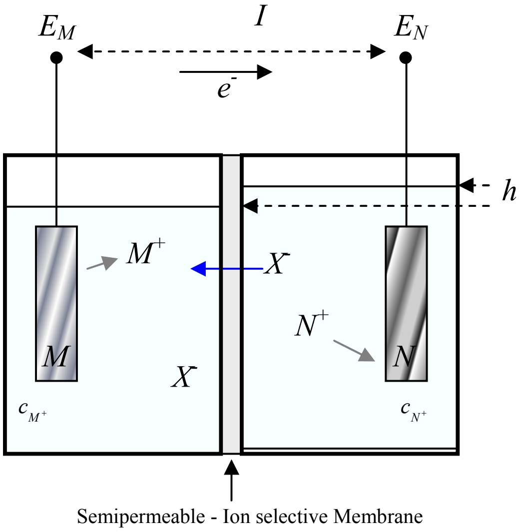


Fig. 12. Schematic diagram of osmotic cells

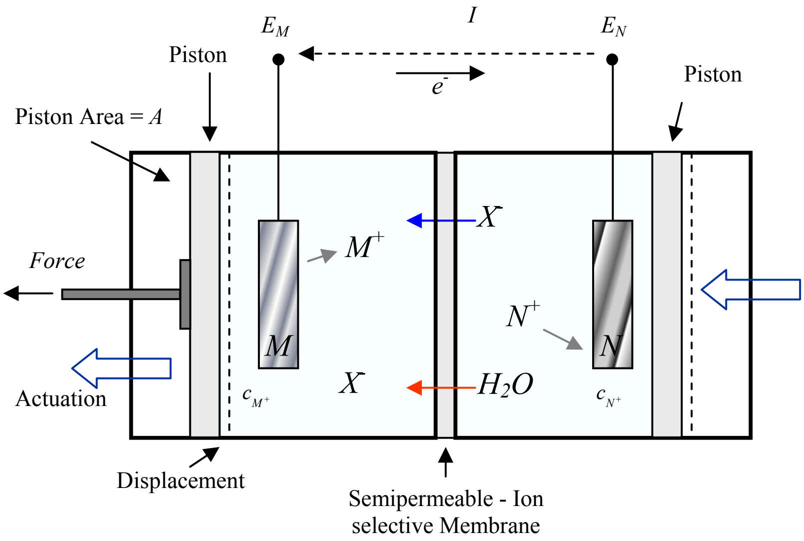


Fig. 13. Schematic of hypothetic electro-osmotic actuator

In the simple case of a cylinder with a base of surface *A* the calculation of force achievable by the mechanism is trivial, being the force *F* given by:

$$F = \pi \cdot A \tag{5}$$

In a cylinder with a base of 1cm^2 in absence of friction, 2.5 MPa of pressure can produce 25 Kg of force. In this proposed study, the displacement of the "piston" happens because the water flows through the semi-permeable membrane from the cell with minor concentration to the cell with greater concentration, decreasing in this way the concentration differences between cells. This process occurs until the concentration in the two cells becomes the same or until the external forces balance the actual osmotic force.

In order to control the displacements and the forces generated by such kind of osmotic actuator it is necessary to control the ion concentrations on the cells. This goal can be achieved by using electrochemical reaction. If the membrane that connects the two cells allows the passage of some kind of ions (not simply a semi-permeable membrane), thus the cells can work as a battery (it can generate an electrical current) or conversely work as electrolytic cells (applying a suitable external voltage). Fig.14 shows the different steps of the electro-osmotic mechanism.

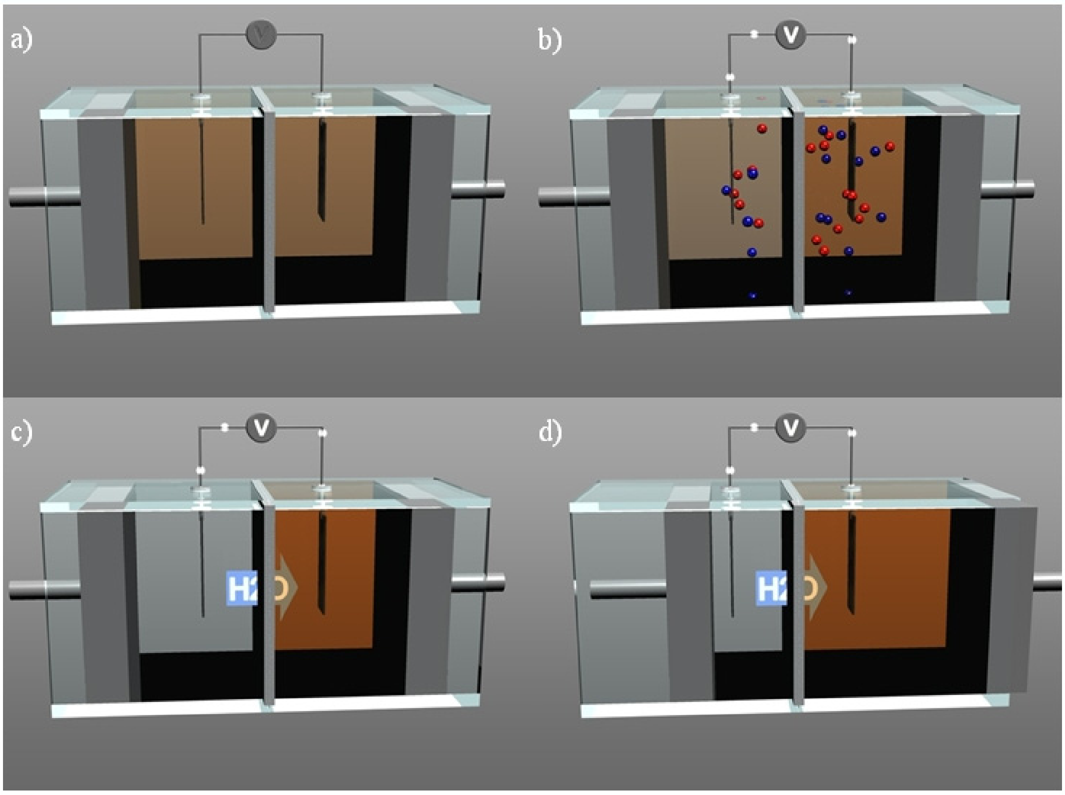


Fig. 14. a) The two cells have the same ion concentration; **b)** Electrolytic process induces a change in ions concentration; c) Osmotic pressure is induced across the two cells; d) The osmotic pressure drives the actuation.

The two reduction semi-reactions involved in the process are:

$$M^{+} + e^{-} \rightarrow M \tag{6}$$

$$N^{+} + e^{-} \rightarrow N \qquad \left(E_N^0\right) \tag{7}$$

The electric potential across the two electrodes ΔE is given by the Nerst equation [21]:

$$\Delta E = E_M - E_N = \left(E_M^0 - E_N^0\right) + \frac{RT}{F}\ln\left(\left[c_{M^+}\right]/\left[c_{N^+}\right]\right) \tag{8}$$

where E_M^0 and E_N^0 are the semi-reaction standard red-ox potentials, and *F* is the Faraday constant (*F* = 96,485.34 s A mol^{-1}).

Providing a suitable current to the cells it is possible to control the concentrations of the electrolytes. The electrical potential needed to induce a current in the cells is in the order of few Volts (at considered concentrations). The induced variation of electrolyte concentration within a cell, after application of a current *I* for a time *t*, is given by the following equation:

$$c_{M^+}(t) - c_{M^+}(0) = \frac{I \cdot t}{F \cdot V} \tag{9}$$

where *V* is the volume of the cell.

It is important to underline that in front of the variation of concentration of the active electrolytic chemical species (in this case the cations M^+ and N^+) due to the red-ox process, the ionic transport of counter-ions (in this case X^-) must be allowed (otherwise electrode gets polarized), by means of a

ion selective membrane. Clearly the active ionic species must be blocked by this membrane otherwise a net variation of concentration is not achievable.
The current flowing within the two cells depends ideally from the Ohm Low

$$I = \left(\Delta E_{ext} - \Delta E - E_{sovr}\right) / R \qquad (10)$$

where ΔE_{ext} is the external potential applied, E_{sovr} is the over potential (that depends from electrodes type, geometry and work conditions) and R the resistance of the cell. The applicable external potential on the cells is limited, because a too high voltage can create collateral unwanted reactions (e.g. water electrolysis). The resistance of the cells depends both from the ionic concentration (ionic transport mechanisms) and from the resistance across the membrane, but in the operative conditions the membrane resistance is much more important (usually it is very high). For this reason it is not possible to apply an arbitrary current. Esteemed applicable currents range from mA to nA. However, any inferior limit for the applicable current is theoretically foreseen.
To create by electrolysis a concentration of 1M in a cell of 1 cm^3 of volume, in term of total charge must be applied 0,0005 Faraday (considering the counter-ion transport, which corresponds to 48.25 Coulomb or 13.4 mA h. 1 Faraday = 96485 Coulomb). In order to obtain this quantity of charge, a current of 1 mA spends 13.4 h.
The time required to exploit the actuation does not depend only by the generation of a difference of concentration between the electro-osmotic cells: in fact, once the difference of osmotic pressure is generated, the water contained into the cell at greater pressure flows through the osmotic membrane into the cell at minor pressure. In particular the water flow through the membrane J_W (m^3/s) is given by the follow equation (Ludwigat al., 2002).

$$J_W = K \cdot A \cdot \left(\rho \Delta \pi - \Delta P\right) \qquad (11)$$

where K (m/s Pa) is the permeability of the membrane respect to water, A the effective surface area of the semipermeable membrane, ρ the dimensionless reflection coefficient of the membrane, $\Delta \pi$ is the osmotic pressure difference and ΔP the hydrostatic pressure difference. A very few studies and researches on membranes specifically designed for osmosis are available. In this proposal, it was necessary to examine reverse osmosis membranes. In particular, a realistic reverse osmosis membrane coefficient should be in the order of 10^{-13}-10^{-14} m/s Pa. On the base of this coefficient it is possible to estimate roughly the time required to perform an actuation displacement of 1 mm in the ideal actuator showed in Fig.7, in presence of a difference of concentration of 1M, corresponding to an osmotic pressure of 2.5 MPa (opposite forces are supposed to be null). In these ideal conditions the actuation is performed in 10-100 hours, depending from the osmotic membrane coefficient.

These very preliminary calculations show that the two processes (the electrolytic change of concentration and the osmotic water flow) can have a comparable timescale; in this condition the limiting process (the slower) is essentially due to the working conditions like pressures required and applicable current.

Electro-osmotic actuation main issues and preliminary experimental results

The idea of the electro-osmotic actuator is presented and evaluated in a preliminary way on the base of simplified theoretical models shown. However, in order to verify the effective feasibility of the proposed solution a number of issues need be investigated in detail. The most important of these issues are now discussed in details on the base of the work carried out until now.

→ Study on suitable chemicals species to use on the electrolytic process
The choice of the chemical species to be used in the electrolytic process is a key point for the realization of the electro-osmotic actuator. It is necessary to find a couple of red-ox substances that can generate a fully reversible electrolytic process. The red-ox reactions involved in this electrolytic process must avoid the generation of by-products, gaseous substances and high aggressive

substances (e.g. strong acids). The red-ox potentials involved must be enough low to avoid the water hydrolysis (with consequent development of gas).

On the base of these considerations the best electrochemical solution is the use of concentration cell based on electrodes of the first kind, in which the red-ox reaction involves only electrons and not other chemical reactions (as previously introduced $M^+ + e^- \to M$).
In this sense a suitable electrode is lead (Pb).
Pb reacts electrochemically in the following way:

$$Pb^{2+} + 2e^- \to Pb \tag{12}$$

The potential standard reduction for the electrode (E^0_{Pb}) at 25°C for this semi-reaction is -0.126 V. The same reaction is used in both the cells; depending to the sign of the applied potential, in one cell will happen the reduction ($Pb^{2+} + 2e^- \to Pb$), while in the other one will happen the oxidation ($Pb \to Pb^{2+} + 2e^-$). The voltage necessary to induce the reaction (V_R) depends from the concentration of Pb^{2+} in the two cells. In particular, substituting the reaction parameter in equation 8 and summing the two semi-reaction we obtain:

$$V_R = E_{Pb} - E_{Pb} = \left(E^0_{Pb} - E^0_{Pb}\right) + \frac{RT}{nF}\ln\left([Pb]_A/[Pb]_B\right) = \frac{0.059}{2}\log\left([Pb]_A/[Pb]_B\right) \tag{13}$$

To avoid the electrolysis of the water it is important that V_R is less that the red-ox potential of the water V_{H_2O}. This potential is given from the two semi-reaction potentials:

$$H_2O \to \frac{1}{2}O_2 + 2H^+ + 2e^- \qquad E^0_{OH^-/O_2} = +4.01V \tag{14}$$

$$\frac{1}{2}H_2 + OH^- \to H_2O + e^- \qquad E^0_{H^+/H_2} = 0V \tag{15}$$

In this case the two semi-potentials are depending from H^+ and H^- concentrations (and thus from pH), but V_{H_2O} is constant, in fact:

$$\begin{aligned} V_{H_2O} &= E_{OH^-/O_2} - E_{H^+/H_2} = \left(E^0_{OH^-/O_2} - E^0_{H^+/H_2}\right) + \frac{RT}{nF}\ln\left(1/\left[H^+\right]\left[OH^-\right]\right) = \\ &= 0.401 + 0.059\log\left(1/\left[H^+\right]\left[OH^-\right]\right) \end{aligned} \tag{16}$$

being $\left[H^+\right]\cdot\left[OH^-\right] = K_W = 10^{-14}$ (17)

we obtain $V_{H_2O} = 0.401 + 0.826 = 1.227V$ (18)

Tacking into account equations 14 and 19 it is possible to calculate the maximum concentration difference (or better ratio) between the two lead cells, and thus obtain the intrinsic limit of the electro-osmotic actuation.
A simple calculation shows that this limit ratio is so low , $([Pb]_A/[Pb]_B) < 10^{-40}$, that practically the water electrolysis happens only when the reaction is quantitative and the Pb^{++} is not present in one of the two cells anymore.

Concerning the counter-ion for the solution, the choice is conditioned by some main factors:

- ion red-ox potentials: the possible interfering semi-reaction potentials must be less (more negative) of the potential of the Pb++/Pb semi-reaction, to avoid unwanted reaction.
- ion volume: the counter-ion must be large to facilitate the osmotic process
- ion mobility: the ion must be mobile to facilitate the charge transfer through the ion-selective membrane, reducing the resistance of the solution

On the base of these considerations a good choice to implement the system seems to be $Pb(ClO_4)_2$ or in alternative $PbSO_4$.

→ Study on suitable membranes to use on the electro-osmotic process

Membranes are the last (but not the least) critical point to evaluate to the feasibility of the electro-osmotic actuation. Suitable membranes should have double functionalities: they should have both ion-selective membrane characteristics (to allow the ionic transport) and osmotic permeability features for water transport. On the base of the type electrolytic reaction used, the membrane must be selective to the cations or to the anions. If it was not possible to combine all these features in a membrane, the use of a composite membrane will be evaluated.
The most important parameters that lead the choice of the membrane are:

- Electrical resistance of the membrane: it affects the maximum ionic flow achievable; lower values of resistance allow higher values of currents and more quick variation of electrolytic concentrations;
- Permeability of the membrane: it affects the water flow rate and thus the velocity of actuation of the actuator.

→ Exploitation of electro-osmotic principle
The most important issue to investigate is the exploitation of the proposed electro-osmotic principle, which consists in transforming the pressure generated by osmosis into a mechanical actuation. Studies have to be carried out in order to find the best solution in terms of mechanisms, geometry and materials. Ratio between active surface and cells volume must be carefully considered to optimise forces and displacement. Effects of cells expansion (or compression) during the actuation must be also taken in consideration, because it alters directly the concentration of the electrolytic solutions and consequently the generation of forces.

Some very preliminary experiments have been performed in order to check the performances of some kind of membrane, both iono-selective and osmotic. In particular membranes of two different manufacturers have been tested: Sterlitech for the osmotic membranes and Residion for the anionic membranes.
From the test the most suitable membranes resulted the ones used for reverse osmosis (RO); different kind of RO membranes have been tested:
- YMCESP3001 CA (Cellulose Acetate) RO CE Membrane
- YMCFSP3001 CA (Cellulose Acetate) RO CF Membrane
- YMADSP3001 Polyamide RO AD Membrane
- YMAGSP3001 Polyamide RO AG Membrane

The Cellulose Acetate membranes are the most used in the desalinization applications and are characterized by a relatively high water flow, but they present not so large rejection sizes (92-97%). Conversely the polyamides membranes have greater filtration capability (99.5%), but are characterized by a lower flow rate.
The osmotic membranes have been tested in order to evaluate the capability to reach high differential pressure between two chambers separated by the membranes. In a first set of experiments performed on these membranes we have obtained that with 2 chambers of 15 x 60 x 60 mm, 1 molar solution of NaCl in H_2O and 12.5 mm^2 exchange surface, the highest pressure has been obtained with Cellulose Acetate RO CE membrane, 11 atm reached in 3 hours. This pressure is comparable with the growing pressure generated by the plant roots. Similar test will be performed with lead in order to evaluate the maximum pressures obtainable with a controlled process.

Concerning the anionic exchange membranes, two types have been taken into account:
- IONAC MA-3475
- IONAC MA-7500

Both the membranes are characterized by a thickness of 0.4mm. They show a good selectivity response and relatively low resistance (allowing reasonable ionic flows), making them useful for our application.

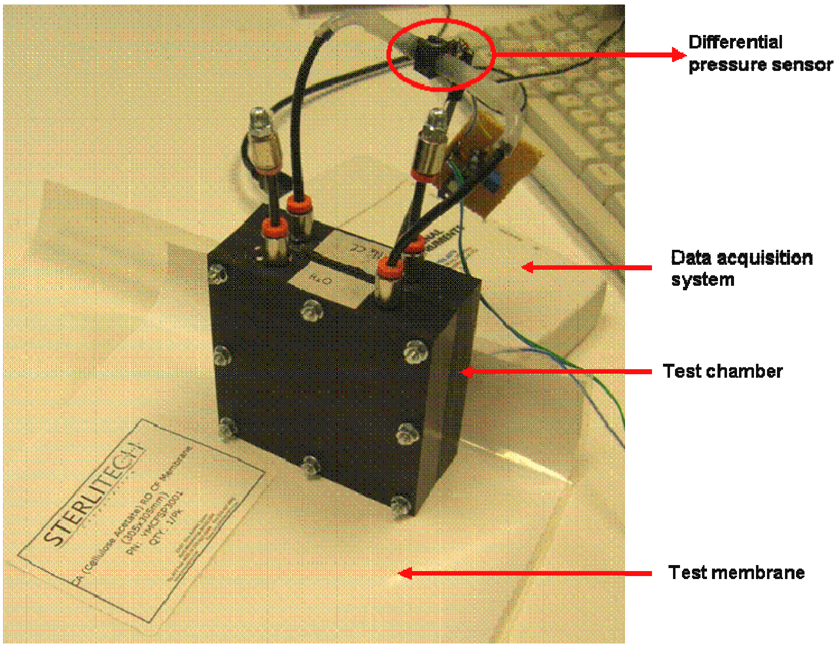


Fig. 15. The experimental set-up for testing the functionalities of the selected membranes.

→ Space constrain considerations and possible solutions

In order to make feasible the use of the electro-osmotic actuator in space related scenarios several considerations need to be done. Materials and working principle must be space-compliant and must suit the application target.

Dramatically different conditions can be found between the environments introduced in the Scenario paragraph. As a consequence, each specific operation environment should be considered in order to define the concept and possibly preliminary designs of the plantoid actuators.

Temperature and pressure, in particular, are the primary parameters to take into account for the electro-osmotic principle. Not only because lower the temperature value is lower the osmotic pressure, according to eq. (4), but also because environment conditions causing the chemical solution to freeze can occur, causing interruption of any actuation dynamics. Pressure is another factor affecting liquid state. Using water as actuation mean in the osmotic process it is quite straighforward to conclude, according to significant temperature and pressure values introduced for each of the three scenario and reported in Fig. 9, that osmotic chambers need to be pressurized and a system heating technology or some anti-freezing compound (demonstrating of course this does not affect the osmotic principle, or eventually it does only partially) need to be introduced. Mars is the less demanding environment from this point of view, offering an ambient quite similar to that present on Earth. A pressurization of about 5 times the Mars atmosphere and a temperature increasing of about 60-80 K should be enough to keep pure water at the liquid state. For South polar craters on the Moon and asteroids the requirements would be much higher: total pressurization of the roots up to at least 10^3-10^4 Pa and an increasing of at least 200 K, for the Moon, and about 100 K, for the asteroids, during the most favourable conditions, as explained in the Scenario paragraph.

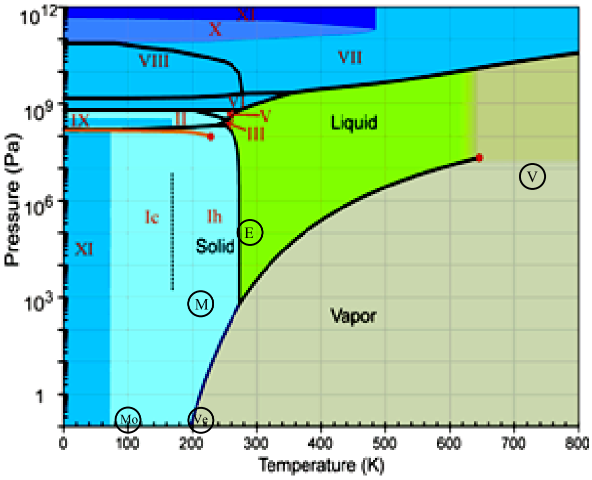


Fig. 16. Phase diagram of water, with the mean surface conditions on Earth (E), Mars (M), and Venus (V), and most favourable condition on south polar Moon crater (Mo) and on Asteroid surface, e.g. Vesta (Ve).

Several anti-freeze compounds can be found in the technical state of the art. For example, Ethylene Glycol is widely used. Commercial antifreeze formulations, in 1:1 dilution can lower the water freezing point down to -40°C (at 1 atm). For instance, at least in principle, the addition of an antifreeze substance to the electro-osmotic cells should not interfere with the osmotic process.

Being the system inspired by plant, it is interesting here just to observe that one of the most serious problems that natural plants have to face is, actually, water ice crystal formation in the cells when temperature decrease, e.g. in winter time. This results in plant tissue death. Plants have two strategies to face freezing: avoid it or tolerate it. Following the former strategy, a plant has several different ways to evade freezing. It can build up insulation, has its stem close to the ground, use the insulation from snow cover, and supercool. When supercooling, water is able to remain in its liquid state down to -38°C. After water reaches -38°C, it spontaneously freezes and plant tissue is destroyed. This is called the nucleation point. The nucleation point can be lowered if dissolved solutes are present.

If a plant chooses the latter strategy, i.e. tolerance, it can apply several different techniques to tolerate freezing. Some plants allow freezing by allowing extracellular, but not intracellular freezing. Plants let water freeze in extracellular spaces, causing a high vapor deficit that pulls water vapor out of the cell. This process dehydrates the cell and allows it to survive temperatures well below -38°C.

While the second strategy cannot be useful for the plantoid, supercooling could be an interesting bio-inspired strategy deserving further study.

The plantoid could manage cold environment during the day, while letting actuators freeze during night and de-freeze during the morning (actuation membranes can be switched off as soon as temperature goes down: their expansion could compensate expansion of the water ice without hopefully damaging the osmotic and anionic membranes).

Review on soil penetrating systems

In principle we find two different needs to penetrate the surface of, e.g., a planet. The first application lies simply in anchoring spacecraft tightly to the ground in order to withstand lateral loads such as strong winds, but also the negative effects of low gravity, i.e. bouncing off. Another reason for ground penetration is mission-related: probing the ground allows collecting a broad variety of data or even samples and hence, is in many cases the core of the mission. In this chapter, we will give a brief and by any means comprehensive overview of common ground penetrating solutions that serve as a benchmark for the SeedBot concept.

Anchoring

Although generally important, pure anchoring systems are not well represented within the scientific literature available in the space sector. Mostly they consist of metallic thorns or nail like structures that extrude rigidly from the landers' landing pads and are rammed into the ground during landing. A student team of the University of Idaho has worked together with the NASA/AMES-Research centre on a more elaborated anchoring-concept. They evaluated a row of ideas and finally focussed on the most feasible ones for a further investigation. They finally propose a screw-like driller which is deployed from the landing pad penetrating the ground (Fig. 17). In general, their design choices are based on existing technologies and a rather short time frame for full realization.

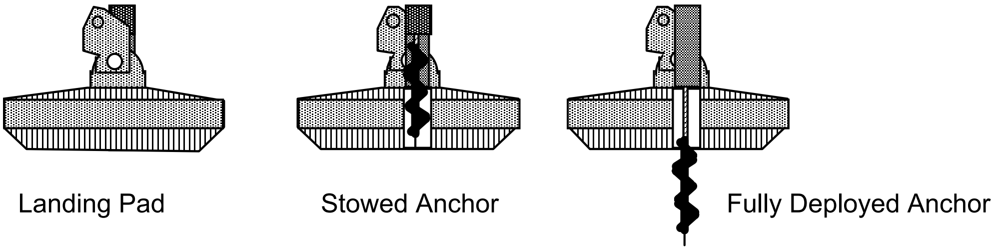


Fig. 17. Integrated landing pad and anchoring for Lunar and Martian missions (Clinton et al., n.a.).

Probing, taking samples

There is a broad variety of ground penetrating systems that have been developed and even used in past and present missions. Again, their design bases on existing technologies and on the demand of technical realization within a short time-frame. The techniques used for penetrating the ground involves screw-like drilling (Fig. 18), hammering (Fig. 19), or ultrasound rock-cracking (Fig. 20). A concept for probing the ground up to 100m depth - called SUBEX (Fig. 21) – is derived from the MOLE driller (Fig. 20).

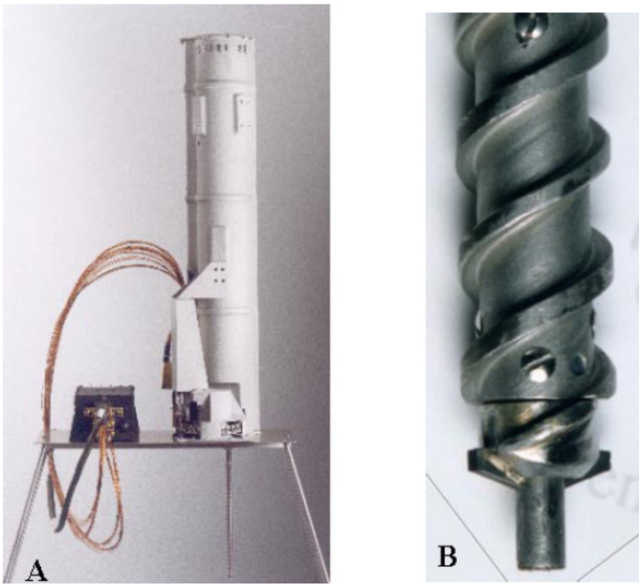


Fig. 18. Drill, sample and distribution system SD2 (RoLand-ROSETTA) (e.g. Coste, 1993).

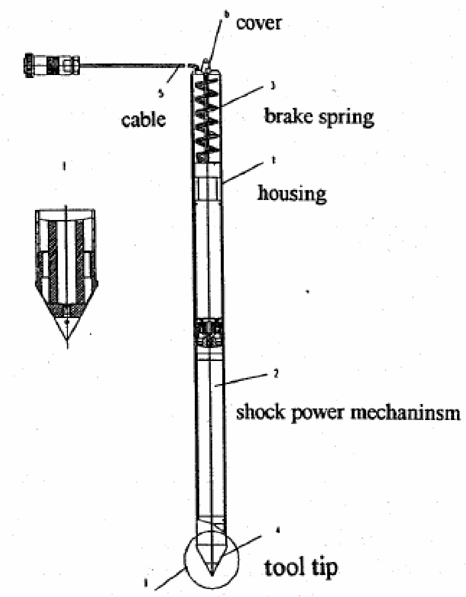


Fig. 19. Mobile penetrometer MOLE (Pluto/Beagle/Mars Express). The penetration is achieved via a shock power mechanism, hammering the tool into the ground.

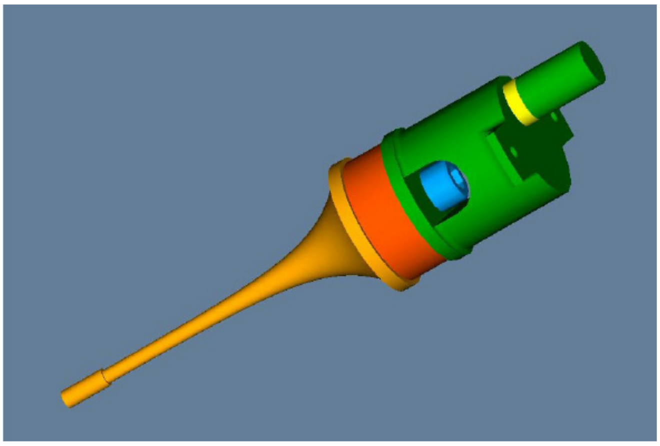


Fig 20. Ultrasonic drill for planetary applications (Sims et al.).

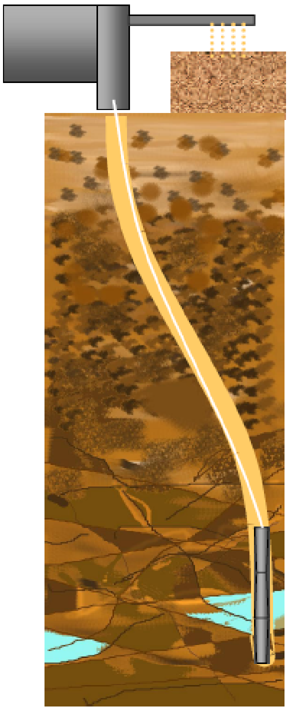


Fig. 21. Schematic drawing of Mars sub-surface exploration system SUBEX.

All rigid anchor systems face the problem of performance over a longer time frame. If the soil creeps, reliable anchoring will not be granted any more. Also the sampling and probing techniques will face similar issues. Natural anchoring and sampling as exhibited by growing plants have the ability of continuous adaptation by longitudinal and radial extension, including changing of growth direction. A technical transfer of root growth may seem visionary but is desirable in terms of improved performance.

WP4. Main potential show-stoppers for space application

This chapter is aimed to resume and highlight the main show-stoppers involved in the development of plant-inspired robot for space applications. Possible show-stoppers concern mainly the actuation mechanism and the capability of soil penetration by the roots.

The actuation mechanism based on the electro-osmotic principle

Although the electro-osmotic principle offers very appealing features for space application, namely low power consumption and generation of high force/pressure, the choice of the chemical species to be used in the electrolytic process and their “space-compatibility” are critical issues. For the proper actuation functionality in space, it is mandatory the chemical compound could withstand very low temperature and pressure. This is a severe constraints, since it drastically reduces the choice range of the chemicals and it forces to accept comprises in performance by adding chemicals able to reduce the freezing point of the chemical compound and/or by heavily pressurizing the root internal volume.

The soil penetration capability

Standard drillers exploit the tip rotational movement in order to move forward into the terrain, digging and ejecting upward the eroded powder. This is not-only a relatively high power demanding action, but it is also responsible of heat generation, which can degrade or destroy significant information about the soil characteristics. On the contrary, the plant-inspired root should penetrate the soil by exerting a simple slow push. This strategy, however, requires:

1) strong push capability (and thus also the possibility for the robot to counter-balance the reaction). In the following, a basic estimation of this is reported.
2) soft soil to penetrate, e.g., loose and silty sand. A similar soil can be probably found only in the very superficial layers of the space terrain to be explored (Mars and Moon), thus the probability to find an underneath non-permeable layer for the plantoid root could be significant.

WP5. Biomechatronic design

Fig. 22 shows a schematic representation of the following robot's actions performed in the very final phases of the landing after the descent procedure (that could be very similar to those performed by the Mars rover mission):

- Rocket ignition to further decrease the speed;
- Bridle cut when close to the surface (few meters if it is possible a fine control) and free fall;
- Solar cell opening and activation of internal control modules ;
- Extension and orientation of the robot's roots.
-

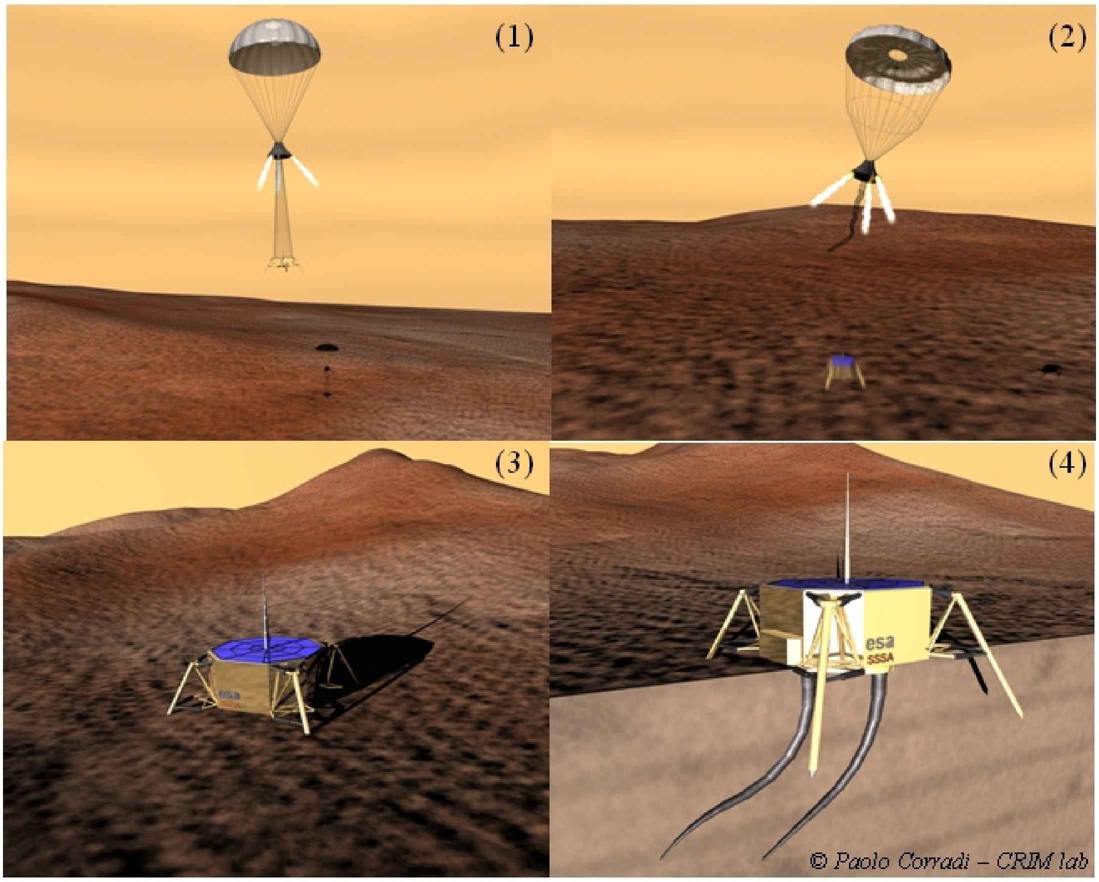


Fig. 22. Final sequence of the plantoid landing: (1) rocket ignition; (2) bridle cut and free fall; (3) anchoring into the soil and system initiation; (4) extension and orientation of the robot's roots.

As a mechatronic system, the robot will be built on a modular scheme. The plantoid can be divided in two major sections: a first part, which corresponds to the trunk and leaves and it is the upper section of the robot, located out of the soil; a second part, which represents the roots, able to move in the soil.

In particular, the upper part of the plantoid aims at:
- acquiring energy from the sun for the working of the robot;
- storing the energy;
- transmitting the data concerning the soil analysis to a remote station;
- managing the plant at high level;
- eventually storing the "fuel" (water or other) of the osmotic process for root growth.

Figure 23 shows a block diagram of the components constituting the plantoid and a different artistic impression of the plantoid on Mars.

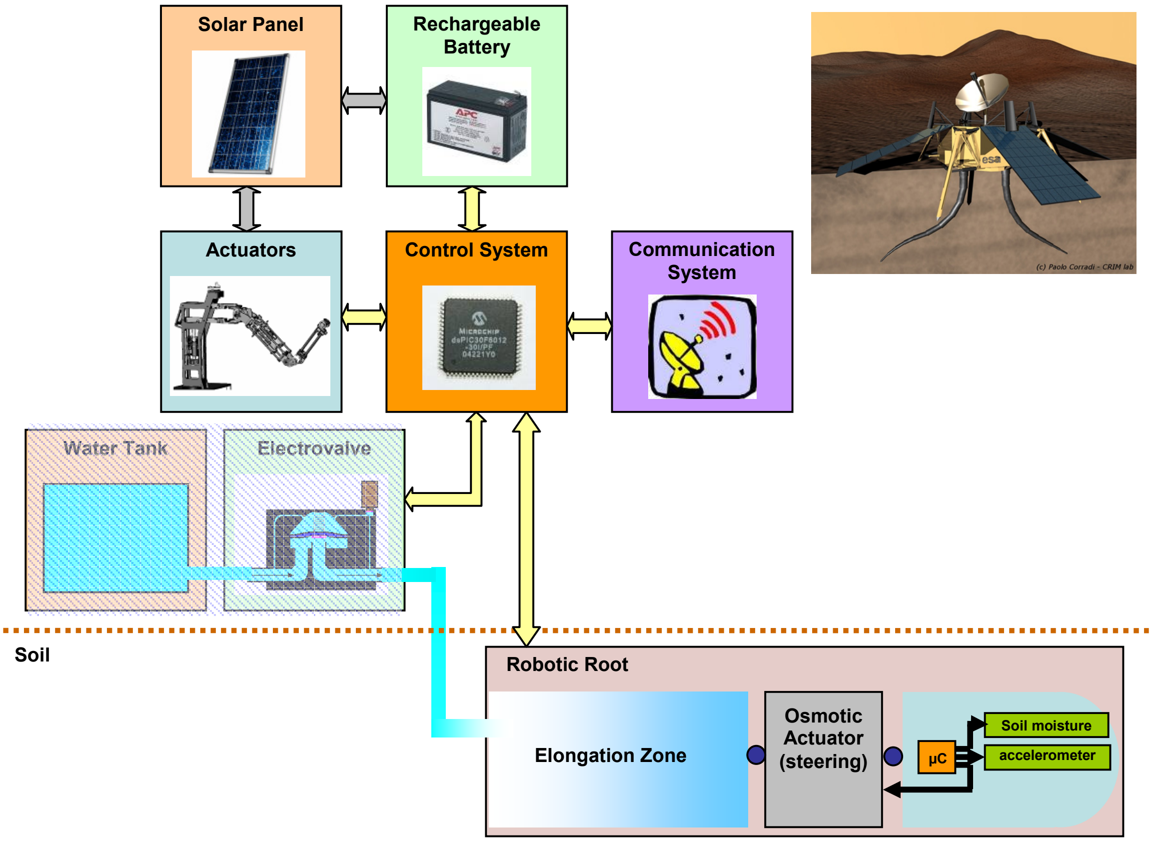


Fig. 23. A schematic representation of the plantoid

The energy is collected by solar panel; the surface of this panel is quite small (depending on the solar radiation on the planet) because the power required by the osmotic actuators is very limited. In particular, for the root apex the current consumption (osmotic actuator + microcontroller and sensors) can be estimated around few mA with a supply voltage of few volts. For the entire root, e.g. composed of 10 modules, it can be roughly estimated a power consumption on the order of tens of mW. The major consumption comes from the transmission block; nevertheless, the data to be sent are not many (the plant movement is very slow and, consequently, the soil analyzed is limited and the data to be acquired are few) and they can be transmitted in a unique solution during the hour of major solar irradiance. The power consumption depends on the type of transmission. For example, the power necessary to transmit to a base located on the planet surface is different from the power necessary to transmit to an orbiting spacecraft. However, these issues are well known in the space field.

The energy collected by the solar panels is stored in a battery in order to have energy during the absence of solar radiation. Moreover in the “trunk” of the robot, there are located also the actuators for commanding the pull out of the solar panels and the tank of “fuel” for the osmotic process.

These components are managed by a microcontroller, which performs several functions:

- It controls the high level tasks of the plantoid;
- it collects the data coming from the roots and it uses them to indicate to the roots the portion of soil that must be analyzed;
- it manages the transmission module;
- it commands the actuators for the solar panels;
- it manages the osmotic tank in order to indicate to the roots when growing.

The lower part of the plantoid is located into the soil and it is composed by the robotic roots. The plantoid roots will be able to grow following different stimuli, such as gravity direction, in order to explore the environment in terms of presence of a variety of chemical-physical parameters and life signatures.

Each root is formed by an apex that comprises sensors and the control part electrically connected to the main microcontroller in the plant body, and by an elongation zone that connects mechanically the apex and the trunk of the plantoid. Each apex embeds a microcontroller module for the emulation of the roots behaviour through the local implementation of networks using as models the real apexes behaviour. By imitating the plants strategy, the robot will move slowly, exploring efficiently the environment and showing high actuation forces and low power consumption. The plantoid apex will grow and move into the ground through the new electrochemical actuators, based on the variation of the osmotic pressure in a liquid, controlled by small electrical signal application (electro-osmotic actuators). These actuators allow a root movement on the plant time scale, applying relative high forces with low power consumption. The plantoid will be able to expand and actuate its roots, implementing *de facto* a biomimetic growing mechanism.

All the roots of the plants are then connected to the central body to realize a network that drives the growing of the roots in preferential directions, driven by the information acquired by the sensors on the apices.

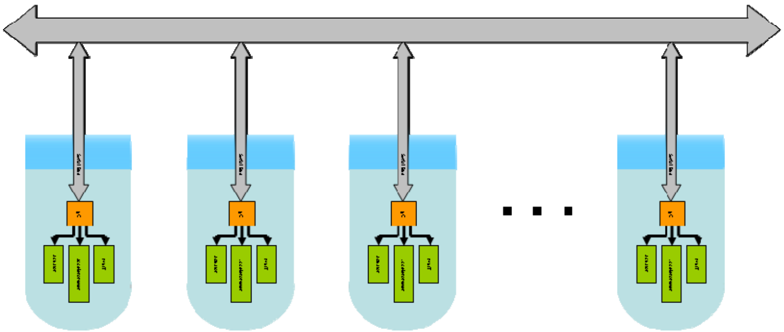


Fig. 24. Plantoid root apex interconnection scheme

Plantoid sensors

As already described, the roots and in particular the root apexes reply to the changes in the environment through mechanisms that are known as tropisms. In detail, the apex has sensors for the gravity (statolith), for the soil moisture and chemicals, which produce respectively gravitropism, hydrotropism and chemotropism.

In order to imitate the plant behaviour, the following components are considered:

- an accelerometer to replicate the capability of the root to follow the gravity;
- a soil moisture sensor to follow the possible gradient of humidity in the soil;
- a microcontroller to realize the distributed control of the plant (every apex is an independent unit);
- a number of actuators (osmotic) for the steering and the penetration of the root in to the soil;
- other sensors to perform chemical analysis of the soil.

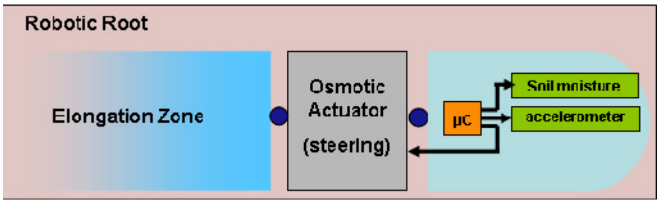


Fig. 25. A schematic representation of a plantoid root apex

Mechanical design

The root should be composed of two basic topologies of mechanical modules:

1) The apex module capable of steering actuation, in order to fix the direction of the soil penetration.
2) The root module working as an axial piston in order to push forward the apex for the soil penetration.

In the following, two concept designs are reported for the two mentioned modules.

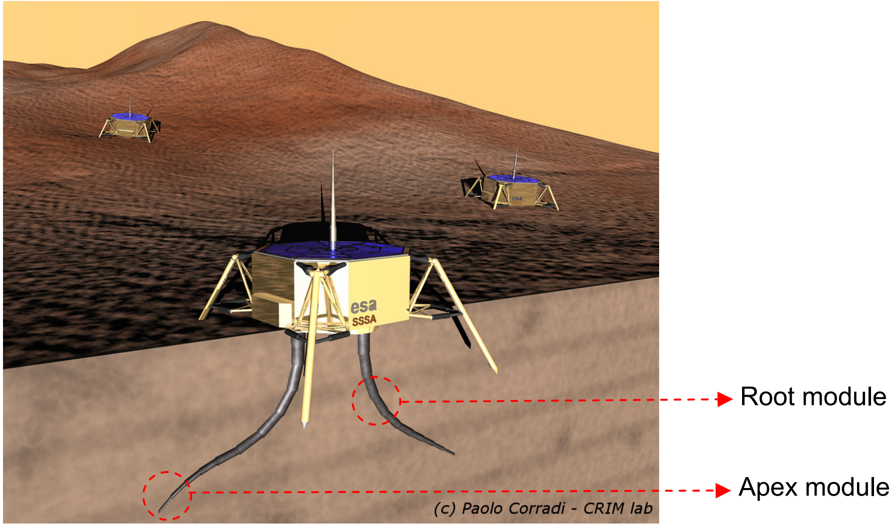


Fig. 26. The basic concept of the plantoid roots. In order to counter-act the penetration push of the roots the plantoid should anchor itself; in the concept four rigid penetrometers are used and made penetrate the soil at the touchdown. Several small plantoids are thought to be disseminated on the planetary surface.

Several solutions can be exploited for achieving the steering of the root by the osmotic actuator. The easiest way is to use directly the osmotic cells with two pistons to move the apex in a plane (Fig. 27).

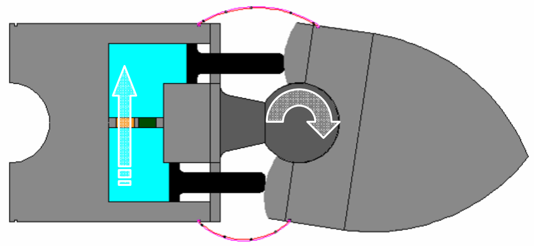


Fig. 27. A basic concept of the osmotic actuator for the steering of the plant root in a bi-dimensional case; the transparent straight arrow states for the water flowing. The red curved lines schematically represents a surrounding membrane placed between the osmotic chamber and the apex tip, aimed at preventing the soil going in between the piston mechanism, thus blocking the actuation.

Considering square chambers with dimensions d x d, the force that the chamber can generate on the piston is $F = d^2 * P$ with P pressure in the chamber. In this way with a d=10mm, for instance, and a pressure generated in chamber P = 6 atm we can obtain a F = 60N on the root apex.

One important issue in the mechanical design of the root apex is the friction coefficient between the root and the soil. Various studies report that the friction coefficient of a metallic structure that penetrates into the soil is much greater than the friction of a root of the same dimension. This is due to a sort of natural lubrication of the root, which during the growth sloughs cells from the cap decreasing this component of friction. Taking inspiration from the secretion of mucilage that plants produce to easy the penetration of the roots in the terrain, this study suggests the use self-lubricant systems, keeping in mind that contaminant chemicals cannot be released in the soil in order to prevent any ambiguous detection. A possible strategy makes use of a Teflon based apex and root or a membrane coming out from the base of the root apex, deployed automatically during the root extension and also by friction with the soil. This would drastically help the root growth, because the entire root can slide inside this self-forming Teflon membrane, focusing the actuation force to contrast the friction with the soil at the root apex, while drastically decreasing the so-called sleeve friction forces that can become even more significant than the forces generated by the cone resistance depending on the length of the moving root into the soil. Moreover, the root apex advances while the Teflon membrane is deployed from its base, and consequently the membrane itself tends to unroll toward the opposite direction for friction with the soil.

The root module can be envisioned as a “cylinder-piston” system as that drawn in Fig. 14 or, in order to reach higher integration, the piston itself can include the two different osmotic and anionic membranes as in Fig. 28 (in this way the disadvantage is that part of the pushing surface of the piston is lost). The piston is rigidly connected to a piston rod that goes out of the cylinder and axially pushes over the following root module by means of an intermediate module that works as a spherical joint whose rotation and stiffness can be controlled by osmotic actuation, and which is basically the same structure proposed for the steering actuation (Fig. 28).

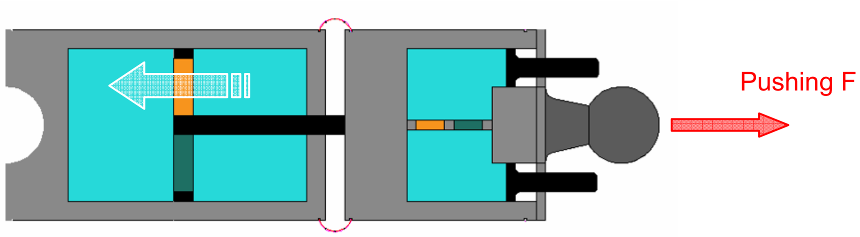


Fig. 28. A basic concept of the osmotic actuator for the root modules; the transparent white arrow states for the water flowing, while the red arrow states for the pushing force direction.

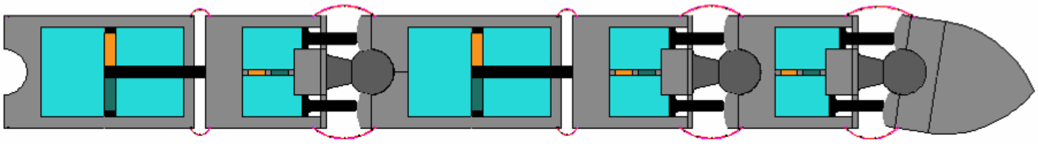


Fig. 29. A basic concept of the final part of the root with the apex and two root modules: the system has an intrinsic modular nature.

The force generated by the osmotic actuation principle itself directly relies on the cross-section area of the root, because the force generated equals the product of the piston section area and the generated pressure. With a diameter of few centimetres (e.g. around 5-6 cm) of the cylinder, a root module could exert forces on the order of 1000 N (around 100 Kgf on Earth) with typical pressures of 5 atm in the actuators. While from a mechanical viewpoint it is possible to decrease the diameter of the root down to a couple of cm (and maybe even further), in order to have comparable and useful forces it is necessary to increase much more the internal pressure. Nevertheless, high pressures of course need stronger and thus larger mechanical parts. As a conclusion there should be a sort of compromise or optimization between the root cross-section area and the actuation force generated, which will depend on the particular soil probed. Hence, it seems reasonable to face the final root design starting with penetration-tests of Martian/Moon-like soil using several dummy apex/roots with different cross-sections and using different controlled pushing forces (corresponding to the possible actuation forces generated by such a root cross-section area), in order to collect results to define the best compromise.

Some preliminary practical considerations can however be done. As said, penetration capability is deeply affected by the soil nature. Depending on this, the root needs to exert a relatively strong push in order to penetrate the soil. In order to simplify a preliminary analysis, it can be considered the root is penetrating straight and perpendicularly to the terrain surface, in a way similar to a strategy currently adopted in civil engineering test (Jacobs, 2004), the Cone Penetration Test (CPT), which is an in-situ test to measure the strength or bearing capacity of soils and soft rocks . It uses a cylindrical cone, with a typical standardized diameter of 35.7 mm, which is pushed vertically from a rig (generally a mobile laboratory-truck) into the ground at a constant rate of penetration of 20 mm/s. During penetration, several geotechnical measurements are recorded at the cone head or along the sleeve as schematically reported in Fig. 30. At the cone head the cone resistance (q_c) is recorded (generally in Pa), which expresses the resistance of the sediments to penetration. Along the cone the sleeve friction (f_s) is recorded (in Pa), which is indicative for the adhesive strength of the material. From the cone resistance and the sleeve friction the friction ratio (R_f) can be calculated as $R_f = [(f_s/q_c)\times 100]$. Numerous analyses of data have lead to an empirical relationship between R_f and inferred lithology. Another important parameter is the measured waterpore pressure (u) that is function of the permeability of the soil and that is also used to correct the cone resistance values in the “corrected tip stress” $q_t=q_c+(1-a_N)u$, where a_N is the net area of the used probe, which is typically around 0.7-0.8. Pore water pressure refers to the pressure of groundwater held within a soil or rock, in gaps between particles (pores). For example, in a high permeability soil, the pressure would be close to hydrostatic in no flow conditions. While in sands can be neglected in first approximation, in clay can be significant. Extracted data correlate the variation of the measured parameters with penetration depth. Depths of up to 100 m can be reached, nevertheless it must be considered that, in order to do that, the push is exerted by extremely strong pushing mechanisms on board heavy trucks (on Earth), which are also consequently able to counter-balance the reaction of the penetrating cone.

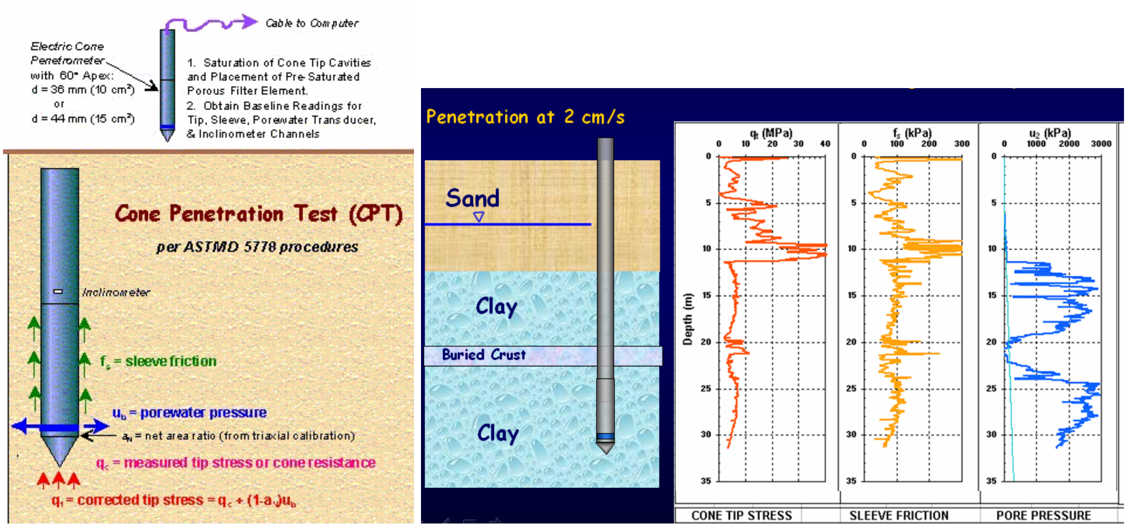


Fig. 30. Cone Penetration Test and typical data plotted (Courtesy of In-Situ Testing Group of Georgia Institute of Technology, GA, US).

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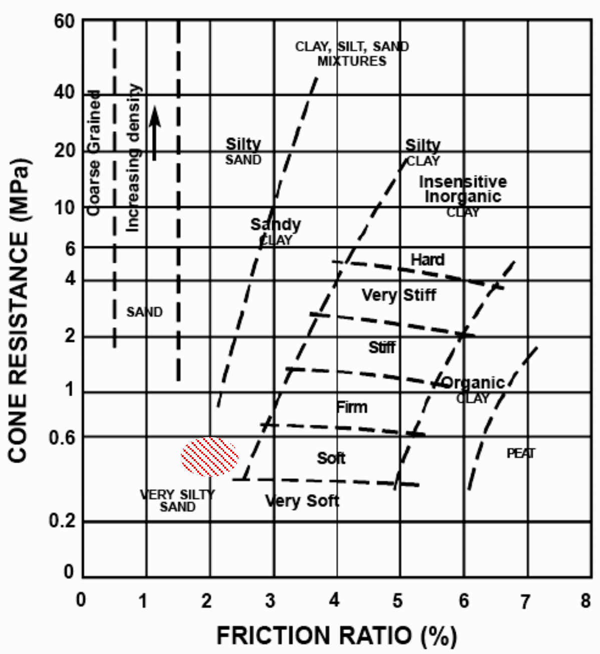

Fig. 31. Guide for Estimating Soil Type (courtesy of Fugro Enginering Services Ltd.). The striped red area indicates the soil characteristics taken into consideration for the following calculations.

From experimental data we can generally classify a soil in:

- Sand if
  - insertion of the cone into sand will give a high end resistance
  - low friction ratio
  - low pore pressure - quick dissipation of water (high permeability)

- Clay if
  - insertion of the cone into clay will give a low end resistance
  - high friction ratio
  - high pore pressure - slow dissipation of water (low permeability)

Considering a Mars soil, it is likely to expect a sandy-like response of the soil during the initial penetration of the root, while later on the response could change in a more clay-like response according to the soil model of Fig. 2. In first approximation we consider so far the pore pressure as negligible and thus $q_t = q_c$.
Considering the root performs a straight elongation, perpendicularly to the surface, and considering it is actuated by axial-piston actuators as in Fig. 28, and exhibits roughly a 60°cone-tip, the following rules of thumb can be obtained by deriving experimental data from the CPT literature, where soil probing is performed in these initial conditions.
The cone resistance forces on the cone tip of the penetrating root are:

$$F_c = q_c \cdot \frac{\pi d^2}{4} \tag{19}$$

Where d is the diameter of the cylindrical root/apex. As clear they decrease with the square of the diameter exactly like the axial forces *Fos* is possible to theoretically generate with the osmotic actuation of the actuator of Fig. 28.

$$F_{OS} = P \cdot \pi d^2 / 4 \tag{20}$$

Where P is the osmotic pressure.
The friction sleeve forces F_s sum to the F_c, which decrease linearly with the root diameter d and depends on the total moving surface of the root:

$$F_s = f_s \cdot \pi d \cdot H \tag{21}$$

Where H is the length of the part of the root that is penetrating.
Although the f_s are generally almost two order of magnitude lower than the q_s (as it is also visible in Fig. 30), the total sleeve force F_s becomes comparable to the F_c as soon as the moving part of the root is roughly an order larger of the diameter of the probe.
In order to have penetration of the root/apex in the terrain it has to be satisfied from (19), (20) and (21):

$$Fos > Fc + Fs \;\Rightarrow\; q_c \cdot \frac{\pi d^2}{4} + f_s \cdot \pi d \cdot H < P \cdot \pi d^2 / 4 \tag{22}$$

And thus: $$d > \frac{4 f_s}{P - q_c} \cdot H \tag{23}$$

From (23) as well as from (19) and (20) it comes that necessary condition is that $P > q_c$ (tipically q_c ranges around 1-8 MPa for sandy terrain, that means about 10-80 atm, while it can be less than 1 MPa for clay).
Considering for Mars, for instance:

- a loose and dry silty sand, consequently accepting an optimistic q_c of around 0.5 MPa, and f_s of about 0.01 MPa (that means a friction ratio around 2%)
- a maximum length of the root of 1 m (that means a maximum H = 1 m),
- an achievable osmotic pressure P of about 1MPa (=10 atm)

it follows that the diameter d of the root/apex should be larger than 80 mm in order to push the whole 1 mm-long root. Less deep the penetration lower the sleeve friction and thus lower can be the diameter d (in the present example, in order to penetrate the first 25 cm, a d = 20 mm could be enough).
In order to gain actuation power, it is possible to think to use larger modules at the beginning of the root and smaller modules towards the apex. The extension of the modules could be contemporary, being the force applied by the strongest root module the resulting force, while the total course would correspond to the sum of the course of each extended module (admitting each module can extend itself in the soil). On the other hand it would be more convenient to actuate the root modules close to the apex at the beginning of the penetration (where less force is required to dig) and keep the largest and strongest modules for the actuation of the final steps. However, as a negative consequence, the last actuated modules will have to push all the extended root, which means stronger sleeve friction will occur.
It has to be considered, however, that the used values for q_c and f_s were derived from data measured with a specific size of the probe (d=35.7mm) and also in dynamic condition of penetration (20 mm/s) and not in the quasi-static penetration that will occur with the plantoid root.

Some tests were also carried out in our laboratory by means of an Instron testing machine 4464 with 1kN load cell. Several cylindrical probes ending in a 60°-aperture cone with different diameters (namely 50 mm, 35.7 mm, 22 mm, and 8 mm) were used for the penetration into a mixed clay-sand soil, firstly in a “wet” condition and afterwards in “dry” condition. Tests were performed keeping the penetration speed constant at 1 mm/s. The resistance force to the soil depth was plotted by means of a LabVIEW program receiving data from the Instron machine. Before each test, the soil was extracted from the container, mixed and re-deposited in the container and a standard pre-load applied before the test, in order to make the tests as repeatable as possible.

The tests demonstrated that soil penetration down to 200 mm in common terrain can be achieved with the osmotic actuation with suitable pressure (< 5 atm) using probes with different size.

Miniaturized probes (e.g., a diameter < 20 mm) demonstrated to require higher pressures for the penetration compared to larger probes (e.g., diameter > 30 mm), in particular in the early stages of the penetration. However, these conclusions are valid only for depth of 180-200 mm.

Larger probes give a monotone trend in the curve, while smaller probes show trend inversions (as visible in Fig. 32): this is because smaller the size of the probe more the probe penetration is affected by the local characteristics of the soil.

Anyway, the optimal size is likely to depend on the particular terrain (and also the same terrain in different condition, e.g. wet or dry).

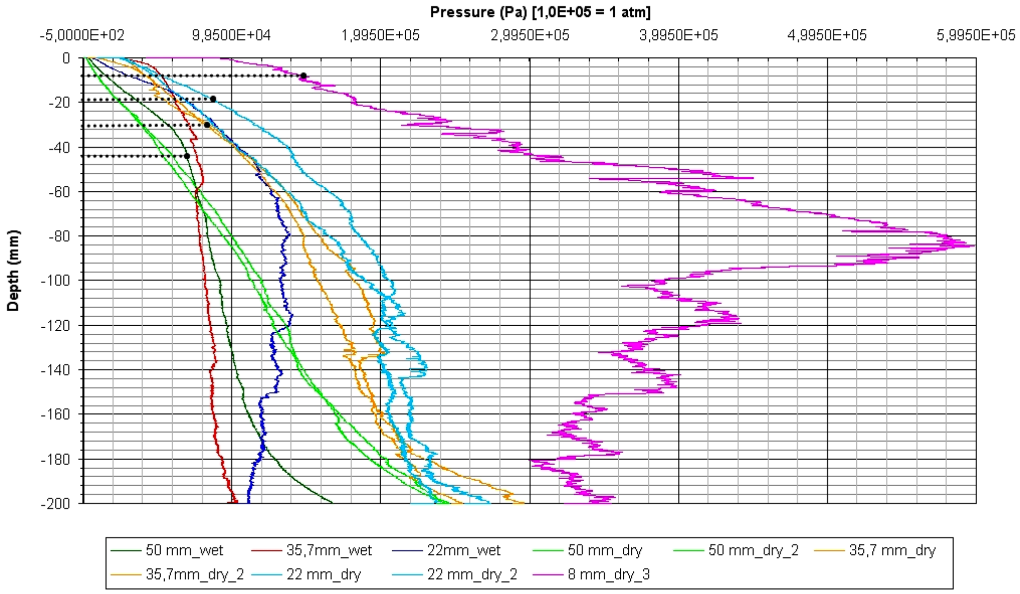

Fig. 32. Results from the laboratory tests using an Instron machine; each coloured line represents the P=P(depth) curve obtained with a probe having a diameter indicated in the underneath box, relatively to the condition of the mixed sand-clay terrain ("wet" or "dry").

By a further research in the literature it is also possible to find cone penetration test performed on Mars-like terrain. For instance, in (Kegege, 2007) two Mars-like terrain (JSC Mars-1: feldspar, Ti-magnetite, with minor olivine, pyroxene and glass; JSC Mars-2: 45% clay, 45% basalt, 10% iron oxide) were investigated and probed by penetrating spikes of 19.05 mm and 12.7 mm. Using the data acquired by the authors by using a simple penetrometer, it results that the necessary osmotic pressure to reach 15 cm depth in the Mars-like soil with a root diameter of 19.05 mm is up to $P \sim$ 0.7 MPa (with JSC Mars-1) and $P <$ 0.08 MPa ~ 0.8 atm (with JSC Mars-2). Considering the data obtained with the spike with a diameter of 12.7 mm the required osmotic pressure would be even less: $P \sim$ 0.6 MPa (with JSC Mars-1) and $P <$ 0.06 MPa ~ 0.6 atm (with JSC Mars-2).

These results are quite promising for the proposed osmotic actuation, even if only penetration tests customized on the plantoid structure and steering capabilities can give an accurate response.

Finally, it must be said that the main problem concerning the osmotic approach would probably still remain the apex steering capability. Relying on the same actuation principle of the root, a thin apex would be hopefully able to steer only at the very beginning of the sandy-soil surface. Going deeper, the steering momentum requested in order to move the surrounding sand could be too large. The steering actuation would occur dynamically, that means progressively during the quasi-static forward penetration of the root. In order to evaluate the steering capability, tests in soil are definitely necessary, because all the experimental experience that is possible to gain from CPTs looses its value in these different conditions.

In addition, the penetration of a bended root is actually expected to be even more difficult, because the pushing force will not be entirely propagated down to the apex if we consider to always push the root from the surface, but only a component of the force will contribute to move forwards the apex. On the other hand, in order to compensate this problem, the root penetration can be conceived as composed by the following steps:

- an initial straight insertion of several root modules into the terrain (as in the CPTs);
- a serial actuation of the underground modules from the upper stages down to the apex, in order to avoid to push all the already extended modules and thus decreasing the problem of advancing all the root through a bended path (that means also decreasing the sleeve friction while the root is penetrating).

It must also be considered that on Mars the gravity is about one third the gravity on Earth and the mean surface level atmospheric pressure is less than 0.01 the Earth pressure. This should be a more favourable condition to the soil penetration compared to Earth.

Conclusions

The project started from considering and investigating plants as source of bio-inspiration for novel actuation and soil probing strategies.

Plants are able to follow several gradients in the soil, showing a growth velocity of 0.5 - 4 mm/h and performing a growth pressure of about 1 MPa. Plants apply the osmotic principle for steering and growing into the terrain.

A quick survey of several actuation technologies has been carried out, highlighting their weak points in comparison with the proposed plant-inspired osmotic-based actuation (whose main advantages are low voltages, very low power consumption and high force/pressure) and keeping in mind the applications in planetary soil probing. Conventional actuators require higher voltages and/or higher power, while among the actuators typically used in biomimetic robotics, high voltages (electronic EAP), low forces (ionic EAP), high power (SMA), low displacement (Piezoelectric), and the required actuation architectures seem to make them unsuitable for replicating the plant root penetration capabilities.

Osmotic principle can be used to fabricate a plant inspired actuator, using anionic (IONAC MA-3475, IONAC MA-7500) and osmotic membranes (YMCFSP3001 CA -Cellulose Acetate- RO CF Membrane), lead nitrate or lead perchlorate, producing a pressure of 0.5 – 1 MPa, thus comparable to the pressure generated by plants.

A robotic plant inspired root can be preliminary designed, with a diameter of few tens of millimeters, for soil with resistance of less than 0.5 – 1 MPa. Penetration tests of cylindrical probes (spikes) on Mars-like terrain, which have been found in literature, reported data that can be encouraging depending on the model of the terrain used. Custom penetration tests, on the base of the standardized process of the cone penetration test (CPT), need to be carried out in order to better define optimal size and capability of the plantoid root.

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