



UNIVERSITY OF LEEDS

This is a repository copy of *Shoot and root branch growth angle control - the wonderfulness of lateralness.*

White Rose Research Online URL for this paper:  
<http://eprints.whiterose.ac.uk/85922/>

Version: Accepted Version

---

**Article:**

Roychoudhry, S and Kepinski, S (2015) Shoot and root branch growth angle control - the wonderfulness of lateralness. *Current Opinion in Plant Biology*, 23. 124 - 131. ISSN 1369-5266

<https://doi.org/10.1016/j.pbi.2014.12.004>

---

(c) 2014, Elsevier. Licensed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International  
<http://creativecommons.org/licenses/by-nc-nd/4.0/>

**Reuse**

Unless indicated otherwise, fulltext items are protected by copyright with all rights reserved. The copyright exception in section 29 of the Copyright, Designs and Patents Act 1988 allows the making of a single copy solely for the purpose of non-commercial research or private study within the limits of fair dealing. The publisher or other rights-holder may allow further reproduction and re-use of this version - refer to the White Rose Research Online record for this item. Where records identify the publisher as the copyright holder, users can verify any specific terms of use on the publisher's website.

**Takedown**

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing [eprints@whiterose.ac.uk](mailto:eprints@whiterose.ac.uk) including the URL of the record and the reason for the withdrawal request.



[eprints@whiterose.ac.uk](mailto:eprints@whiterose.ac.uk)  
<https://eprints.whiterose.ac.uk/>

## **Shoot and root branch growth angle control - the wonderfulness of lateralness**

Suruchi Roychoudhry and Stefan Kepinski

Centre for Plant Sciences, University of Leeds, Leeds, LS2 9JT, UK

### **ABSTRACT**

The overall shape of plants, the space they occupy above and below ground, is determined principally by the number, length, and angle of their lateral branches. The function of these shoot and root branches is to hold leaves and other organs to the sun, and below ground, to provide anchorage and facilitate the uptake of water and nutrients. While in some respects lateral roots and shoots can be considered mere iterations of the primary root-shoot axis, in others there are fundamental differences in their biology, perhaps most conspicuously in the regulation their angle of growth. Here we discuss recent advances in the understanding of the control of branch growth angle, one of the most important but least understood components of the wonderful diversity of plant form observed throughout nature.

### **Introduction**

The growth angle of branches and other lateral organs is a fascinating topic in developmental biology. For the most part root and shoot branches grow at angles that are non-vertical, a crucial adaptation required for the effective capture of resources both above- and below-ground. The spatial regulation of growth angle is manifest all through the plant kingdom in the form of characteristic species-specific patterns of branch angle control, most strikingly in the wonderfully diverse branching patterns of trees. The fact that deciduous trees are identifiable in the winter by the angle, rather than the number, of their branches emphasises that these differences are genetic and reflects the extent to which growth angle regulation is an important component of the developmental biology of individual lateral organs and of the plant as a whole.

### **Non-vertical growth in lateral branches: gravitropic setpoint angles or just angles?**

Despite the fundamental importance of non-vertical branch growth it is a phenomenon that is only now becoming understood. We can distinguish two broad classes of growth angle control; branch angles that are maintained, independently of other parts of the plant, with respect to gravity and those that are not. For this first class, where growth angle is set and maintained with respect to gravity, that angle of growth is known as the gravitropic setpoint angle or GSA. In this system, an organ being maintained parallel with the gravity vector will have a GSA of  $0^\circ$  if it is growing downwards (e.g. a primary root), and a GSA of  $180^\circ$  if it is growing upwards (e.g. a primary shoot). Organs being maintained at angles to the gravity vector will have GSAs between these two values (for example, an organ growing perpendicular to the gravity vector would have a GSA of  $90^\circ$ ) (Figure 1, Box 1). The GSA concept, introduced by Digby and Firn [1], is a wonderful way of thinking about gravity-dependent growth because it distinguishes sharply between growth angle, which is a physical quantity, and gravitropism, which is a growth process. This distinction

ensures that GSA and gravitropism are not conflated, bringing an analytical clarity to the task of determining how a particular growth angle might be generated.

The second class of growth angles, where the organ in question is not being actively maintained relative to gravity, is necessarily a much more disparate group. This is because it takes in every other angle that does not conform to the simple yet strict definition of a GSA, and includes growth angles for which response to gravity plays no part. It is against these classifications of GSA-based and non-GSA-based growth that we will explore recent studies on growth angle regulation in non-woody higher and attempt to reconcile existing approaches to thinking about growth angle control that are really quite different.

### **Shoot branch angle control in dicots**

The non-vertical growth of shoot branches attracted the interest of some of the earliest plant developmental biologists and physiologists [2,3]. The principal focus of these researchers was in branches that were themselves gravitropic, perhaps because that made the angled growth they observed all the more interesting [2,4]. Branches with non-vertical GSAs present an intriguing problem; the simple tilting of a plant, to say 30°, immediately places some root and shoot branches more vertical than their GSA and others less. All of them will grow back toward their GSA and crucially, some shoot branches will have to grow downwards to do this, and similarly, some lateral roots must grow upwards. This means that the non-vertical growth of these organs cannot be merely because they are not able to 'do' gravitropism as well as the primary shoot and root; there must be another mechanism that drives this downward growth in lateral shoots and upward growth in lateral roots.

In trying to understand how gravity-dependent non-vertical growth might be generated it helps first to think about how vertical GSAs are maintained. For the vertical primary root-shoot axis, GSA control is readily accounted for by the so-called starch-statolith hypothesis and the well-supported model for auxin-mediated tropic growth originating with Cholodny and Went; shifts from the vertical, detected by the sedimentation of starch-rich statoliths, result in asymmetries in auxin distribution that drive tropic, bending growth (Box 1 and Figure 1) [5, reviewed in 6]. Most of the work aiming to understand gravity-dependent angled growth has begun with the idea that these fundamental mechanisms of gravity perception and response should not be excluded from initial models for non-vertical GSA control. Indeed, there are good reasons for accepting this assumption, not the least of which is that in many species the removal of the primary shoot apex (by herbivores or gardeners for example) causes the sub-apical branch to transition to a near-vertical GSA as it becomes the new primary shoot, a state where, as discussed, GSA control is attributable to Cholodny-Went-based gravitropism [7]. The question then is of whether this basic gravitropic response was operating in the lateral branch while it was growing at a non-vertical GSA or if it was only induced following removal of the shoot apex. Recent research using clinostats to eliminate a stable gravitational stimulation suggests that it is the former [7]. This work showed that following the omnilateral gravitational stimulation that occurs during horizontal clinorotation, *Arabidopsis* lateral shoots generate pronounced upward/outward growth curvatures, a pattern of growth that is never observed in primary shoots. These data led the authors to propose that the outward bending of lateral shoots was the manifestation of a growth component (termed the antigravitropic offset or AGO) that under normal conditions acts in tension with gravitropic response to generate net, non-vertical growth, an idea that can be traced back to the pioneering work of De Vries in the late 1800s [2,4].

Following a series of experiments, including the demonstration that the AGO activity required active auxin transport, the authors proposed a model for GSA maintenance based on the antagonistic interaction of two balancing auxin-dependent growth components; gravitropism and the AGO. In this model there are important differences in the regulation of gravitropic and AGO components: for a given GSA the magnitude of the AGO component is constant. In contrast, the magnitude of gravitropic component is continuously variable according to the orientation of the branch in the gravity field, the further it is from the vertical, the greater the magnitude of graviresponse (see Box 1, Figure 2). This difference in the control of the magnitude of gravitropic and AGO components is sufficient to account for the robust maintenance of GSA against displacements to angles either more- or less vertical (Figure 2). In this model, branch GSA is dependent on the magnitude of this AGO; a stronger AGO induces less vertical growth and vice versa. This work also showed that auxin negatively regulates AGO activity and that this regulation is effected specifically in the gravity sensing cells of the shoot (and root, see below) [7]. Thus auxin is involved both in regulating the GSA of a given branch (by controlling the magnitude of the AGO) *and* in driving the gravitropic and anti-gravitropic growth that generates that branch's GSA [7]. These mechanistically distinct roles for auxin suggest a significant capacity for the auxin-mediated self-organisation of growth angle throughout development [7].

This central role for auxin in regulating shoot branch GSA is reflected in studies of other *Arabidopsis* mutants. For example, loss-of-function of members of the *INDETERMINATE DOMAIN (IDD)* transcription factor family *IDD14*, *SGR5/IDD15* and *IDD16* in *Arabidopsis* and the rice ortholog of *SGR5/IDD15* *LOOSE PLANT ARCHITECTURE1* display less vertical branch GSA phenotypes in *Arabidopsis* (and in rice, less vertical tiller angles, Table 1). These genes affect the distribution of auxin through their regulation of the auxin biosynthesis genes *YUCCA5*, *TAA1* and the auxin efflux transporter *PIN1* [8,9,10].

Recent work has also identified a role for orthologs of the rice gene *LAZY1* in the control of branch angle in *Arabidopsis*. *atlazy1* mutants have less vertical shoot branch phenotype, similar to reported effects of loss of *LAZY1* function in rice (see below; [11,12, Table 1]). Although the *AtLAZY1* protein localises to both the plasma membrane and nucleus, nuclear targeting was shown not to be required to complement the branch angle phenotype of *atlazy1* mutant [11]. In 90° reorientation assays the primary inflorescence of *atlazy1* mutants is gravitropic, albeit with slightly slower kinetics. Given that the *atlazy1* primary shoot can maintain a vertical GSA it is quite possible that the non-vertical growth in lateral branches reflects a true GSA phenotype [11].

Another regulator of branch angle control in dicots was identified in a very interesting study aimed at understanding the regulation of branching angle in peach trees [13]. In contrast to *LAZY1*, the *AtTAC1* gene promotes horizontal growth in *Arabidopsis* inflorescence branches and comes from a distinct clade of genes from the same gene family as *AtLAZY1*. Structurally, *AtTAC1* differs from *AtLAZY1* in that it lacks a conserved C-terminal EAR-like LxLxL transcriptional repression domain, a motif indicative of some sort of nuclear function for *AtLAZY* [11,13, Table 1]. Although it is tempting to speculate that a *TAC1-LAZY1* module may act antagonistically either upstream or downstream of auxin transport and signalling to modulate gravitropic and/or antigravitropic growth components, no links between *TAC1* and auxin signalling have been identified thus far. In addition, as the authors of the *TAC1* study point out, models based solely in variation in gravitropic response do not seem to be able to account for the role of *TAC1* in shoot growth angle control in trees where the

angles of higher order secondary branches appear to be set without reference to gravity.

### Shoot branch angle control in monocots

Much of the work on shoot branch angles over the last decade has been done in rice. In monocots and grasses in particular, lateral branches in the form of tillers arise from a primary or main culm. A significant step in the domestication of rice was a shift from the prostrate growth habit of wild rice species to the more erect tiller growth of modern cultivated rice varieties. In 2008, two groups working independently identified *PROSTRATE GROWTH1 (PROG1)* as an important regulator of this transition to more vertical tiller growth [14,15, Table 1]. *PROG1* contains a single C2H2 type Zinc finger motif suggesting that the protein may function as a transcription factor, a hypothesis supported by its predominantly nuclear localisation. *PROG1* is highly expressed in unelongated basal internodes of the culm from where tillers are produced, and loss-of-function mutation in *PROG1* leads to a phenotype of significantly less vertical tiller angle.

The first mechanistic insights into the regulation of tiller angle came from the analysis of the rice mutant *lazy1 (la1)*. *la1* has a prostrate growth habit with tillers growing less vertically than wild-type. *LAZY1* encodes what was initially thought to be a grass-specific protein although deeper analysis has revealed apparent functional orthologues in dicots ([12,16 and see above). In addition to the less vertical tiller phenotype, loss of *LAZY1* function also causes a much reduced gravitropic response at earlier stages of development in coleoptiles and young seedlings. Experiments with coleoptiles of *la1* showed that the formation of the gravity-induced lateral auxin gradient across organ is impaired. Interestingly, it appears that the capacity to transport auxin along the length of the main axis of the coleoptile is increased in *la1* [11,13]. This raises the question of whether the reduced capacity to generate a lateral auxin asymmetry in gravistimulated rice coleoptiles is due to a defect in lateral transport *per se* or in the upstream signalling events required to trigger that transport. Further insights into the role of auxin transport in tiller angle regulation also came from other studies showing that misexpression of the *OsPIN1* and *OsPIN2* auxin efflux transporters lead to changes in tiller angle in rice [17,18, Table 1].

A role for another protein related to *LAZY1* called *TILLER ANGLE CONTROL1 (TAC1)* was identified by QTL analysis. [19, Table 1] In contrast to *LAZY1* and *PROG1* mutants, *tac1* mutants have a smaller, more vertical tiller angle than wild-type plants. *TAC1* is also expressed at the base of the culm and has been proposed to play an antagonistic role to *LAZY1* in dicots (13 and see above). The *TAC1* orthologue in maize was reported to regulate leaf branching angles, and was highly expressed in leaf sheath pulvini [20]. Whether or not there are links between *TAC1*- and *PROG1*-mediated tiller angle regulation and auxin signalling and/or transport remains unknown.

Analysis of the maize mutant *prostrate stem (ps)*, which has a spreading, prostrate growth habit showed that this phenotype was the result of a mutation in the maize orthologue of *LAZY1*. Similar to the rice mutant, the *Zmlazy1* mutant also has increased levels of basipetal auxin transport and is unable to generate an asymmetrical auxin gradient across gravitropically stimulated coleoptiles or shoots of young seedlings. The *ZmLAZY1* protein has one transmembrane domain and two NLs domains, all of which appear functional and the protein was found to localise to the plasma membrane and nucleus. Interestingly, in yeast two hybrid assays,

ZmLAZY1 interacted with the Aux/IAA protein ZmIAA17 in maize, and a member of the protein kinase C (PKC) family [21, Table 1]. Because the polarity of PIN auxin efflux carrier proteins appears to be regulated by their phosphorylation status, these interactions with PKC family kinases raise the possibility that LAZY1 might play a role in regulation of the subcellular localisation of PIN proteins in response to gravitropic stimuli.

More recently, a genetic screen to identify suppressors for *lazy1* (SOLs) in rice led to the identification of a role for strigolactones in regulating rice tiller angles. Loss-of-function of *DWARF3* (*d3*), a signalling component essential for strigolactone perception, was found to suppress the spreading tiller phenotype of *la1* [22, Table 1]. Subsequent analysis of several strigolactone signalling and synthesis mutants revealed that these mutants had enhanced gravitropic responses in seedling shoots. The authors proposed that this enhanced gravitropism was a result of increased auxin accumulation at the base of gravistimulated seedlings relative to wild type, and also that strigolactones were able to modulate gravitropic responses independently of *LAZY1* expression. While lateral auxin transport is unimpaired in these strigolactone mutants, the seedlings themselves contain higher levels of free IAA compared to wild-type and *la1* seedlings [22]. Given that gravitropic bending is dependent on the generation of auxin asymmetry and that *la1* is defective in lateral auxin transport it is not immediately clear how increased auxin levels would suppress the *la1* tiller angle phenotype. A central question here is whether or not the tiller angles that were the focus of all the studies described here are GSAs. If they are, this indicates that differences in gravitropic capacity in *la1* and strigolactone mutants might not be the *cause* of the observed variation in non-vertical growth but rather a *symptom* of a change or defect in a more sophisticated growth angle control mechanism. In this case, the apparent effects of elevated auxin levels on tiller angle noted in the *SOL*/strigolactone study could be considered to have parallels with effect of auxin on shoot branch angle in *Arabidopsis* [7].

If tiller growth angles are not GSAs, how might reduced gravitropism lead to sustained growth at a non-vertical angle? There are several possibilities. For example, it may be that these mutants with reduced gravitropic capacity are unable to exceed a given angle with respect to gravity. Alternatively, because tiller angle is highly dependent on curvature at the tiller base another possibility is that tiller angles are largely determined by the amount of curvature developed in a finite period of gravitropic competence in those basal tissues. Further work will be required to understand which, if any, of these explanations is most relevant to the control of tiller angle in cereals.

### **Root branch growth angle control**

While the primary root typically maintains a near-vertical growth direction, in several monocot and dicot plant species secondary lateral and adventitious roots arising from the primary root or shoot base grow away from the primary axis at non-vertical angles. The mechanisms regulating establishment and maintenance of non-vertical growth in both monocot and dicot root systems have only recently begun to be understood.

Recent insight into regulation of monocot root growth angle has come from an interesting study based on the characterisation of a deep-rooting wild rice variety Kinandang Patong (KP). This work identified a major QTL named *DEEPER ROOTING 1* (*DRO1*) as an important regulator of root growth angle in rice [23,24, Table 1]. Working with a near-isogenic line derived from KP (*Dro1-nil*), the authors found that compared to *Dro1-nil*, root gravitropism kinetics are slower in the shallow-

rooting IR64 rice variety bearing a truncated *DRO1* loss-of-function allele. *DRO1* expression is repressed by auxin and in gravitropism assays, increased auxin levels at the bottom half of gravistimulated roots are sufficient to reduce *DRO1* levels, even in the presence of the protein synthesis inhibitor cycloheximide. From these data it is unclear how auxin-mediated *DRO1* repression contributes to growth angle control in rice roots given that *DRO1* seems to be a positive regulator of gravitropic response in seedling roots [23]. One possibility is that the expression of full-length *DRO1*, as a positive regulator of cell elongation, allows the development of a greater asymmetry in growth between upper and lower sides of graviresponding roots in KP and *Dro1-nil* compared to IR64. This might be expected to be reflected differences in root elongation between these lines, a phenomenon that was not apparent [24]. It is also the case that the growth angle phenotypes of the shallow- and deep-rooting varieties that were the focus of this study are sustained for weeks while differences in the kinetics of gravitropic response between these lines (albeit assayed in young seedling roots) were of the order of hours. Again, establishing whether or not these root growth angles are GSAs will be informative in better understanding the role of *DRO1* in growth angle control.

The GSA biology of *Arabidopsis* lateral roots was first characterised in detail by Mullen and Hangarter [25]; lateral roots emerge perpendicular to the primary root but almost immediately grow downward attaining a GSA of approximately 75° within the first 0.2 mm of growth [25]. After this brief initial downward curvature, lateral roots maintain a programme of gravity-dependent non-vertical growth that becomes increasingly vertical over the course of several days [7,25,26]. Importantly, throughout this period of non-vertical growth lateral roots can reorientate their growth both upwards and downwards to maintain their prevailing GSA [7,25]. These data indicate that this pattern of lateral root growth is most accurately described as a continuum of increasingly vertical GSA states.

Recent work has described the central role of auxin and auxin transport in lateral root GSA maintenance. Consistent with the effect of auxin on shoot branch GSA, increased auxin levels were shown to induce more vertical growth in *Arabidopsis* root systems [7,26] as well as those of rice and bean [7]. Two studies also characterised the expression of PIN auxin efflux carriers during lateral root development noting dynamic changes in the expression of PIN3, PIN4 and PIN7 [26,27]. Ruiz-Rosquete et al. went on to examine the lateral root GSA- and auxin distribution phenotypes of *pin3-4*, *pin4-3* and *pin7-2* single and multiple mutants. This analysis led them to propose a model in which lateral root growth angle in *Arabidopsis* is explained by reduced gravitropic competence of laterals, attributed principally to the repression of PIN4 and PIN7 in the gravity-sensing columella cells of young laterals [26]. They also confirmed that establishment of the elongation zone is required gravitropic response in *Arabidopsis* lateral roots. These studies provide important molecular details about the components that are likely contributing to GSA control in roots but on their own cannot account for the patterns of non-vertical growth observed in *Arabidopsis* roots. This is because, as outlined above, models based solely on differences in gravitropic competence between the primary and lateral roots do not explain the spatio-temporal pattern of lateral root growth over periods of days (see Box 1) nor the ability for lateral roots to reorientate upwards to regain their GSA.

The model of balancing, auxin-dependent gravitropic and antigravitropic offset (AGO) growth components described above for shoot branch GSA control applies equally to lateral roots. Using the same techniques, it was shown that following the removal of a stable gravity reference during horizontal clinorotation lateral root growth is shifted upwards, a response that is also dependent on auxin transport. Again, this finding can account for the maintenance of non-vertical GSAs in lateral roots (Figure 2). In

addition to exogenous auxin inducing more vertical growth in lateral roots [7,26], the analysis of a panel of mutants with higher or lower levels of auxin or TIR1/AFB-Aux/IAA-ARF-mediated auxin response indicated that as in the shoot, auxin controls GSA by negatively regulating the magnitude of the AGO component. This regulation is effected in the gravity-sensing columella cells because alteration in TIR1/AFB-Aux/IAA-ARF-mediated auxin signaling in just these cells is sufficient to modulate lateral root, but not primary root, GSA [Table 1].

The effects of auxin on lateral root growth and the AGO-based model of Roychoudhry et al. raise the possibility that the growth profile of lateral roots could be accounted for by a gradual and discontinuous increase in auxin concentration and/or auxin sensitivity in the tips of lateral roots as they mature. Thus, the increasingly vertical growth of laterals would be attributable to the decreasing magnitude of the AGO component. It is important to point out that this model does not exclude the findings on PIN protein expression during lateral root development described above [26,27] and that at all times, the GSA of the lateral root is the product of the interaction between gravitropic and AGO growth components.

This model of auxin-dependent balancing gravitropic and antigravitropic growth components also offers a way to see how environmental signals might be integrated to shape lateral branch growth. For example, low nitrogen and phosphorus conditions have recently been shown to lead to more vertical crown and lateral root GSA in maize and Arabidopsis [28,29]. For the low phosphate response in Arabidopsis at least, it is possible that these changes are effected through TIR1-dependent auxin signalling, as phosphate deficiency has been shown in increased TIR1 expression in Arabidopsis roots [30] and the effect of low phosphate on lateral root GSA is diminished in the *tir1-1* background (Roychoudhry and Kepinski, unpublished).

## Conclusions

The regulation of growth angle is a fascinating and complex problem with the potential for multiple intrinsic and extrinsic factors to contribute to the final angle of growth. Happily, mechanistic understanding is beginning to emerge but there is still a spectacularly vast amount that isn't known about growth angle control. Here we have focused on the regulation of growth angles that are in some way gravity-dependent. Of course, where significant gradients of other important environmental stimuli such as light or water occur, there will often be a shift in growth angle arising from the interaction between gravitropism and phototropism (typically in the shoot) and gravitropism and hydrotropism (typically in the root), the extent of which is dependent on the relative magnitude of the tropic responses involved [31-33]. The precise nature of the interactions between phototropic, hydrotropic, and GSA control mechanisms remain to be determined but it seems likely that the understanding of their molecular basis may well provide new insights into processes underlying the individual tropic growth components as well as their action in combination.

One common theme to the various models of non-vertical branch growth that have been proposed is the central role of auxin and auxin transport. This alone leaves several important open questions. For example, PIN-mediated auxin transport appears to be required for both upward and downward growth in lateral root and shoots. It has been suggested that antagonistic regulation of the polarity of PIN activity in gravity-sensing cells could account for this control [7] but more work is required to understand these mechanisms and of course, why this apparent



additional regulation (whatever form it takes) is absent or significantly suppressed in the primary organs with near-vertical GSAs.

Part of the wonderfulness referred to in the title of this article stems from the fact that in lateral branches there is a specific requirement to do things differently to the primary axis and these differences have the potential to provide important new insights into the biology of GSA control and gravitropic response. Indeed, it is possible that research focused on gravitropism in the vertical main root and shoot may sometimes have been attempting to rationalise the molecular and cell biological features of graviresponse without consideration of the strong adaptive value of *not* growing vertically upwards and downwards. It is also the case that given the importance of non-vertical branch angles for the capture of the resources required for plant growth it would not be at all surprising if more than one mechanism has arisen that allows gravity-dependent non-vertical growth and in particular, the maintenance of GSAs.

### **Acknowledgements**

We wish to thank Ryan Kaye for assistance in the preparation of the figures and the BBSRC and EPSRC for their support of relevant projects in the Kepinski lab (BB/K10956/1; EP/G039496/1).

### **References:**

1. J. Digby and R. Firn  
The Gravitropic Setpoint Angle: Identification of an important developmentally controlled variable governing plant architecture.  
Plant, Cell and Environment (1995) 18: 1434-1440
2. H. De Vries  
Ueber einige Ursachen der Richtung bilateralsymmetrischer Pflanzentheile.  
Arb.bot.Inst.zu Wuerzburg. (1872) Bd.II:223-277.
3. J. Baranetzsky  
Ueber die Ursachen welche die Richtung der Aeste der Baum- und Straucharten bedingen (1901). Flora 89: 138-239.
4. C.J. Lyon  
Auxin factor in branch epinasty.  
Plant Physiology (1963)
5. B. Nemeč  
Über die Wahrnehmung des Schwerkraftreizes bei den Pflanzen.  
Jahrbucher fuer Wissenschaftliche Botanik (1901) 36: 80-178
6. M.T. Morita  
Directional gravity sensing in gravitropism.  
Annual Review of Plant Biology (2010) 61: 705-720
7. S.Roychoudhry, M. Kieffer, M. Del Bianco, S.Kepinski  
•• Auxin controls gravitropic-setpoint angle in higher plant lateral branches.  
Current Biology (2013) 5: 1497-504  
Describes a model for the maintenance of non-vertical growth angles in lateral shoots and roots in Arabidopsis based on the antagonistic interaction of two auxin-dependent growth components: gravitropism and an antigravitropic offset component, the magnitude of which is regulated in the

gravity sensing cells of the root and shoot via Aux/IAA-TIR1-ARF dependent auxin signalling

8. D. Cui, J. Zhao, Y. Jing, M. Fan, J. Liu, Z. Wang, W. Xin, Y. Hu  
The Arabidopsis IDD14, IDD15 and IDD16 cooperatively regulate lateral organ morphogenesis and gravitropism by promoting auxin biosynthesis and transport.  
PLOS Genetics (2013), 9: e1003759
9. X. Wu, D. Tang, M. Li, K. Wang, Z. Cheng  
Loose Plant Architecture1, an INDETERMINATE DOMAIN protein involved in shoot gravitropism, regulates plant architecture in rice.  
Plant Physiology (2013) 161: 317-329
10. M.T. Morita, K. Sakaguchi, S. Kiyose, K. Taira, M. Nakamura, M. Tasaka  
A C2H2-type zinc finger protein, SGR5, is involved in early events of gravitropism in Arabidopsis inflorescence stems.  
The Plant Journal (2006) 47 (4): 619-628
11. T. Yoshihara, E. Spalding, M. Lino  
•AtLAZY1 is a signaling component required for gravitropism of the Arabidopsis thaliana inflorescence.  
The Plant Journal (2013), 74: 267-279  
The authors identified a role for an ortholog of the rice gene *LAZY1* in the control of branch angle in Arabidopsis. *atlazy1* mutants have less vertical shoot branch phenotype. Although the AtLAZY1 protein was found to localise to both the plasma membrane and nucleus, nuclear targeting was shown not to be required to complement the branch angle phenotype of *atlazy1* mutant.
12. T. Yoshihara and M. Lino  
Identification of the gravitropism related gene rice LAZY1 and elucidation of LAZY1 dependent and independent signalling pathways.  
Plant and Cell physiology (2007) 48: 678-688
13. C. Dardick, A. Callahan, R. Horn, K.B. Ruiz, T. Zhebentyayeva, C. Hollender, M. Whitaker, A. Abbot, R. Scorza  
•• PpeTAC1 promotes the horizontal growth of branches in peach trees and is a member of a functionally conserved gene family found in diverse plant species.  
The Plant Journal (2013) 75: 618-630  
Describes the functional conservation of the *TAC1* gene in promoting non-vertical growth in peach tree and Arabidopsis inflorescence branches. The Arabidopsis *attac1* loss-of-function mutant displays more vertical branch growth angles than wild-type. The authors report that *TAC1* and *LAZY1* are both part of the same *IGT* gene family. The gene structures of *TAC1* and *LAZY1* differ by the presence of an additional EAR repression motif in the *LAZY1* gene, indicating the capacity for *LAZY1* to act as a transcriptional repressor. The authors propose that *IGT* genes may play a conserved role in modulating plant architecture through a *TAC1-LAZY1* module.
14. J. Jin, W. Huang, J. Gao, J. Yang, M. Shi, M. Zhu, D. Luo, H. Lin  
Genetic control of rice plant architecture under domestication.

Nature Genetics (2008) 40:11, 1365-1369

15. L. Tan, X. Li, F. Liu, X. Sun, C. Li, Z. Zhu, Y. Fu, H. Cai, X. Wang, D. Xie, C. Sun  
Control of a key transition from prostrate growth to erect growth in rice domestication.  
Nature Genetics (2008) 40:11, 1360-1364
16. P. Li, Y. Wang, Q. Qian, Z. Fu, M. Wang, D. Zeng, b. Li, X. Wang, J. Li  
LAZY1 controls rice shoot gravitropism through regulating polar auxin transport.  
Cell Research (2007) 5: 402-410
17. M. Xu, L. Zhu, H. Shou, P. Wu  
A PIN1 gene family OsPIN1 involved in auxin dependent adventitious root emergence and tillering in rice.  
Plant Cell Physiology (46): 1674-1681
18. Y. Chen, X. Fan, W. Song, Y. Zhang, G. Xu  
Over-expression of OsPIN2 leads to increased tiller numbers, angle and plant height through suppression of OsLAZ1.  
Plant Biotechnology Journal (2012), 10: 139-149
19. B. Yu, Z. Lin, H. Li, X. Li, J. Li, Y. Wang, X. Zhang, Z. Zhu, W. Zhai, X. Wang, D. Xie, C. Sun  
TAC1, a major quantitative trait locus controlling tiller angle in rice.  
The Plant Journal (2007): 52, 891-898
20. L. Ku, X. Wei, S. Zhang, J. Zhang, S. Guo, Y. Chen  
Cloning and characterization of a putative TAC1 ortholog associated with leaf angle in maize (*Zea mays* L.)  
PLOS One (2011) 6: e20621
21. Z. Dong, C. Jiang, X. Chen, T. Zhang, L. Ding, W. Song, H. Luo, J. Lai, H. Chen, R. Liu, X. Zhang, W. Jin  
Maize LAZY1 mediates shoot gravitropism and inflorescence development through regulating auxin transport, auxin signaling and light response.  
Plant Physiology, 2013, 163, 1306-1322
22. D. Sang, D. Chen, G. Liu, Y. Liang, L. Huang, X. Meng, J. Chu, X. Sun, G. Dong, Y. Yuan, Q. Qian, J. Li, Y. Wang  
•Strigolactones regulate rice tiller angle by attenuating shoot gravitropism through inhibiting auxin biosynthesis.  
PNAS (2014) 111: 11199-11204  
Through screening for suppressors of the *lazy1* (*la1*) tiller spreading phenotype in rice, the authors identify the *dwarf3* strigolactone signalling mutant that partially rescues the *la1* phenotype. Further, they show that strigolactone signalling and synthesis mutants have increased auxin levels and show enhanced gravitropic curvature compared to wild type and *la1* seedlings. In contrast to *la1*, strigolactone mutants do not show defects in lateral auxin transport leading the authors to propose that strigolactones

regulate shoot gravitropism through auxin biosynthesis via a pathway that is genetically independent from that of *LAZY1*.

23. Y. Uga, K. Okuno, M. Yano  
Dro1, a major QTL involved in deep rooting of rice under upland field conditions.  
J. Exp. Botany (2011) 62:8, 2485-2494
24. Y. Uga, K. Sugimoto, S. Ogawa, J. Rane, M. Ishitani, N. Hara, Y. Kitomi, Y. Inukai, K. Ono, N. Kanno, H. Inour, H. Takehisa, R. Motoyama, Y. Nagamura, J. Wu, T. Matsumoto, T. Takai, K. Okuno, M. Yano
  - Control of root system architecture by *DEEPER ROOTING 1* increases rice yield under drought conditions.  
Nature Genetics 2013 (45):9 -1097-1102  
The authors characterize an important regulator of seminal root growth angle in rice based on phenotypic differences between IR64 shallow rooting and Kinandang Patong deep rooting rice varieties. The *DRO1* gene allele in the IR64 variety contains a single base pair deletion in exon 4 leading to a premature stop codon. *DRO1* is expressed in the root tip and the basal region of the shoot. The *DRO1* promoter contains a single TGTCTC auxin response element and *DRO1* expression was repressed within 30 minutes of auxin treatment. Because auxin transport is not impaired in the IR64 background, the authors propose that reduced gravitropic curvature resulting from the truncated *DRO1* allele in this background may result from defects in cell elongation in the distal elongation zone of gravistimulated roots.
25. J.L. Mullen, R.P. Hangarter  
Genetic analysis of gravitropic setpoint angle.  
Adv. In Space Research (2003), 10: 2229-36
26. M. Ruiz-Rosquete, D. von Wangenheim, P. Marhavy, E. Barbez, E.H.K. Stelzer, E. Benkova, A. Maizel, J. Kleine-Vehn
  - An auxin transport mechanism restricts positive orthogravitropism in lateral roots.  
Current Biology (2013), 9: 817-822  
This work describes a model for GSA establishment in young lateral roots of *Arabidopsis* based on the repression of auxin efflux transporters *PIN4* and *PIN7*. *PIN3* is highly expressed in columella young emerging lateral roots in *Arabidopsis* and has weaker expression in older roots, while *PIN4* and *PIN7* expression is absent or low in young lateral roots but becoming stronger in older roots. The authors propose a model wherein the ability of a lateral root to grow vertically downward is limited by a deficiency in auxin transport through sequential repression initially of *PIN4* and *PIN7* and then *PIN3*.
27. S. Guyomarc'h, S. Leran, M. Auzon-Cape, F. Perrine-Walker, M. Lucas, L. Laplace
  - Early development and gravitropic response in lateral roots of *Arabidopsis thaliana*.  
Philos. Trans. Royal Soc. London B. Biol. Science (2012), 367: 1509-1516  
The authors describe the onset of gravitropic curvature and statolith development and in the columella cells of young lateral roots. Gravitropic curvature of lateral roots begins soon after emergence, while statoliths are not detectable until 1-day post emergence. The auxin efflux transporters *PIN3*, *PIN4* and *PIN7* show differential expression patterns in primary and lateral

roots which the authors suggest may play a role in gravitropic response of lateral roots.

28. S. Trachsel, S.M. Kaeppler, K.M. Brown, J.P. Lynch  
Maize root growth angles become steeper under low N conditions.  
Field Crops Research 140: 18-31
29. H. Bai, B. Murali, K. Barber, C. Wolverton  
Low phosphate alters lateral root setpoint angle and gravitropism.  
Am. J. Botany (2013), 1: 175-182
30. C. Perez-Torres, J. Lopez-Bucio, A. Cruz-Ramirez, E. Ibarra-Laclette, S. Dharmasiri, M. Estelle, L. Herrera-Estrella  
Phosphate availability alters lateral root development in Arabidopsis by modulating auxin sensitivity via a mechanism involving the TIR1 Auxin receptor.  
Plant Cell (2008) 20: 3258-3272
31. P. Galland  
Tropisms of *Avena* coleoptiles: Sine law for gravitropism, exponential law for photogravitropic equilibrium.  
*Planta* (2002) **215**(5):779–784.
32. Y. Miyazawa, H. Takahashi  
How do Arabidopsis roots differentiate Hydrotropism from Gravitropism?  
Plant Signal. Behav. (2007) 2: 388-389
33. T. Moriwaki, Y. Miyazawa, A. Kobayashi, , H. Takahashi,  
Molecular mechanisms of hydrotropism in seedling roots of Arabidopsis thaliana (Brassicaceae)..  
Am. J. Bot. (2013) 100: 25-34
34. J. Friml, J. Wisniewska, E. Benkova, K. Mendgen, K. Palme  
Lateral relocation of auxin efflux transporter PIN3 mediates tropism in Arabidopsis.  
Nature (2003) 415: 806-809
35. J. Kleine-Vehn, Z. Ding, A.R. Jones, M. Tasaka, M.T. Morita, J. Friml  
Gravity-induced PIN transcytosis for polarization of auxin fluxes.  
PNAS (2010) 107: 22344-22349
36. J.Z. Kiss, K.M. Miller, L.A Ogden, K.K. Roth.  
Phototropism and gravitropism in lateral roots of Arabidopsis.  
Plant Cell Physiol. (2002) 43:35–43.
37. Ottenschlager, P. Wolff, C. Wolverton, R. Bhalerao, G. Sandberg, H. Ishikawa, M. Evans, K. Palme

- Gravity-regulated differential auxin transport from columella to lateral root cap cells.  
PNAS (2003) 100: 2987-2991
38. L.R. Band, D.M. Wells, A. Larrieu, J. Sun, A.M. Middleton, A.P. French, G. Brunoud, E.M. Sato, M.H. Wilson, B. Peret, M. Oliva, R. Swarup, I. Sairanen, G. Parry, K. Ljung, T. Beeckman, J.M. Garibaldi, M. Estelle, M.R. Owen, K. Vissenberg, T. C. Hodgman, T.P. Pridmore, J.R. King, T. Vernoux, M.J. Bennet  
Root gravitropism is regulated by a transient lateral auxin gradient controlled by a tipping point mechanism.  
PNAS (2012) 109: 4668-4773
39. Sachs J.  
Über orthotrope und plagiotrope Pflanzenteile.  
Arb Bot Inst Würzburg. (1882) ;2:226–284
40. J.L. Mullen, C. Wolverton, H. Ishikawa, M.L. Evans  
Kinetics of constant gravitropic feedback stimulus in Arabidopsis roots using a feedback system.  
Plant Physiol. (2000) 123: 665-670
41. J. Dumais  
Beyond the Sine Law for plant gravitropism.  
PNAS (2012) 110 (2): 391-391
42. M. Iino, Y. Tarui, C. Uematsu  
Gravitropism of maize and rice coleoptiles: Dependence on the stimulation angle.  
*Plant Cell Environ* (1996) **19**(10):1160–1168
43. L.J. Audus  
Geotropism and the modified sine rule: An interpretation based on the amyloplast statolith theory.  
*Physiol Plant* (1964) **17**:737–745.
44. P. Larsen  
The optimum angle of geotropic stimulation and its relation to the starch statolith hypothesis.  
*Physiol Plant* (1969) 22:469–488

### **Box 1. Gravitropism and GSA control**

In non-woody tissues, bending growth to or away from a stimulus is driven largely by asymmetries of auxin concentration and response. In the case of gravitropism, this asymmetry is generated by changes in the subcellular distribution of PIN auxin efflux carriers within the gravity-sensing cells, or statocytes, of the shoot and root (Figure 1) [34,35]. A predominantly downward polarity of PIN-mediated auxin flow out of these cells appears to be linked, in ways that are still entirely unknown, to the sedimentation of starch-rich amyloplasts within each statocyte. Through these

biophysical and molecular events, auxin accumulates on the lower side of a gravitropic organ [34-36]. In roots, this auxin accumulation inhibits cell elongation causing the root tip to grow downwards while in the shoot the reverse is true, auxin accumulation on the lower side of the organ drives cell elongation and so upward growth. Importantly, the magnitude of gravitropic response (as degrees of bending per unit time) increases as the organ is tilted further from the vertical [37,38]. This angle-dependent variation in the magnitude of graviresponse was first analysed by Sachs in the late 1800s [39]. He proposed the so-called sine rule or sine law which states that rate of gravitropic bending is proportional to the sine of the angle of displacement [39]. Subsequent work in a range of species has shown that while the sine rule has limitations, in general it holds well for angles of displacement up to 90° from the vertical [31, 41-44].

The gravitropic setpoint angle, or GSA, is the angle at which an organ is maintained with respect to gravity. The capacity to maintain a GSA has to be inherent in the organ itself; an organ would not be considered to have a GSA if its growth relative to gravity was being maintained by virtue of gravitropic response in another part of the plant to which it is attached. If there is ever any doubt as to whether a particular growth angle is a GSA or not a simple reorientation test will immediately reveal the answer: if upon tilting an organ to a more vertical or less vertical orientation it grows back close its original angle of growth with respect to gravity it is a GSA, if it doesn't, it isn't. Although gravitropism and GSA will often be considered together it is important not to conflate the two. Where stable growth at non-vertical GSAs is maintained for periods of days or even weeks then it is not reasonable to expect differences in the kinetics of graviresponse between primary and laterals branches that are of the order of minutes or hours to account for that sustained period of angled growth.

## Figure legends

### **Figure 1. Gravity-sensing tissues and graviresponse in dicot and monocot plant organs**

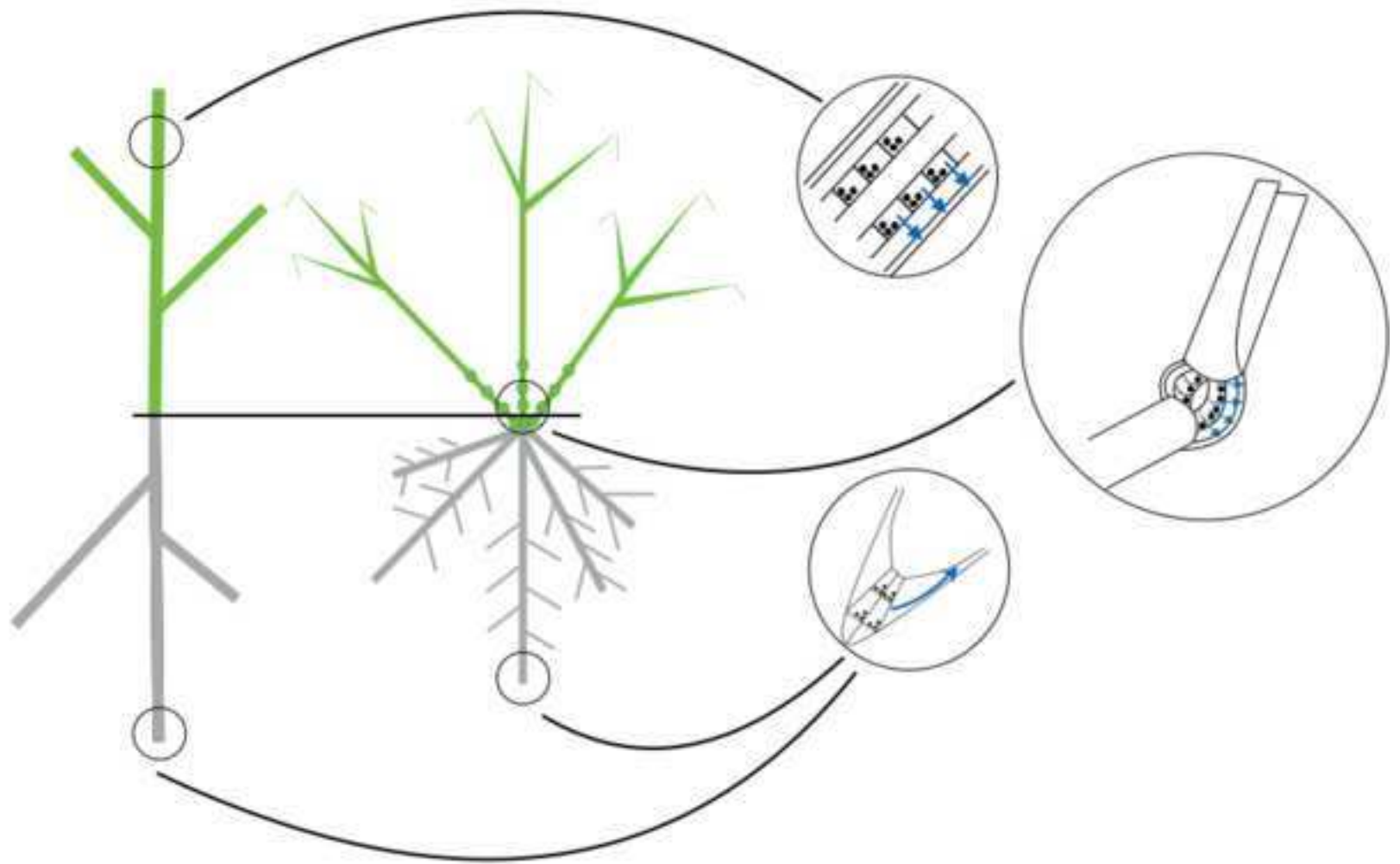
Gravity sensing occurs in specialised cells known as statocytes in the endodermis of dicot shoots, the pulvini of rice tillers and leaves, and the columella cells of dicot and monocot roots. In these cells gravity is perceived by the sedimentation of dense starch-filled bodies known as amyloplasts. When an organ is moved away from the vertical, the amyloplasts sediment onto the new physical bottom of the cell. This triggers the lateral, downward flow of the plant hormone auxin (blue arrows) leading to the accumulation of auxin in the bottom half of the gravistimulated organ. This increase in auxin concentration leads to differential cell elongation across the top and bottom of the organ ultimately causing upward curvature in the shoot and downward curvature in the root.

### **Figure 2. Non-vertical GSAs are maintained by means of antagonistic interaction between auxin-dependent gravitropic and antigravitropic offset components**

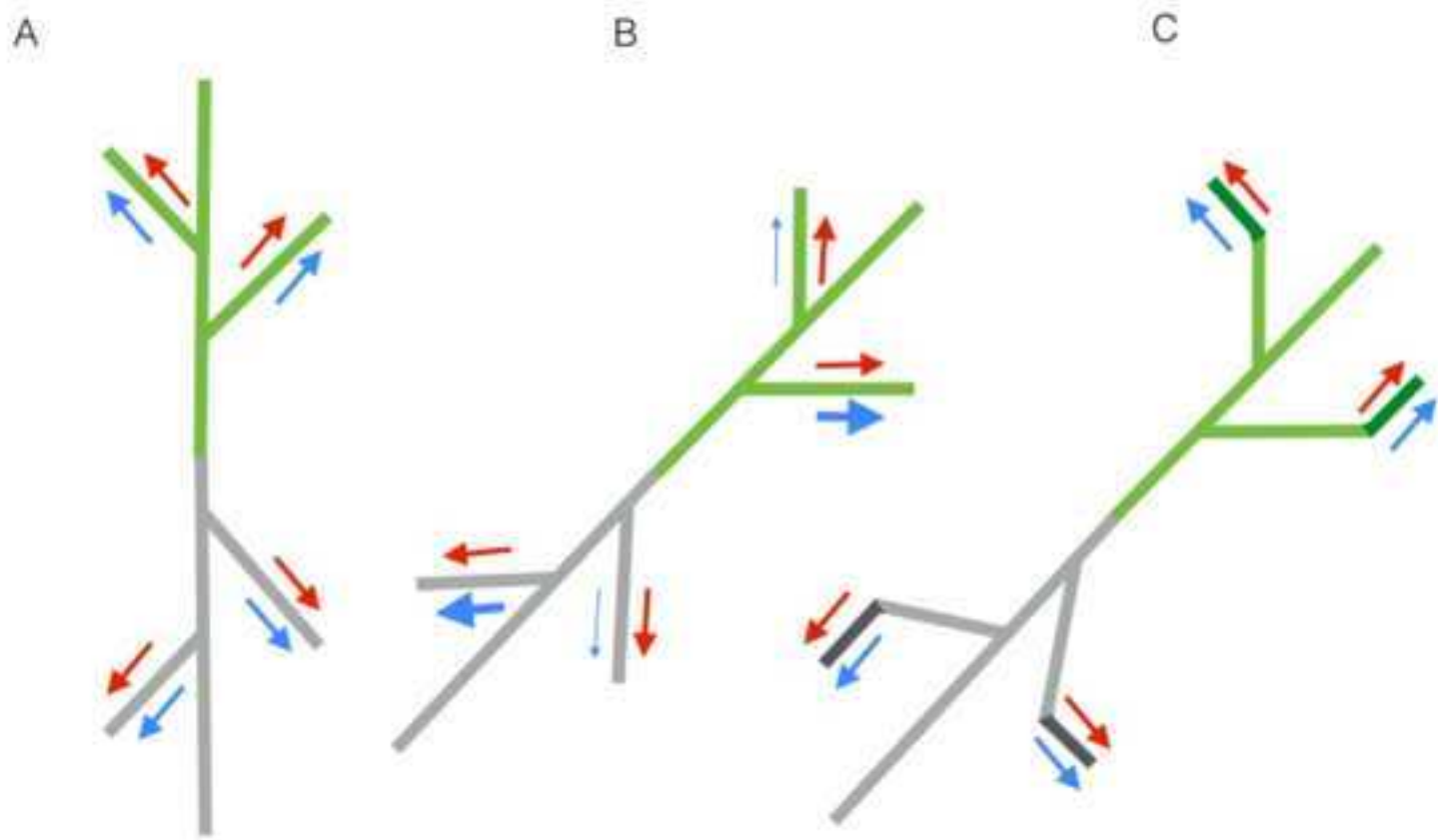
(A) Lateral roots and shoots are maintained at their GSAs by antagonistic interaction of two opposing auxin-dependent growth components: gravitropic growth (blue arrow) that would otherwise cause bending to the vertical and counteracting antigravitropic offset (AGO) growth (red arrow). Stable non-vertical growth occurs when the gravitropic and AGO components are in equilibrium. (B) When an organ is moved to an angle more vertical than its GSA, the magnitude of the gravitropic component is reduced (see Box 1) while the AGO remains constant. This relative magnitude of the AGO then promotes growth in a direction that returns the organ

back towards its original GSA (C). In contrast, when an organ is moved to an angle that is less vertical than its GSA, the magnitude of the gravitropic component increases (Box 1) while again, the antigravitropic offset remains constant. This increase in gravitropic response brings the organ back towards its GSA (C). In all cases, the orientation of the branch in the gravity field is continuously monitored by events within the gravity-sensing cells, with the magnitude of the gravitropic growth component being adjusted accordingly as the organs approach their GSA.





**Figure 1: Gravity-sensing in dicot and monocot plant organs**



**Figure 2: Non-vertical GSAs are maintained by means of antagonistic interaction between a sine-law dependent gravitropic auxin flux and an anti-gravitropic offset component**

Table

| Protein                 | Structure  | Species     | Mutant(s)  | Phenotype  |
|-------------------------|--|-------------|--|--|
| IDD14, IDD15, IDD16     | Conserved ID domain with four zinc finger motifs   | Arabidopsis | <i>idd14-1</i><br><i>idd15-5/sgr5</i><br><i>idd16-RNAi</i> | Loss-of-function mutations have significantly less vertical lateral branch GSAs  |
| LPA1                    | Ortholog of <i>AtIDD15</i> . Conserved ID domain with four zinc finger motifs. Also contains a EAR domain conferring transcriptional repression activity | Rice        | <i>lpa1</i>  | Loss-of-function mutants have increased tiller and leaf angles   |
| AtLAZY1                 | Two NLS domains with a single LxLxL EAR motif  | Arabidopsis | <i>lazy1</i>   | Loss-of-function mutations have significantly less vertical lateral branch GSAs and reduced gravitropism   |
| OsLAZY1                 | Single transmembrane domain and NLS domain   | Rice        | <i>la1</i>   | Loss-of -function mutants have increased tiller and leaf angle   |
| ZmLAZY1/PROSTRATE STEM1 | Single transmembrane and NLS domain  | Maize       | <i>Zmla1/ps1</i>   | Loss-of-function mutant has significantly reduced shoot gravitropism   |
| AtTAC1                  | Conserved IGT motif in domain II, belongs to same gene family as <i>LAZY1</i>  | Arabidopsis | <i>tac1</i>  | Loss-of-function mutants have significantly more vertical lateral branch GSAs  |
| OsTAC1                  | Ortholog of <i>AtTAC1</i> containing conserved IGT motif   | Rice        | <i>Ostac1</i>  | Loss-of-function mutants have increased tiller angles  |
| PROG1                   | C2H2 type Zinc finger motif  | Rice        | <i>prog1</i>   | Loss-of-function mutants have increased tiller angle   |
| DWARF3                  | F-box component of SCF E3 ubiquitin ligase complex for strigolactone perception  | Rice        | <i>dwarf3/d3</i>   | Identified in a screen for suppressors of the <i>lazy1</i> phenotype, loss-of-function mutants have short compact tiller and show enhanced gravitropic responses in seedlings shoots |
| DRO1                    | Contains two putative N-myristoylation sites   | Rice        | <i>dro1</i>  | Loss-of-function mutants have a shallow rooting angle  |

|      |   |             |               |   |
|------|---|-------------|---------------|---|
|      | associated with lipid modification  |             |               | and reduced root gravitropism in seedlings                    |
| TIR1 | F box auxin receptor that forms part of the SCF <sup>TIR1/AFB</sup> E3 ubiquitin ligase complex | Arabidopsis | <i>tir1-1</i> | Loss-of-function mutations have a less vertical GSA phenotype |

**Table 1:** List of proteins with described structures that are known to regulate root and shoot branching angles across a range of dicot and monocot plant species. (NLS = Nuclear Localisation Signal, EAR = Ethylene-responsive element binding factor-associated amphiphilic repression)