

7. Herrnstein, R.J. (1990). Levels of stimulus control: A functional approach. *Cognition* 37, 133–166. [https://doi.org/10.1016/0010-0277\(90\)90021-B](https://doi.org/10.1016/0010-0277(90)90021-B).
8. Benedict, L.M., Heinen, V.K., Welklin, J.F., Sonnenberg, B.R., Whitenack, L.E., Bridge, E.S., and Pravosudov, V.V. (2023). Food-caching mountain chickadees can learn abstract rules to solve a complex spatial-temporal pattern. *Curr. Biol.* 33, 3136–3144.
9. Krebs, J.R., Sherry, D.F., Healy, S.D., Perry, V.H., and Vaccarino, A.L. (1988). Hippocampal specialization of food-storing birds. *Proc. Natl. Acad. Sci. USA* 86, 1388–1392. <https://doi.org/10.1073/pnas.86.4.1388>.
10. Sherry, D.F., and Vaccarino, A.L. (1989). Hippocampus and memory for food caches in black-capped chickadees. *Behav. Neurosci.* 103, 308–318.
11. Pravosudov, V.V., and Clayton, N.S. (2002). A test of the adaptive specialization hypothesis: Population differences in caching, memory, and the hippocampus in black-capped chickadees (*Poecile atricapilla*). *Behav. Neurosci.* 116, 515–522. <https://doi.org/10.1037/0735-7044.116.4.515>.
12. Croston, R., Kozlovsky, D.Y., Branch, C.L., Parchman, T.L., Bridge, E.S., and Pravosudov, V.V. (2016). Individual variation in spatial memory performance in wild mountain chickadees from different elevations. *Anim. Behav.* 111, 225–234. <https://doi.org/10.1016/j.anbehav.2015.10.015>.
13. Wright, A.A., Kelly, D.M., and Katz, J.S. (2021). Same/different concept learning by primates and birds. *Learn. Behav.* 49, 76–84. <https://doi.org/10.3758/s13420-020-00456-z>.
14. Katz, J.S., and Wright, A.A. (2021). Issues in the comparative cognition of same/different abstract-concept learning. *Curr. Opin. Behav. Sci.* 37, 29–34. <https://doi.org/10.1016/j.cobeha.2020.06.009>.
15. Wasserman, E.A., Kain, A.G., and O'Donoghue, E.M. (2023). Resolving the associative learning paradox by category learning in pigeons. *Curr. Biol.* 33, 1112–1116.e2. <https://doi.org/10.1016/j.cub.2023.01.024>.
16. Wasserman, E.A., and Young, M.E. (2010). Same–different discrimination: The keel and backbone of thought and reasoning. *J. Exp. Psychol. Anim. Behav. Process.* 36, 3–22. <https://doi.org/10.1037/a0016327>.
17. Hoeschele, M., Cook, R.G., Guillette, L.M., Hahn, A.H., and Sturdy, C.B. (2012). Auditory same/different concept learning and generalization in black-capped chickadees (*poecile atricapillus*). *PLoS One* 7, e47691. <https://doi.org/10.1371/journal.pone.0047691>.
18. Magnotti, J.F., Katz, J.S., Wright, A.A., and Kelly, D.M. (2015). Superior abstract-concept learning by Clark's nutcrackers (*Nucifraga columbiana*). *Biol. Lett.* 11, 20150148. <https://doi.org/10.1098/rsbl.2015.0148>.
19. Weisman, R.G. (2008). Advice to young behavioral and cognitive scientists. *Behav. Process.* 77, 142–148. <https://doi.org/10.1016/j.beproc.2007.09.004>.

Plant signaling: The sugar-coated story of root growth

Shahram Shokrian Hajibehzad, Andrés Romanowski, and Ronald Pierik*

Plant-Environment Signaling, Department of Biology, Science4Life, Utrecht University, Padualaan 8, Utrecht, 3584 CH, the Netherlands

*Correspondence: r.pierik@uu.nl

<https://doi.org/10.1016/j.cub.2023.06.065>

A new study draws attention to photosynthetically produced sucrose as a major shoot-derived and auxin-dependent regulator of root growth and development in plants.

Beneath the surface, shielded from the sun's direct influence, the first stages of plant life unfold. In these subterranean environments, plants prioritize growth in the embryonic stem — or hypocotyl — over root development. This dark growth pattern, known as skotomorphogenesis, characterizes the early stages of plant life¹. Once the seedling pierces the surface and encounters light, a remarkable transformation ensues. Hypocotyl elongation is halted, and the cotyledons — embryonic leaves — now turn green to enable photosynthesis, marking the shift from skotomorphogenesis to photomorphogenesis². Photomorphogenesis, though instigated above ground, exerts a profound influence underground as well. It shapes the architecture of the root system, promoting the growth of the existing primary root and

stimulating the formation of new lateral roots³. This light-dependent root growth of course involves photosynthates required for any growth, but there are also indications for a long-distance molecular signaling network³.

Prior research has shown that light orchestrates multiple processes, including photosynthesis, photoreceptor signaling, and auxin signaling, to sculpt the root system's architecture⁴. In a recent issue of *Current Biology*, a study by Kircher and Schopfer⁵ from the University of Freiburg suggests that sucrose, generated by photosynthesis and transported through the phloem to the root, serves as the core component for light-dependent lateral root formation.

The authors first explored the influence of light conditions on the lateral root formation in wild-type *Arabidopsis* plants

and mutants deficient in phytochrome and cryptochrome photoreceptors. Results showed that these photoreceptors must be activated to allow lateral root development, suggesting a role for photoreceptors in signal generation. However, some of the mutants may have impaired photosynthesis, as they are characterized by smaller, absent, or inadequately greening cotyledons. A *cop1* mutant that shows light-grown morphogenic traits in darkness⁶ could still not form lateral roots in darkness, confirming that root growth and development indeed rely on photosynthates that are absent in darkness. These observations hint at the presence of a signal originating from light and reliant on photoreceptor and/or photosynthesis for regulation of lateral root formation. The authors aimed to delve further into understanding the



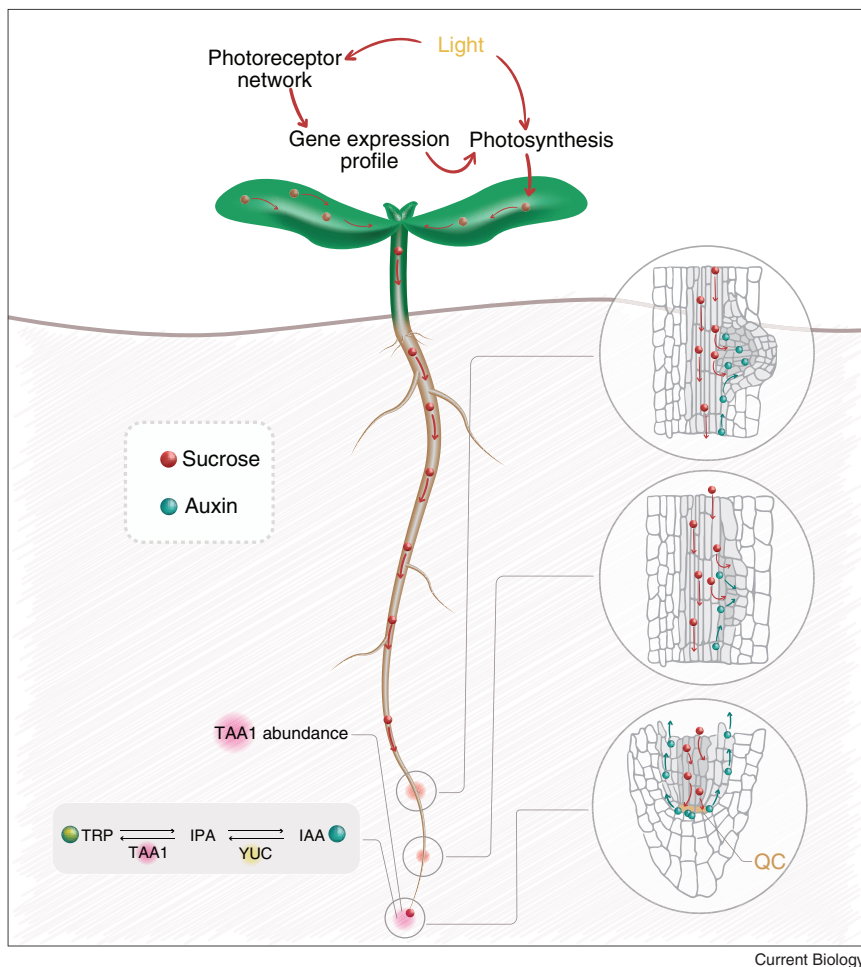


Figure 1. Schematic representation of light-induced sucrose synthesis and its shoot-to-root transport to modulate lateral root formation.

Light-induced activation of photomorphogenic and photosynthetic photoreceptors in the aerial segment of the plant triggers the synthesis of sucrose. This photosynthetically derived sucrose is then translocated from the shoot to the root system. Upon reaching the apex of the root, sucrose promotes TAA1 enzyme accumulation, which could lead to enhanced conversion of tryptophan into IPA and ultimately into IAA (auxin), required for lateral initiation and development. Sucrose also constitutes the carbon and energy required to build new tissue, including lateral roots.

source of this light-mediated shoot-to-root signal.

Kircher and Schopfer conducted a series of experiments to investigate the role of sucrose as a potential shoot-to-root signal in lateral root formation. The authors used a simple yet very elegant adjustment to routine vertical plate assays that allowed them to individually treat root and shoots with droplets of substances, such as sugars or hormones, without cross-contamination. They moved the lamps from above to the side, thus creating a phototropic bending of the shoot horizontally towards the light, perpendicular to the agar medium plane. This way, the shoot tissues would not be

in contact with the medium, and therefore substances would not diffuse through the medium between the different plant organs.

Initially, they observed that lateral root formation ceased in a CO₂-free environment, which impairs photosynthesis, but resumed when CO₂ was reintroduced. In another experiment, the researchers removed the cotyledons, which are a primary source of sucrose in seedlings, resulting in halted root development. Although this effect was only partially mitigated by sucrose supplementation, it suggested sucrose's involvement in root development and implied the presence of other

contributing factors. Furthermore, they found that supplementing sucrose to the aerial parts of dark-grown seedlings promoted formation of lateral roots (Figure 1). These findings indicate that sucrose is important for lateral root formation and can initiate lateral root formation even in the absence of other potential light cues.

The authors proceeded to examine the interaction between sucrose and auxin, a plant hormone fundamental to various developmental processes, including lateral root formation. Auxin regulates every stage from lateral root preparation and initiation to emergence^{7,8}. Their observations hinted at a potential link between these two pivotal elements in regulating lateral root growth. They noted that the anti-auxin AXO could inhibit sucrose-induced lateral root formation, suggesting a connection between sucrose and auxin signaling. Time-lapse imaging of *pDR5:LUC* further revealed that the initiation of sucrose-driven lateral root formation corresponded with shifts in auxin response. Considering auxin — synthesized in the above-ground parts of the plant — can be transported to the roots, the researchers were led to an essential question: what are the respective roles of auxin and sucrose in the transmission of shoot-to-root signals?

Auxin can be transported from the shoots to the roots via two main pathways: cell-to-cell polar auxin transfer or through passive bulk flow via the phloem. Once at the root tip, IAA gets redistributed and moves shootward via the outer cell layers (Figure 1)^{7,8}. However, the specific mechanisms and the role of this shoot-to-root transport in lateral root development remain somewhat ambiguous. Prior research using N-1-naphthylphthalamic acid (NPA) to inhibit polar auxin transport from shoot to root plays a crucial role in regulating lateral root development⁹. However, Kircher and Schopfer did not see a strong effect of this inhibitor when applied at the shoot-to-root junction in their much shorter (2-day) time window of experimentation. Although NPA would inhibit PIN-dependent transport, it would not affect IAA transport in the phloem. Measurements of IAA levels (either directly or via genetically encoded

sensors) could help reveal the efficacy of these pharmacological interventions within the shorter experimental time-frame used here. Experiments using the natural auxin indole-3-acetic acid (IAA) showed that when IAA was applied to the shoot, this did not affect root development, except when very high concentrations (above 10 μ M) were applied that inhibited root development. However, endogenous IAA is considered to promote, not inhibit, lateral root development. Although minimal effects observed here could indicate that auxin from the shoot is less effective in regulating root development than previously thought, the absence of clear effects can also be related to technical aspects of the experiment, such as instability of IAA in the application droplet, suboptimal penetration through the outer leaf tissue or different tissue localization of IAA when applied externally.

The authors then delved into the IPA pathway, wherein TAA1/TARs and YUCCA enzymes catalyze the transformation of tryptophan into IAA. Experiments showed that lateral root formation is significantly augmented in dark-grown seedlings when sucrose and tryptophan are supplied to the shoot and root, respectively (Figure 1). Notably, the influence of tryptophan on lateral root formation was less apparent when applied alone, when combined with sucrose together in the shoot, or when administered solely to the root. The authors proposed that shoot-derived sucrose may stimulate the conversion of tryptophan into auxin within the root. Indeed, sucrose treatment seems to moderately induce abundance at the root tip of the TAA1 enzyme involved in auxin synthesis from tryptophan, suggesting that sucrose may stimulate local auxin production in the root tip, which might in turn promote lateral root formation (Figure 1). To corroborate this hypothesis, future studies would need to assess auxin synthesis and signaling under these experimental conditions. The precise mechanism through which sucrose modulates TAA1, be it transcriptional, post-transcriptional, or post-translational, remains to be elucidated. One option would be a phosphorylation-based mechanism for the regulation of

TAA1, which indeed can influence auxin concentrations in response to various signals¹⁰. Future studies can resolve how light from the shoot regulates TAA1 and auxin homeostasis in the roots, amongst others via sucrose translocation.

Upon distilling the findings detailed in this work, we can appreciate the important role played by photosynthetic sucrose in translocating light-dependent signals and resources from the shoot to the root, thus regulating root system architecture. Future studies are required to disentangle the signaling versus resource functions that sucrose has in this context. Although this study compared dark versus light conditions, plant life is typically characterized by highly variable light conditions, such as fluctuations in photoperiods, light intensity and light composition. These variations of the light climate elicit a multitude of different signals, both local and mobile, to adjust plant development⁷. Future studies will benefit from taking the important role of variations in photosynthate production into account while studying root developmental plasticity to mobile signals derived from these shoot-detected variations in the light environment.

In conclusion, the *de novo* formation of lateral roots requires both sucrose and auxin at the initiation sites. How plants sense the availability of metabolic resources including sucrose and how this is linked to auxin signaling and lateral root growth are unclear. Synergistic effects of photosynthetically derived sucrose and photoreceptor-mediated photomorphogenesis have previously been shown to regulate shoot and root meristem activation^{11,12}. In these studies, the TOR kinase — a central metabolic sensor — emerges as the common point of integration that is affected by both auxin and glucose. Indeed, TOR kinase was recently proposed¹³ to act as a ‘metabolic gatekeeper’, integrating local auxin-dependent pathways with systemic metabolic signals, and modulating the translation of auxin-induced genes to regulate lateral root initiation and development. Unraveling the molecular mechanisms through which plants integrate signaling components, including but not limited to hormones, with metabolic state presents a

challenging and exciting direction in developmental plasticity research.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

- Josse, E.-M., and Halliday, K.J. (2008). Skotomorphogenesis: The dark side of light signalling. *Curr. Biol.* *18*, R1144–R1146. <https://doi.org/10.1016/j.cub.2008.10.034>.
- Gommers, C.M.M., and Monte, E. (2017). Seedling establishment: A dimmer switch-regulated process between dark and light signaling. *Plant Physiol.* *176*, 1061–1074. <https://doi.org/10.1104/pp.17.01460>.
- Gelderen, K. van, Kang, C., and Pierik, R. (2017). Light signaling, root development, and plasticity. *Plant Physiol.* *176*, 1049–1060. <https://doi.org/10.1104/pp.17.01079>.
- Sairanen, I., Novák, O., Pěncík, A., Ikeda, Y., Jones, B., Sandberg, G., and Ljung, K. (2012). Soluble carbohydrates regulate auxin biosynthesis via PIF proteins in Arabidopsis. *Plant Cell* *24*, 4907–4916. <https://doi.org/10.1105/tpc.112.104794>.
- Kircher, S., and Schopfer, P. (2023). Photosynthetic sucrose drives the lateral root clock in Arabidopsis seedlings. *Curr. Biol.* *33*, 2201–2212.e3. <https://doi.org/10.1016/j.cub.2023.04.061>.
- Ma, L., Gao, Y., Qu, L., Chen, Z., Li, J., Zhao, H., and Deng, X.W. (2002). Genomic evidence for COP1 as a repressor of light-regulated gene expression and development in Arabidopsis. *Plant Cell* *14*, 2383–2398. <https://doi.org/10.1105/tpc.004416>.
- Küpers, J.J., Gelderen, K. van, and Pierik, R. (2018). Location matters: Canopy light responses over spatial scales. *Trends Plant Sci.* *23*, 865–873. <https://doi.org/10.1016/j.tplants.2018.06.011>.
- Du, Y., and Scheres, B. (2017). Lateral root formation and the multiple roles of auxin. *J. Exp. Bot.* *69*, 155–167. <https://doi.org/10.1093/jxb/erx223>.
- Reed, R.C., Brady, S.R., and Muday, G.K. (1998). Inhibition of auxin movement from the shoot into the root inhibits lateral root development in Arabidopsis. *Plant Physiol.* *118*, 1369–1378. <https://doi.org/10.1104/pp.118.4.1369>.
- Wang, Q., Qin, G., Cao, M., Chen, R., He, Y., Yang, L., Zeng, Z., Yu, Y., Gu, Y., Xing, W., et al. (2020). A phosphorylation-based switch controls TAA1-mediated auxin biosynthesis in plants. *Nat. Commun.* *11*, 679. <https://doi.org/10.1038/s41467-020-14395-w>.
- Li, X., Cai, W., Liu, Y., Li, H., Fu, L., Liu, Z., Xu, L., Liu, H., Xu, T., and Xiong, Y. (2017). Differential TOR activation and cell proliferation in Arabidopsis root and shoot apices. *Proc. Natl. Acad. Sci. USA* *114*, 2765–2770. <https://doi.org/10.1073/pnas.1618782114>.

12. Hajibehzad, S.S., Silva, S.S., Peeters, N., Stouten, E., Buijs, G., Smeekens, S., and Proveniers, M. (2023). *Arabidopsis thaliana* rosette habit is controlled by combined light and energy signaling converging on transcriptional control of the TALE homeobox gene ATH1. *New Phytol.* 239, 1051–1067. <https://doi.org/10.1111/nph.19014>.
13. Stitz, M., Kuster, D., Reinert, M., Schepetilnikov, M., Berthet, B., Reyes-Hernández, J., Janocha, D., Artins, A., Boix, M., Henriques, R., et al. (2023). TOR acts as a metabolic gatekeeper for auxin-dependent lateral root initiation in *Arabidopsis thaliana*. *EMBO J.* 42, e111273. <https://doi.org/10.15252/embj.2022111273>.

Cognition: Crows are natural statisticians

Claudia A.F. Wascher

School of Life Sciences, Anglia Ruskin University, East Road, Cambridge CB1 1PT, UK

Correspondence: claudia.wascher@aru.ac.uk

<https://doi.org/10.1016/j.cub.2023.06.073>

A new study shows that carrion crows use memorized reward associations in a combinatorial way to apply relative probabilistic information to optimize reward outcome. This for the first time shows that a corvid species can flexibly apply statistical inference during decision making.

Statistical inference refers to the ability to use patterns, correlations, and probabilities to reason about the world and anticipate future events and was long thought of as a uniquely human cognitive ability¹. However, in a lot of contexts, statistical inference is potentially also beneficial to non-human animals to make predictions and decisions based on incomplete or uncertain information², for example when navigating environments to find food and avoid predators or when responding to social dynamics³.

In a new discovery by Johnston *et al.*⁴ reported in this issue of *Current Biology*, crows learned to associate reward probabilities with arbitrary stimuli in a choice task and applied this knowledge to maximise the rewards they gained during the task, indicating they are capable of true statistical inference. Two crows were trained on a touch screen to associate nine unique stimuli with reward probabilities ranging between 10–90%. The crows learned the associated reward probability for each stimulus in about 10 days of training and over 5,000 trials. During the test, when presented with a choice between two of the stimuli they reliably chose the higher reward probability, indicating an ‘optimal choice’ (Figure 1). This was also the case when conditions included an option with high ($\geq 50\%$) reward probability and they made more optimal choices when the distance between the two stimulus

options increased. The crows did show statistical inference in the first session of the test and performance only slightly improved over the sessions, indicating that optimal choice was not based on associative learning. They also memorized the stimulus associations for at least one month, without exposure to the stimuli⁴. Taken together, these results provide compelling evidence that crows use reward associations in a combinatorial way to apply relative probabilistic information in order to optimize their reward outcome.

A strength of the new study is that it allows an investigation of the exact cognitive mechanism at play and whether animals use simple rules, such as avoiding the least rewarding stimulus, rather than statistical inference. The study shows that the latter is the case in crows, as individuals use the relative rather than absolute reward frequency during decision making. The crows receive the same absolute amount of reward from each stimulus. For example, the stimulus for 40% reward probability is presented twice as often during the test as the stimulus resulting in 80% of reward probability; however, crows still make the optimal choice, namely choosing the stimulus for 80% reward probability more often.

Similar levels of statistical inference have previously only been described in different great ape species and keas

(*Nestor notabilis*), a parrot species. When presented with mixtures of different ratios of preferred and non-preferred food great apes were able to infer the optimal choice, namely choosing the option which more likely leads to receiving the preferred reward type⁵. In a similar experiment, keas had to choose between different proportions of rewarding and unrewarding tokens and showed evidence of statistical inference allowing them to optimize their choice⁶. In these previous studies, statistical inference has been drawn from population-to-sample inference, meaning that a conclusion about the sample is drawn from population information that is visible to the subject at the time of decision making. The apes and keas in their respective experiments have been presented with visual information about the relative reward frequency, i.e., the rewards or tokens representing different reward types were shown to the test subjects. In contrast to this, the crows in the present experiment made sample-to-population inferences, meaning that decisions need to be drawn from mentally stored information, i.e., the symbol–reward associations learned during the training phase of the experiment. This means that the cognitive demands experienced by the crows are higher compared to previous experiments in other species.

The current study by Johnston *et al.*⁴ adds to a growing body of literature