

# Chapter 2

## Nutation in Plants

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**Abstract** This chapter aims to explore and describe the physiological aspects of oscillating growth patterns in rapidly elongating plant organs, such as roots, hypocotyls, shoots, branches and flower stalks. After a brief description of the phenomena, the theories and models proposed to explain circumnutation are reported, focusing largely on the internal oscillator model and the gravitropic overshoot model. The former is derived from the intuition of Charles Darwin, the first to suggest that circumnutatory movements are mediated by an endogenous oscillator, i.e. the driving and regulating apparatus responsible for circumnutation is internal. By contrast, the latter theory proposes a gravity-dependent model to account for circumnutations, essentially consistent with the Cholodny-Went theory, thus interpreting oscillations as being a continuous series of over-compensatory responses of the plant to the changing orientation of its gravisensory apparatus relative to the Earth's gravity vector. A revised two-oscillator model is also reported, which is based on a combination of the above-mentioned two models. In this combined model, circumnutational movement involves a gravitropic reaction acting as an externally driven feedback oscillator, together with an endogenous or intrinsic oscillator which sends a rhythmic signal to the feedback system. The role of hormones will be finally discussed, with particular attention to the effect of ethylene in controlling nutation.

### 2.1 Overview of Nutations: Definition and Kinematic

More than a century ago, plant physiologists were already aware that rapidly elongating plant organs—roots, hypocotyls, shoots, branches, flower stalks—rarely grow in only one direction. Mean growth direction may be maintained for long

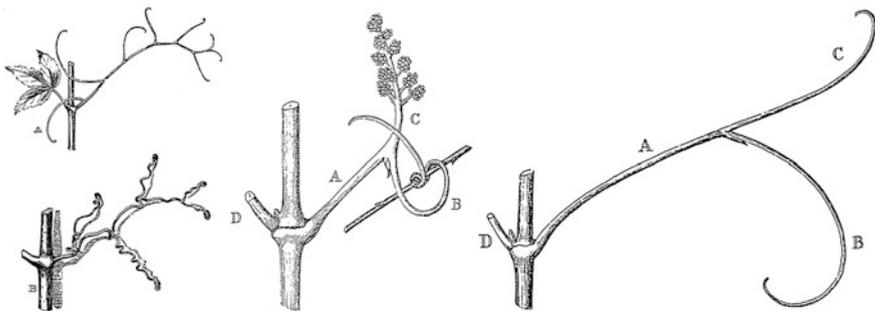
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intervals, but the organ's instantaneous growth direction usually oscillates slowly around that mean. From a distal viewpoint, the plant organ tip, or an elongating cylindrical plant organ, describes an ellipse, a circle- or pendulum-like movements about the plumb line, which can alternate between a clockwise and counterclockwise direction. The axes of the ellipse can vary: at one extreme, the ellipse approximates a line and, at the other, a circle. As the organ grows, its tip advances and (in three dimensions) traces an irregular helix (Migliaccio et al. 2009). This oscillating growth pattern was well known to nineteenth-century plant scientists as 'revolving nutation' until the Darwins (father and son, Darwin and Darwin 1880) introduced the term 'circumnutation', used to this day (Fig. 2.1). Thus, circumnutational oscillations are manifestations of the radially asymmetric growth rate typical of elongating plant organs (Fig. 2.2). These do not include tropic processes occurring in response to a directional cue, such as gravity or light, or nastic movements, which occur in response to external factors but are independent of the position, i.e. the closing of leaves at night. These various forms of movements usually occur together; for example, it has been shown that gravity amplifies the circumnutatory response in *Arabidopsis thaliana* (Johnsson et al. 2009).

Darwin's (1875) close observation of the behaviour of 'climbing plants', which tendrils appeared to 'search' for some upright support, led him to widen his investigation to a large variety of species in which, however, he found no exception to his generalization that circumnutations must be a universal kind of plant movement (Darwin and Darwin 1880). Indeed, today we know that the widespread occurrence of circumnutations is even greater than Darwin had ever suspected. It not only occurs in dicots and monocots (Brown 1993) but also is well established for gymnosperms, fungi (*Basidiomycetes*), bryophytes (*Ceratodon purpureus*, Kern et al. 2005) and algae (*Spirogyra*, Kim et al. 2005). Even some colonial forms of bacteria (*Acetobacter xylinum*) exhibit oscillating growth patterns which kinematically resemble higher plant circumnutations (Hoiczzyk 2000).

Although circumnutatory movements are of obvious use to twining plants seeking mechanical support, in other cases the movements appear to have no useful purpose. The amplitude, period and shape of circumnutation depend on the plant



**Fig. 2.1** Some sketches illustrating Darwin's close observation of the behaviour of 'climbing plants' (extracted from Darwin 1875)

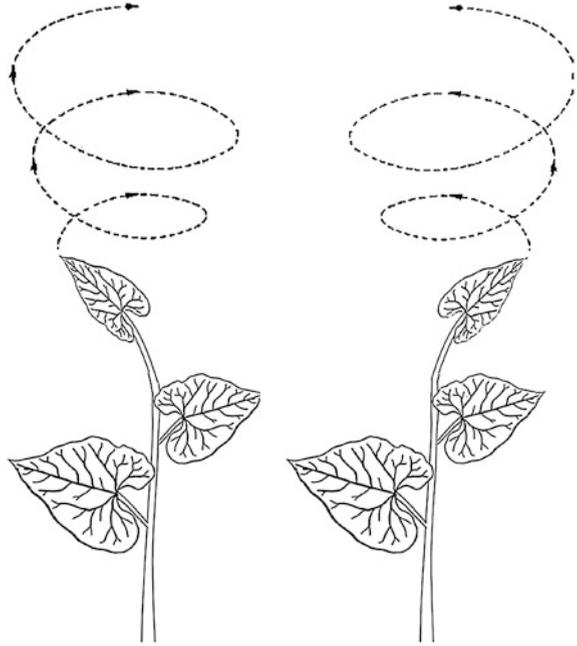
**Fig. 2.2** *Brassica oleracea* and circumnutation of the hypocotyl and cotyledons (extracted from Darwin and Darwin 1880)



species, the plant organs involved and the developmental stage of growth. Shoots of climbing plants (e.g. *Dioscorea batatas*, *Ipomoea quamoclit* and *Phaseolus vulgaris*) circumnutate very regularly in circular orbits (Baillaud 1962; Millet et al. 1984). By contrast, such regular circumnutation can rarely be found in more common non-climbing plants such as *Arabidopsis* (hypocotyls, Schuster and Engelmann 1997), rice (Yoshihara and Iino 2005), *Triticum* (coleoptiles, Joerrens 1959) and tulip (peduncles, Hejnowicz and Sievers 1995).

Researchers have regarded these phenomena both as oddities of plant growth and also as an outward manifestation of some important processes involved in the elongation of plant organs. Circumnutation is a growth movement, its expression depending closely on growth: whatever interferes with growth reduces or inhibits circumnutation—when tissues mature and elongation ceases, so do circumnutations. Moreover, circumnutations do not necessarily persist throughout the entire time course of organ growth. The oscillations may be interrupted by periods of straight growth, some lasting several hours, alternating with periods of vigorous oscillations. Plant organs (shoots and roots) may oscillate either clockwise or counterclockwise (Fig. 2.3). The same organ may stop oscillating while continuing to elongate; later, it may resume circumnutating but in the opposite direction or, without any pause, its tip may trace a figure of eight which accomplishes the reversal. Most circumnutational oscillation frequencies are in the range of 50  $\mu\text{Hz}$  (periods of about 20–300 min). In some cases, the oscillating rhythm is connected with circadian cycles, as shown in *Helianthus* (Niimura et al. 2005; Stolarz et al. 2008; Stolarz 2009). Therefore, appropriate methods are needed to fully reveal the

**Fig. 2.3** Clockwise and counterclockwise oscillations of *Phaseolus vulgaris* L



high incidence of circumnutational behaviour in growing plant parts. In higher plants, kinematic patterns of circumnutation are unique for each organ of a given plant. Different shoots often do not oscillate in phase and usually have different periods of oscillation.

Various mechanical stimuli can exert a dominant influence on circumnutational behaviour. Pressure (mechanical distortion), mechanical shock, subsonic vibrations and even gentle tactile stimulation can sometimes suppress the vigour of circumnutations. It may be significant that these effects can occur only within a few minutes, often less than that needed for auxin to be transported from an organ tip to the growth region. This observation may be used as an argument in favour of a growth-control process which is local, rather than occurring in the remote tip region of the growing organ.

Beginning about 60 years ago, speculations about how plants grow and respond to tropistic stimulations were dominated by the Cholodny-Went theory (Cholodny 1926; Went 1926), according to which both the plant's environmental gravity-force detectors (statocytes) and the site of production of the growth 'hormone' are located in the apex of the responding organ. As originally proposed, the Cholodny-Went theory was chiefly concerned with the role of a chemical growth regulator in transport and its influence on the growth phases of a plant's tropistic response to a gravitational stimulus. The Cholodny-Went theory served as a guide for several generations of plant physiologists to examine and to revise. More recently, however, other naturally occurring growth regulators have been found, arguing against

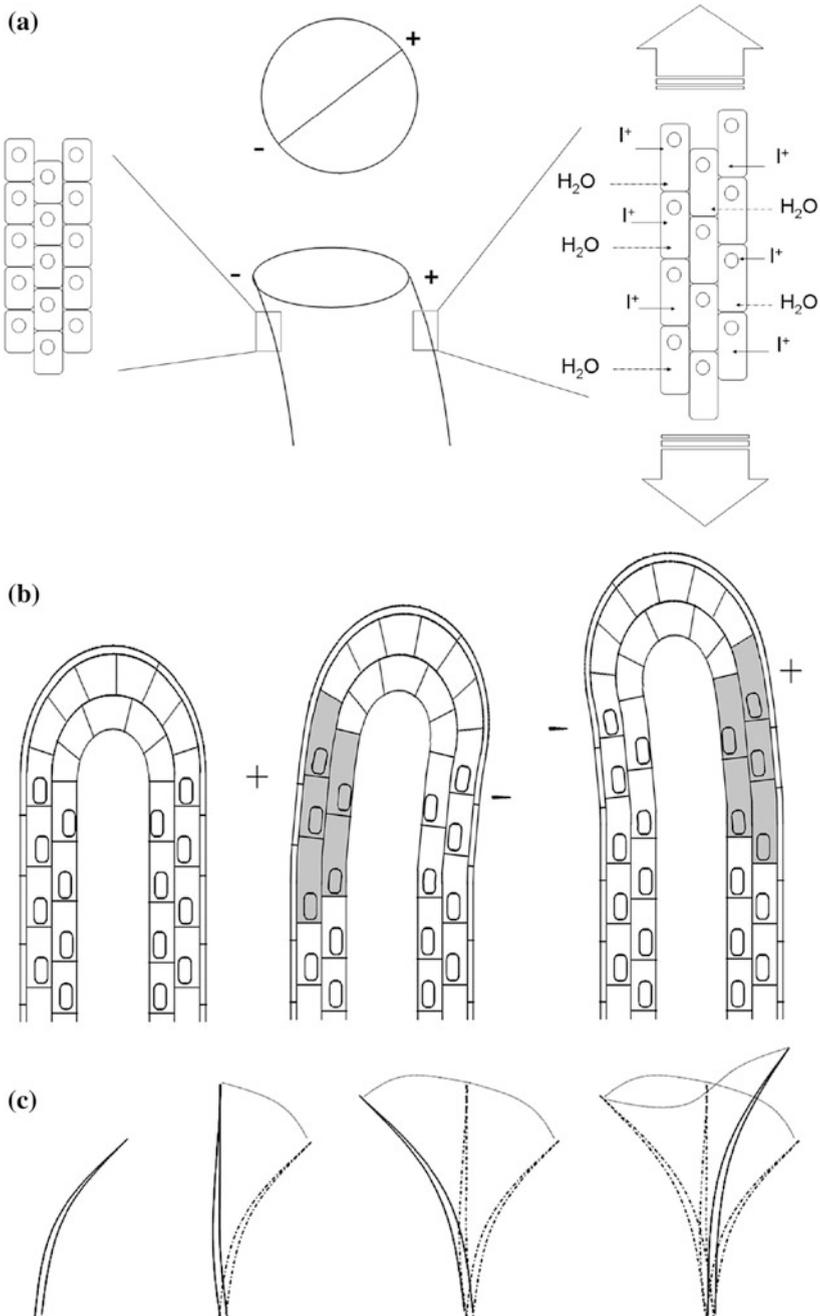
the ‘comfortable’ simplicity of views long existing in this research area. As fundamental departure from the simplified Cholodny-Went theory, evidence has been accumulating in support of a local-control theory whereby the tropistically responding region, especially of the shoot, plays a dominant role in determining the kinematics of its own response.

## 2.2 Circumnutation Can Be Explained by Theories and Models

Unequal rates of growth on different sides of an organ represent the primary process behind circumnutation: the side with the most rapid growth pushes the apex of the organ over, bending it towards the least active side (Migliaccio et al. 2013). The active area then proceeds around the organ, typically along a helical path, which can be right-handed or left-handed (Johnsson 1997). Circumnutation is therefore the consequence of helical growth (Brown 1993) and reversible volume variations occurring in the cells of the moving part of the stem (the bending zone below the apex; Caré et al. 1998). These variations seem to be caused by the difference in water content between the convex and concave sides of the bending zone, associated with turgor and ion concentration differences between opposite sides of the stem (Fig. 2.4; see Forterre (2013) for an extensive review). Possibly, a turgor wave rotating around the stem during circumnutation drives a helical, likely acidic growth of the stem (Hejnowicz and Sievers 1995), which we can see as stem bending. The helical growth is hypothesized to be a mechanism which increases the stability of the hypocotyls (Schuster and Engelmann 1997) during cell wall loosening (Cosgrove 2000) accompanying elongation. This is also associated with defects in microtubule patterning (microtubules are transversely oriented in spirals with respect to the longitudinal axis of the cell in elongating root cells), which can cause the epidermal and cortical layers to lag in expansion rates such that cell file rotation increases (Roy and Bassham 2014). It has also been suggested that turgor changes are generated by endogenous, spontaneous oscillations. As a consequence, oscillatory growth and movement are generated (Van den Driessche 2000).

The cells of the bending zone communicate via plasmodesmata (Brown 1993), ion channels (Badot et al. 1990) and aquaporins (Comparot et al. 2000). Unlike pulvinar cells which are highly specialized (Engelmann 1996), no particular structure has been identified for cells in the bending zone. Circumnutations occur temporarily in young growing shoots, in the cells at a given distance from the apex, or rather in a certain developmental stage (Van den Driessche 2000). The movements also strongly depend on light intensity, photoperiod (Buda et al. 2003), mechanical stress and temperature (Anderson-Bernadas et al. 1997).

Currently, two main models for circumnutation have been proposed.



**Fig. 2.4** Schematic representation of cellular turgor changes (a), differential growth rate (b), and at two opposite sides of a shoot and its effects on circumnutation (c)

### 2.2.1 Model I: 'Internal Oscillator' Model

Charles Darwin and his son Francis suggested that circumnutatory movements are mediated by an endogenous oscillator. Darwin tried to explain (in terms of nineteenth-century science) why the potential for circumnutating is ubiquitous. He considered that circumnutation is not only universal but also a fundamental process which would 'be modified for the good of the plant' to accomplish tropistic or other growth responses. The Darwinian internal oscillator model is more a concept than a model, connected with the biological clock mechanism (Thain et al. 2002). Operationally, this means that the driving and regulating apparatus responsible for circumnutation is internal. Because circumnutation is patently advantageous to the plant only in a small minority of cases, researchers are not inclined to consider that it has endured only because it confers some evolutionarily significant advantage—quite the contrary—there must be something fundamental about the growth process which endows growing plant organs with the ability to circumnutate, an ability commonly displayed.

There are different hypotheses concerning the nature of an endogenous oscillator. Arnal (1953) advanced the argument that the circumnutation of coleoptiles is due to periodic variations in auxin fluxes from the tip. Moreover, Joerrens (1959) proposed that the sensitivity of the elongating cells to auxin changes periodically. Heathcote and Aston (1970) considered a hypothetical 'cellular nutational oscillator', situated in each cell and having a period equal to the periodicity of the circumnutational movement. A recently proposed model relates to the existence of an intrinsic 'oscillator'. This model is based on the observation of strong correlations between nutation and rhythmical patterns of ion fluxes in the elongation region of corn roots (Shabala and Newman 1997; Shabala 2003). The authors noted that, when maize roots showed rhythmical movements,  $H^+$  and  $Ca^{2+}$  fluxes also changed rhythmically, with the same average period and amplitude; when root movement was periodic, so were ion fluxes; moreover, when root growth was absent or very slow, no oscillations in ion fluxes occurred, and no nutation was observed. Shabala (2003) found that correlations between flux oscillations and root circumnutation could also be extended to include  $K^+$ . As  $K^+$  is a major osmotic agent in plant cells and, accordingly, a main factor responsible for differential growth of root cells, an efflux of  $K^+$  results in a loss of turgor within the cell and a consequent 'slumping' of the cell. The non-turgid cells cause asymmetric rigidity in the root, which consequently bends to the side with less turgor (Shabala and Knowles 2002). This was further supported by direct evidence of  $K^+$  flux oscillations closely associated with root circumnutations (Shabala 2003), the fluxes being in reversed phases when measured from opposite sides of a vertically growing root.

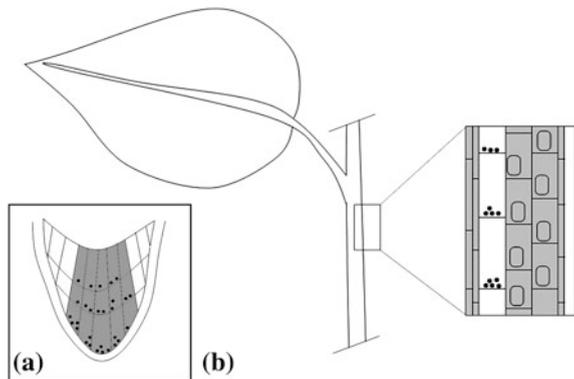
Circumnutation and circadian rhythms have been well studied, and there are some reports of relationships between circumnutation and biological rhythms. Schuster and Engelmann (1997) reported that *Arabidopsis* seedlings showed a very wide range of circumnutation rhythms. In *Helianthus annuus*, circumnutation speed and trajectory length exhibit daily modulation under 16-h light/8-h dark (Buda et al.

2003). Niimura et al. (2005) demonstrated that the modulation of circumnutation speed in *Arabidopsis* inflorescence stems is regulated by a circadian clock, pointing to the existence of an internal oscillator which regulates the speed of circumnutation. Experiments with two loss-of-function mutants, *TOC1* (mutant which shortens the period for all circadian processes analysed to date) and *ELF3* (mutant which causes arrhythmic circadian outputs under constant white light conditions, with an almost constant nutation speed), demonstrated genetically that the circadian clock controls circumnutation speed. These results strongly confirm the hypothesis that rhythmical membrane transport processes play a key role in plant circumnutation, showing a genetic-based control.

### 2.2.2 Model II: ‘Gravitropic Overshoot’ Model

When Israelsson and Johnsson (1967) proposed a gravity-dependent model to account for circumnutations, their reasoning was essentially consistent with the Cholodny-Went theory, and their theory about circumnutations proved to be an attractive explanation of how oscillations might be driven and controlled specifically by gravity. Basically, they interpreted the oscillations as being a continuous series of over-compensatory responses of the plant to the changing orientation of its gravisensory apparatus relative to the Earth’s gravity vector. By interpreting the oscillations as gravity driven, their model described circumnutation as a special kind of tropistic behaviour (Fig. 2.5). The model also was consistent with the modern version of the Cholodny-Went theory for gravitropic responses, according to which both the plant’s gravity detectors (statocytes) and the site of production of IAA are located in the apex of the responding organ. Nevertheless, the localization of gravisensing is much more pronounced in root tips than in shoot tips, which has to be taken into account when we try to explain circumnutations in shoots. Experiments performed under microgravity conditions aboard the Spacelab (Brown 1993) and the ISS (Correll and Kiss 2008; Paul et al. 2012), however, revealed that

**Fig. 2.5** Diagram of a root (a) and a stem (b), illustrating the regions of gravity perception



gravity is not an absolute requirement either for the initiation or for the continuation of circumnutatory movements, leading to the conclusion that the inner oscillator and gravitropism seem to operate independently. The patterns of circumnutation observed during microgravity experiments clearly show that gravity is not an essential component of the mechanism that drives this type of root growth (Paul et al. 2012). It has been hypothesized for a long time that circumnutation is primarily a result of a correlation between gravitropism and the tactile interaction with a surface (thigmotropism) (Simmons et al. 1995). And although the specific metabolic drivers are not fully known, differential expression among cultivars has proposed the existence of a certain amount of unknown candidate genes that may contribute to cultivar-specific phenotypes, as well as confirming several known to be involved with circumnutation (Vaughn and Masson 2011). Regardless of the underlying influences, the statement that gravity contributes to circumnutation deserves therefore reconsideration.

Kitazawa et al. (2005) demonstrated that gravisensing endodermal cells are required for shoot circumnutation in morning glory (*Pharbitis nil*). They identified a gene, *PnSCR*, regulating circumnutation: the insertion of a single amino acid into the VHIID motif caused a loss of PnSCR function, resulting in an abnormal development of the endodermis required for gravisensing in the shoots of dicotyledonous plants, and suggesting that circumnutation is a gravity-dependent morphogenetic phenomenon. However, it remains obscure whether endodermis-mediated gravisensing is the sole prerequisite for circumnutation. To solve this issue, Kitazawa et al. (2005) analysed the shoot circumnutation of two agravitropic mutants of *Arabidopsis*, *sgr2* and *zig/sgr4*, which have endodermal cell layers with abnormal amyloplast sedimentation (Kato et al. 2002), finding that inflorescence stems of these mutants were defective in nutational movement. In addition, an earlier study demonstrated that circumnutation in an *Arabidopsis* mutant, *pgm*, known to show reduced gravitropism caused by the loss of starch granules, was smaller than that of the wild type (Hatakeda et al. 2003). Together, these data corroborate the hypothesis that gravisensing and circumnutation are interlinked, demonstrating also that gravisensing cells or the endodermis-mediated graviresponse is essential for circumnutation in morning glory. The identification of *PnSCR* as the gene responsible for gravitropism in climbing plants has provided a molecular basis for elucidating the detailed mechanism of the relationship between gravisensing/graviresponse and circumnutation.

### 2.2.3 The ‘Mediating’ Model

To overcome the differences between the models previously described, Johnsson et al. (1999) proposed a revised model which combines the two models: a two-oscillator model to explain the phenomenon of circumnutation. In this model, circumnutational movement involves a gravitropic reaction which acts as an externally driven feedback oscillator, together with an endogenous or intrinsic

oscillator which sends a rhythmic signal to the feedback system. The problem remains that there has been no direct evidence yet for the involvement of the graviresponse as an external oscillator in circumnutation. Indeed, this is rather controversial, as the following discussion demonstrates.

The hypocotyls of space-flown sunflowers show circumnutation in microgravity, although the period and amplitude of the movements are relatively small (Brown 1993). Recently, Yoshihara and Iino (2005, 2006) supported the existence of a close relationship between gravitropism and circumnutation in dark-grown rice coleoptiles: (1) circumnutation was interrupted by a gravitropic response and re-initiated at a definable phase after gravitropic curvature; (2) circumnutation can be re-established by submergence and a brief gravitropic stimulation in coleoptiles which have stopped nutating in response to a red light treatment. Moreover, *lazy* mutants show no circumnutation.

Inconsistent with these results, however, Yoshihara and Iino (2006) report cases in which gravitropism and circumnutation could be separated. Firstly, the non-circumnutating *lazy* coleoptile showed nearly a wild-type level of gravitropic responsiveness in its upper half, although this part was an active site of both gravitropism and circumnutation in wild-type coleoptiles. Secondly, coleoptiles could nutate without overshooting the vertical when developing phototropic curvature. The authors concluded that gravitropism influenced, but is not directly involved in the process of circumnutation. They also suggested that a gravity signal, shared with gravitropism, contributes to the maintenance of circumnutation.

## 2.3 Root Circumnutation

Although there have been only occasional suggestions in earlier literature that circumnutation may aid underground organs in soil penetration (e.g. Fisher 1964), this idea has more recently gained experimental confirmation in a study on rice (*Oryza sativa*) varieties (Inoue et al. 1999). Rice, an aquatic plant in origin, must have evolved to acquire traits for securing seedling establishment under flooded conditions. However, many modern varieties fail to become established under such conditions. Inoue et al. (1999) demonstrated that varietal differences in seedling-establishment percentage were attributable not to seminal root elongation rate nor apparent weight of the seed in water but rather to differences in the penetrating ability of the seminal root into soil. To examine whether root tip circumnutation could have been a facilitator of soil penetration by the root, a spectrum analysis of the root tip rotations of various varieties of rice seedlings was performed. Those seedlings which circumnutated with a frequency of 2.0–3.4 cycles per day showed the highest seedling-establishment percentage. From these results, it appears that root tip rotations with large spiral angles are more effective in enabling the root tip to penetrate flooded or very soft soil.

In shoots, the movement has been reported to be irregular (Orbović and Poff 1997), both right- and left-handed. In roots, by contrast, at least of the commonly

studied *Arabidopsis* ecotypes, the movement is helical and right-handed (Simmons et al. 1995). The direction towards which the *Arabidopsis* roots slant during elongation in the wild type is considered to be the right-handed because, when the plant is viewed from above the shoot apex, the root appears to move forwards in clockwise loops—right-handed, as is known in physics. However, it should be remembered that Linnaeus and other scientists (Hashimoto 2002) considered the above movement to be left-handed because they pictured the helix from its interior, in which case the view, logically, is reversed.

In wild-type *Arabidopsis*, root movements are not random at all but rather show a clear right-handedness, i.e. they appear to be animated by a process which could be named ‘chiral circumnutation’. Mullen et al. (1998), investigating the kinetics of the gravitropic response of the *Arabidopsis* mutant *rgr1* (reduced root gravitropism), found that the frequency of the waving pattern and circumnutation was the same in *rgr1* and in the wild type. Thus, the waving/coiling phenomenon is likely governed by circumnutation patterns. The amplitudes of these oscillations may then be selectively amplified by tactile stimulation to provide a directional preference to the slanting.

Recently, *Arabidopsis* root movements were reinterpreted as the combined effect of essentially three processes: circumnutation, gravitropism and negative thigmotropism (Migliaccio and Piconese 2001), albeit with some difficulty in discriminating between these. Piconese et al. (2003), using an RPM (random positioning machine, which subjected the material set at its centre to a general multilateral gravistimulation, approximating space conditions), showed that the observed root pattern depended only on the circumnutating movement, since both gravitropism and negative thigmotropism had been excluded. Using wild-type ecotypes and different gravitropic mutants (auxin transport mutants such as *aux1* and *eir1*; auxin physiology mutants such as *axr1*; handedness mutants such as 1-6C), they observed that wild-type *Arabidopsis* roots made large movements of circumnutation only to the right-hand side, but auxinic mutants, such as *aux1* and *eir1*, showed a lack of regular chiral circumnutation: auxinic mutants are disturbed not only in their gravitropic response (*aux1* and *eir1* are totally agravitropic) but also in their chiral circumnutational movement. Furutani et al. (2000) isolated two mutants, namely *spr1* and *spr2*, which presented a left-handed symmetry with their coils growing anticlockwise, and the left-handed waves dominant in the slanting. The mutant genes were successfully cloned, with the proteins SPR1 and SPR2 identified. When bound to GFP, SPR1 is first localized to the plus end of microtubules, and SPR2 is localized along the length of the cortical microtubules (Sedbrook 2004). The isolation of mutated *spr* genes (*lefty1* and *lefty2*, Marinelli et al. 1997), which encode for  $\alpha$ -tubulin 6 and  $\alpha$ -tubulin 4, respectively, gave an evidence for the involvement of tubulins in guiding the symmetry of the mutants. This result was further confirmed by Collings et al. (2006) on the basis of a research using the *mor1* mutant, which is involved in the control of the synthesis of microtubules arrays, spindle, and phragmoplast, and consequently of the whole karyokinesis process.

The process destroyed in the mutants controls not only gravitropism but also circumnutation: consequently, these seem to have a common basis at the level of

signal transduction (Piconese et al. 2003). Indeed, in an earlier paper, Ney and Pilet (1981) concluded that circumnutation and gravitropism had a common basis because, when the roots were responding to gravitropism, they stopped circumnuting and then resumed the movement at the end of the gravitropic response. Similar results were obtained by the Darwins (Darwin and Darwin 1880) who, on the basis of analogous experiments, stated that gravitropism is a form of modified circumnutation and that all plant movements have a common origin, evolved from the simple (non-chiral) movement of nutation.

The experiments reported by Piconese et al. (2003), however, limited to *Arabidopsis* roots, cannot fully support the above hypothesis, as they show that chiral circumnutation and gravitropism in *Arabidopsis* primary roots seem to depend on auxin transport and/or physiology. This does not imply that the processes of circumnutation and gravitropism in plants are controlled solely by auxin, which probably would be incorrect (Firn et al. 2000), but simply that this hormone seems particularly highly involved, primarily or secondarily, in the circumnuting and tropic responses of plants, as suggested from the very beginning by the pioneers of auxin research (Went and Thimann 1937).

## 2.4 The Role of Plant Hormones: Auxin and Ethylene

Although several hypotheses exist as to what triggers root waving, it is clear that auxin transport and signalling are required to propagate the differential growth response once it has been triggered. Historically, auxin was thought to be transported from the shoot tip to the root, but recent evidence shows that the root tip can also synthesize auxin (Ljung et al. 2005). The asymmetric localization of auxin efflux carriers in the plasma membrane determines the polarity of transport (Galweiler et al. 1998). These carriers relocalize upon environmental stimulation and subsequently alter the overall growth response of the organ (Friml et al. 2002). Mutants of *WAV6/EIR1/AGR1/PIN2*, which encodes a putative auxin efflux facilitator, have defects in gravitropic responses and do not wave when grown on inclined hard agar plates (Okada and Shimura 1990; Luschnig et al. 1998). Mutants of *WAV5/AUX1*, which encodes a putative auxin influx carrier, are also defective in gravitropic responses but form root coils on inclined hard agar plates (Okada and Shimura 1990). Santner and Watson (2006) found that knockout mutants in the *PK3At* gene, which encodes for protein kinase, cause aberrant growth of the primary roots of young seedlings, such that they wave. These genes were renamed *WAG1* and *WAG2*, to connote root phenotypes appearing to move to and from an agar surface.

Recently, the role of ethylene in controlling the circumnutation was explored by several studies. Kim et al. (2011) found that the nutation response was constitutive in *ctr1-2* mutants maintained in air. On the contrary, ethylene-stimulated nutations were eliminated in *ein2-1* mutants and *etr1-7* loss-of-function mutants. When *etr1-7* mutant was transformed with a wild-type genomic *ETR1* transgene, the nutation phenotype was set again, thus supporting a strong requirement for *ETR1*.

Interestingly, loss-of-function mutations in the other receptor isoforms had no effect on nutations in ethylene-stimulated seedlings. On the contrary, they constitutively nutated in air. These evidences lead to the development of a model where all the receptors are involved (but not strictly required) in ethylene-stimulated nutations, but the ETR1 receptor is needed, with a contrasting role from the other receptor isoforms in this nutation phenotype. Further experiments showed a lack of nutations when *Arabidopsis* seedlings were treated with 5 mM AVG, an inhibitor of ethylene biosynthesis. There are also clear differences that can be highlighted between the inhibitory effect of ethylene on hypocotyl growth and its role in stimulating the nutations (Binder et al. 2004a, b). Finally, the chain of biochemical and transcriptional events by which ethylene causes nutation is not well defined and still under investigation. Hatakeda et al. (2003) showed that auxin transport inhibitors, such as NPA, have been shown to block nutations. Similarly, Kim et al. (2011) found that NPA blocks nutations but at the same time has no measurable effect on growth inhibition caused by ethylene. This supports the assertion that, whereas nutations require growth, growth does not require nutations, hypothesizing that ethylene promotes nutations by differentially altering local auxin levels in the zone where nutational bending is observed. Given the complexity of interactions between hormones in plants, it is also likely that other hormones could be involved with ethylene-stimulated nutations. As previously shown, the regulation of microtubule orientation seems to be a mechanism controlling nutations (Furutani et al. 2000). As ethylene directly affects microtubule orientation (Steen and Chadwick 1981), this could be considered another potential mechanism to control nutations by ethylene. However, many other hormones affect microtubules (Shiboaka 1994), and therefore, the interaction between ethylene and microtubules in controlling nutation is not completely defined yet.

## References

- Anderson-Bernadas C, Cornelissen G, Turner CM, Koukkari WL (1997) Rhythmic nature of thigmomorphogenesis and thermal stress of *Phaseolus vulgaris* L. shoots. *J Plant Physiol* 151:575–580
- Arnal C (1953) Recherches sur la nutation des coleoptiles. I. Nutation et croissance. *Ann Univ Sarav* 2:1–2
- Badot PM, Melin D, Garrec JP (1990) Circumnutation in *Phaseolus vulgaris* L. II. Potassium content in the free-moving part of the shoot. *Plant Physiol Biochem* 28:123–130
- Baillaud L (1962) Les mouvements d'exploration et d'enroulement des plantes volubiles. *Handb Pflanzenphysiol* 17:637–715
- Binder BM, Mortimore LA, Stepanova AN, Ecker JR, Bleecker AB (2004a) Short-term growth responses to ethylene in *Arabidopsis* seedlings are EIN3/EIL1 independent. *Plant Physiol* 136:2921–2927
- Binder BM, O'Malley RC, Wang WY, Moore JM, Parks BM, Spalding EP, Bleecker AB (2004b) *Arabidopsis* seedling growth response and recovery to ethylene: a kinetic analysis. *Plant Physiol* 136:2913–2920
- Brown AH (1993) Circumnutations: from Darwin to space flights. *Plant Physiol* 101:345–348

- Buda A, Zawadski T, Krupa M, Stolarz M, Okulski W (2003) Daily and infradian rhythms of circumnutation intensity in *Helianthus annuus*. *Physiol Plant* 119:582–589
- Caré AF, Nefed'ev L, Bonnet B, Millet B, Badot PM (1998) Cell elongation and revolving movement in *Phaseolus vulgaris* L. twining shoots. *Plant Cell Physiol* 39:914–921
- Cholodny N (1926) Beiträge zur Analyse der geotropischen Reaktion. *Jahrb Wiss Bot* 65:447–459
- Collings DA, Lill AW, Himmelspach R, Wasteneys GO (2006) Hypersensitivity to cytoskeletal antagonists demonstrates microtubule–microfilament cross-talk in the control of root elongation in *Arabidopsis thaliana*. *New Phytol* 170:275–290
- Comparot S, Morillon R, Badot PM (2000) Water permeability and revolving movement in *Phaseolus vulgaris* L. twining shoots. *Plant Cell Physiol* 41:114–118
- Correll MJ, Kiss JZ (2008) Space based research on plant tropisms. In: Gilroy S, Masson PK (eds) *plant tropisms*. Blackwell, New York, pp 161–182
- Cosgrove DJ (2000) Expansive growth of plant cell walls. *Plant Physiol Biochem* 38:109–124
- Darwin CA (1875) *The movements and habits of climbing plants*, 2nd edn. Murray, London
- Darwin CA, Darwin F (1880) *The power of movement in plants*. Murray, London
- Engelmann W (1996) Leaf movement rhythms as hands of biological clocks. In: Greppin H, Degli Agosti R, Bonzon M (eds) *Vistas on biorhythmicity*. University of Geneva, Geneva, pp 51–76
- Firn RD, Wagstaff C, Digby J (2000) The use of mutants to probe models of gravitropism. *J Exp Bot* 51:1323–1340
- Fisher JE (1964) Evidence of circumnutational growth movements in rhizomes of *Poa pratensis* L. that aid in soil penetration. *Can J Bot* 42:293–299
- Forterre Y (2013) Slow, fast and furious: understanding the physics of plant movements. *J Exp Bot* 64:4745–4760
- Friml J, Wisniewska J, Benkova E, Mendgen K, Palme K (2002) Lateral relocation of auxin efflux regulator PIN3 mediates tropism in *Arabidopsis*. *Nature* 415:806–809
- Furutani I, Watanabe Y, Prieto R, Masukawa M, Suzuki K, Naoi K, Titamadee T (2000) The SPIRAL genes are required for directional control of cell elongation in *Arabidopsis thaliana*. *Development* 127:4443–4453
- Gälweiler L, Guan C, Müller A, Wisman E, Mendgen K, Yephremov A, Palme K (1998) Regulation of polar auxin transport by AtPIN1 in *Arabidopsis* vascular tissue. *Science*, 282: 2226–2230
- Hashimoto T (2002) Molecular genetic analysis of left–right handedness in plants. *Philos Trans R Soc Lond* 357:799–808
- Hatakeda Y, Kamada M, Goto N, Fukaki H, Tasaka M, Suge H, Takahashi H (2003) Gravitropic response plays an important role in the nutational movements of the shoots of *Pharbitis nil* and *Arabidopsis thaliana*. *Physiol Plant* 118:464–473
- Heathcote DG, Aston TJ (1970) The physiology of plant nutation. I. Nutation and geotropic response. *J Exp Bot* 21:997–1002
- Hejnowicz Z, Sievers A (1995) Proton efflux from the outer layer of the peduncle of tulip on gravitropism and circumnutation. *Bot Acta* 108:7–13
- Hoiczuk E (2000) Gliding motility in cyanobacteria: observations and possible explanations. *Arch Microbiol* 174:11–17
- Inoue N, Arase T, Hagiwara M, Amnao T, Hyashi T, Ikeda R (1999) Ecological significance of root tip rotation for seedling establishment of *Oryza sativa* L. *Ecol Res* 14:31–38
- Israelsson D, Johnsson A (1967) A theory for circumnutations in *Helianthus annuus*. *Physiol Plant* 20:957–976
- Joerrens G (1959) Nutationsbewegungen bei Triticum-Koleoptilen. *Zeitsch Naturforsch* 29:717–724
- Johnsson A (1997) Circumnutations: results from recent experiments on Earth and in space. *Planta* 203:147–158
- Johnsson A, Jansen C, Engelmann W, Schuster J (1999) Circumnutations without gravity: a two-oscillator model. *J Grav Physiol* 6:P9–12
- Johnsson A, Solheim BGB, Iversen TH (2009) Gravity amplifies and microgravity decreases circumnutations in *Arabidopsis thaliana* stems: results from a space experiment. *New Phytol* 182:621–629

- Kato T, Morita MT, Fukaki H, Yamauchi Y, Uehara M, Niihama M, Tasaka M (2002) SGR2, a phospholipase-like protein, and ZIG/SGR4, a SNARE, are involved in the shoot gravitropism of *Arabidopsis*. *Plant Cell* 14:33–46
- Kern VD, Schwuchow JM, Reed DW, Nadeau JA, Lucas J, Skripnikov A, Sack FD (2005) Gravitropic moss cells default to spiral growth on the clinostat and in microgravity during spaceflight. *Planta* 221:149–157
- Kim H, Helmbrecht EE, Stalans MB, Schmitt C, Patel N, Wen CK (2011) Ethylene receptor ETR1 domain requirements for ethylene responses in *Arabidopsis* seedlings. *Plant Physiol* 156:417–429
- Kim GH, Yoon M, Klotchkova TA (2005) A moving mat: phototaxis in the filamentous green algae *Spirogyra* (*Chlorophyta*, *Zygnemataceae*). *J Phycol* 41:232–237
- Kitazawa D, Hatakeda Y, Kamada M, Fujii N, Miyazawa Y, Hoshino A, Iida S, Fukaki H, Morita M, Tasaka M, Suge H, Takahashi H (2005) Shoot circumnutation and winding movements require gravisensing cells. *PNAS* 102:18742–18747
- Ljung K, Hull AK, Celenza J, Yamada M, Estelle M, Normanly J, Sandberg G (2005) Sites and regulation of auxin biosynthesis in *Arabidopsis* roots. *Plant Cell* 17:1090–1104
- Luschnig C, Gaxiola RA, Grisafi P, Fink GR (1998) *EIR1*, a root-specific protein involved in auxin transport, is required for gravitropism in *Arabidopsis thaliana*. *Genes Dev* 12:2175–2187
- Marinelli B, Omarasca SG, Soave C (1997) A pleiotropic *Arabidopsis thaliana* mutant with inverted root chirality. *Planta* 202:196–205
- Migliaccio F, Piconese S (2001) Spiralizations and tropisms in *Arabidopsis* roots. *Trends Plant Sci* 6:594–595
- Migliaccio F, Fortunati A, Tassone P (2009) *Arabidopsis* root growth movements and their symmetry: progress and problems arising from recent work. *Plant Signal Behav* 4:183–190
- Migliaccio F, Tassone P, Fortunati A (2013) Circumnutation as an autonomous root movement in plants. *Amer J Bot* 100:4–13
- Millet B, Melin D, Bonnet B, Ibrahim CA, Mercier J (1984) Rhythmic circumnutation movement of the shoots in *Phaseolus vulgaris* L. *Chronobiol Int* 1:11–19
- Mullen JL, Turk E, Johnson K, Wolverson C, Ishikawa H, Simmons C, Söll D, Evans ML (1998) Root-growth behavior of the *Arabidopsis* mutant *rgr1*. Roles of gravitropism and circumnutation in the waving/coiling phenomenon. *Plant Physiol* 118:1139–1145
- Ney D, Pilet PE (1981) Nutations of growing and georeacting roots. *Plant Cell Environ* 4:339–343
- Niimura K, Someya N, Kimura M, Yamaguchi I, Hamamoto H (2005) Circadian rhythm of circumnutation in inflorescence stems of *Arabidopsis*. *Plant Cell Physiol* 46:1423–1427
- Okada K, Shimura Y (1990) Reversible root tip rotation in *Arabidopsis* seedlings induced by obstacle-touching stimulus. *Science* 250:274–276
- Orbović V, Poff KL (1997) Interaction of light and gravitropism with nutation of hypocotyls of *Arabidopsis thaliana* seedlings. *Plant Growth Regul* 23:141–146
- Paul AL, Amalfitano CE, Ferl RJ (2012) Plant growth strategies are remodelled by spaceflight. *BMC Plant Biol* 12:232
- Piconese S, Tronelli G, Pippia P, Migliaccio F (2003) Chiral and non-chiral nutations in *Arabidopsis* roots grown on the random positioning machine. *J Exp Bot* 54:1909–1918
- Roy R, Bassham DC (2014) Root growth movements: waving and skewing. *Plant Sci* 221–222:42–47
- Santner AA, Watson JC (2006) The WAG1 and WAG2 protein kinases negatively regulate root waving in *Arabidopsis*. *Plant J* 45:752–764
- Schuster J, Engelmann W (1997) Circumnutations of *Arabidopsis thaliana* seedlings. *Biol Rhythm Res* 28:422–440
- Sedbrook JC (2004) MAPs in plant cells: delineating microtubule growth dynamics and organization. *Curr Opin Plant Biol* 7:632–640
- Shabala S (2003) Physiological implications of ultradian oscillations in plant roots. *Plant Soil* 255:217–226
- Shabala S, Knowles A (2002) Rhythmic patterns of nutrient acquisition by wheat roots. *Funct Plant Biol* 29:595–605

- Shabala SN, Newman IA (1997) Proton and calcium flux oscillations in the elongation region correlate with root nutation. *Physiol Plant* 100:917–926
- Shiboaka H (1994) Plant hormone-induced changes in the orientation of cortical microtubules: alterations in the cross-linking between microtubules and the plasma membrane. *Annu Rev Plant Physiol Plant Mol Biol* 45:143–150
- Simmons C, Söll D, Migliaccio F (1995) Circumnutation and gravitropism cause root waving in *Arabidopsis thaliana*. *J Exp Bot* 46:143–150
- Steen DA, Chadwick AV (1981) Ethylene effects in pea stem tissue: evidence of microtubule mediation. *Plant Physiol* 67:460–466
- Stolarz M (2009) Circumnutation as a visible plant action and reaction. *Plant Signal Behav* 4:380–387
- Stolarz M, Krol E, Dziubinska H, Zawadzki T (2008) Complex relationship between growth and circumnutations in *Helianthus annuus* stem. *Plant Signal Behav* 3:376–380
- Thain SC, Murtas G, Lynn JR, McGrath RB, Millar AJ (2002) The circadian clock that controls gene expression in *Arabidopsis* is tissue specific. *Plant Physiol* 130:102–110
- Van den Driessche T (2000) Nutations in shoots and in *Desmodium* lateral leaflets, nyctinastism and seismonastism in *Mimosa pudica*. Comparison and evolution of morphology and mechanism. *Biol Rhythm Res* 31:451–468
- Vaughn LM, Masson PH (2011) A QTL study for regions contributing to *Arabidopsis thaliana* root skewing on tilted surfaces. *G3: Genes, Genomes, Genetics* 1(2):105–115
- Went FW (1926) On growth accelerating substances in the coleoptile of *Avena sativa*. *Proc K Akad Wet Amsterdam* 30:10–19
- Went FW, Thimann KV (1937) *Phytohormones*. MacMillan, New York
- Yoshihara T, Iino M (2005) Circumnutation of rice coleoptiles: its occurrence, regulation by phytochrome, and relationship with gravitropism. *Plant Cell Environ* 28:134–146
- Yoshihara T, Iino M (2006) Circumnutation of rice coleoptiles: its relationships with gravitropism and absence in *lazy* mutants. *Plant Cell Environ* 29:778–792