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New links between auxin and starch

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Auxin was one of the first plant hormones to be identified, but we are still discovering the roles of this "master" hormone. Here we discuss new evidence that links auxin with the synthesis and accumulation of one of humanity's staple food ingredients, starch.

In an exciting development, a recent series of papers reports evidence that the plant hormone, auxin, promotes the accumulation of starch. Prior findings had hinted at such a relationship, but mutations affecting auxin biosynthesis or perception had not been utilised. Now, this definitive approach has been exploited, revealing that specifically disrupting the biosynthesis of endogenous auxin reduces starch content in developing pea seeds[1,2], *Arabidopsis* root tips[3], maize kernels[4], rice endosperm[5,6], and barley pollen[7]. In rice endosperm and *Arabidopsis* root tips, impairing auxin perception has a similar consequence[3,8]. Furthermore, variation in endogenous auxin content as seeds develop is strongly correlated with starch formation in rice and wheat[9,10], indicating a regulatory role for this "classical" plant hormone.

While further research is required, the implications of these papers are far-reaching, from plant physiology to food security. For example, while it is well known that starch granules are involved in auxin-mediated gravity perception by roots, it has now emerged that auxin promotes granule formation in the first place. But perhaps the most important implication relates to food production. Starch is the primary component of cereal crop grains, including maize and rice, and a critical source of carbohydrates in the seeds of legumes such as pea. To address future food security, it is important to fully understand, and potentially exploit, the auxin-starch relationship.

The recent studies have followed the discovery that auxin is mainly produced from the amino acid tryptophan, by a simple, two-step, process. The *TAA1*/*TAR* genes encode the enzymes for the first step, while YUCCA enzymes catalyse the second, resulting in the bioactive auxins, indole-3-acetic acid (IAA), and in developing pea seeds, chlorinated IAA. Mutations in *YUCCA* genes have provided most of the new insight into the auxin-starch relationship in cereals[4–7], while it turns out that the *PsTAR2* gene is a critical one for maturing pea seeds[1,2]. Particularly relevant to starch levels in rice are mutations in the endosperm-localised *YUCCA* genes, such as *OsYUC11*.

In rice, starch typically comprises 60–80% of the grain dry weight, and a major consequence of impaired starch accumulation is decreased grain size[5,8]. A second important consequence is reduced grain quality: disrupting the auxin pathway results in "chalky" grains. Chalkiness is associated with an opaque, rather than translucent, appearance of at least parts of the mutant grains, and reduces palatability and marketability. The phenomenon is attributed to loose packing of the starch, as typically illustrated by scanning electron microscopy of the mature endosperm[5,8], which also shows rounded starch grains characteristic of chalkiness. There are dramatic effects also of auxin deficiency on the size and/or abundance of starch granules in pea seeds and barley pollen[1,7]. (In rice, starch grains contain the much smaller starch granules).

Across the diversity of species involved, three main mechanisms have been invoked to explain the auxin-starch link (Fig. 1). The first of these is that auxin acts by upregulating the expression of genes encoding enzymes that catalyse the formation of starch from sucrose. In all the systems mentioned above, auxin deficiency reduces the expression of at least some starch synthesis genes. Importantly, in developing pea seeds, *Arabidopsis* root tips, and rice endosperm, these genes include ADP glucose pyrophosphorylase (AGPase), considered to be a key enzyme in starch formation[3]. In some cases, the actual starch synthesis enzyme activities, as well as the corresponding transcript levels, have been shown to be reduced in auxin-deficient seeds[1]. The "starch synthesis gene expression" theory was favoured in the earlier papers in the current series[1,3].

Then in 2022, Amanda et al.[7] suggested a second mechanism, namely that in barley stamens, auxin boosts energy generation, producing the ATP required for starch synthesis. These authors studied barley mutants carrying mutations in a *YUCCA* gene, *HvYUCCA4*. Mutant stamens are deficient in auxin, and produce only 15% of the wild-type starch level. Amanda et al.[7] performed a transcriptomic analysis, and discovered that many genes encoding enzymes for energy generation were down-regulated in mutant stamens. In contrast, "only three" starch synthesis genes were similarly affected. The transcriptome data were supported by measurements of the relevant metabolite levels[7].

Amanda et al.[7] suggest that in barley grains, as well as barley pollen, auxin might stimulate energy generation, thereby promoting starch accumulation. Amanda et al.[7] did not present evidence from grains for that suggestion, but it is interesting to note that in the auxin-deficient seeds of maize[4] and rice[5], transcriptome data reveal a down-regulation of some genes involved in energy generation. That the auxin-energy connection might be widespread is an intriguing possibility that requires further research[11].

A third suggested mechanism is that auxin promotes starch accumulation by enhancing the transport of sucrose from source to sink. This theory, suggested more than twenty five years ago[12], has been resurrected recently, based on studies of the rice auxin receptor gene *OsTIR1*[8]. Wu et al.[8] found that in the endosperm of *OsTIR1* mutants both sucrose and starch levels were reduced[8]. In the flag leaves, however, sucrose accumulated to a higher level than in the wild type. This is strong evidence that auxin is required for the transport of sucrose from the source leaves to the developing grain. The disruption of sucrose transport in the mutants appears to occur adjacent to the endosperm[8].

For all three mechanisms, the genes involved have been reported to harbour auxin response elements in their promoters[1,3,7,8]. Importantly, that evidence indicates that auxin acts directly. There is evidence also for the involvement of canonical auxin signalling proteins, especially in *Arabidopsis* and rice[3,8,13], but much remains to be learned about that aspect.

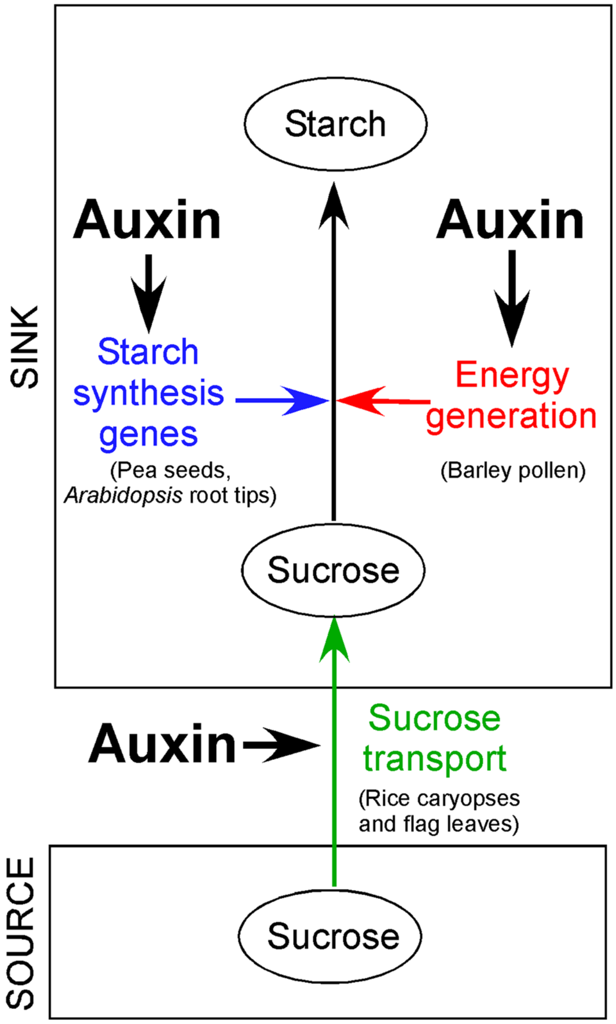

Fig. 1 | A hypothetical model integrating three suggested mechanisms, from different plant systems, by which auxin promotes starch accumulation. Auxin is suggested to promote sucrose transport from source to sink[8]. Within the sink, two suggested mechanisms are shown: up-regulated expression of starch synthesis genes[1,3,5,6], and increased energy generation[7].

Each of the papers in the auxin-starch series tends to focus on just one of the three mechanisms. The possible interconnections between these three could be a profitable topic for future research. The tissue-specificity of expression of the relevant genes can be instructive in this respect. We can already discern, for example, that auxin does not act just on sucrose transport, with the sink-localised mechanisms being mere consequences of that first effect. That is because some of the genes involved, and therefore mutations in those genes, are expressed only in the sink concerned. For example, in barley, *HvYUCCA4* is specifically expressed in stamens, and mutations in that gene are not likely to directly affect sucrose transport into those organs, as noted by Amanda et al.[7] Consistent with that, sucrose accumulated in the mutant stamens[7], an unlikely effect if the mutations reduced sucrose transport. This evidence indicates that auxin can act within the stamens themselves to promote starch accumulation. That is not to say, however, that auxin does not promote sucrose transport into the stamens as well. On that question, the mutations in *HvYUCCA4* are uninformative, since the gene is exclusively expressed in stamens, and not in the sucrose source.

In rice, given the evidence for an effect of auxin on sucrose transport[8], it seems possible that all three mechanisms (Fig. 1) operate concurrently. In that context, we should note that in the Wu et al. study[8], mutation of *OsTIR1* reduced not only sucrose transport, but also the expression of most of the starch synthesis genes tested.

It is relevant also that sucrose can affect the auxin pathway. In developing pea seeds, the influx of sucrose increases the content of trehalose-6-phosphate, which in turn up-regulates the auxin biosynthesis gene *PsTAR2*, increasing auxin levels and thereby the conversion of sucrose to starch[2].

Wu et al.[8] report a finding of considerable practical significance. The overexpression of *OsTIR1* increased the starch percentage of rice grains from approximately 60–85%—a massive increase—and grain dry weight was also elevated. Clearly, the degree of auxin signalling in wild-type rice can be sub-optimal for starch production. This result opens up exciting possibilities for increasing starch synthesis and grain filling, by manipulating the auxin pathway.

However, there are some important caveats to be considered. In the rice *dao* mutant, elevated auxin levels are associated with parthenocarpic seeds that lack starch[14,15], in contrast to the increased starch production in endosperm overexpressing *OsTIR1*[8].

Furthermore, the synthetic auxin, 2, 4-dicholophenoxyacetic acid, was reported to impair, not promote, starch accumulation in lily[16].

Obviously, there is still much to learn about the auxin-starch relationship, and there are copious opportunities for further research. Key areas could be the generality of the auxin-energy link[7], the signalling proteins connecting auxin perception to gene regulation, and the possible connections between the three mechanisms discussed above. It will be intriguing also to discover the extent to which environmental effects on starch content are transduced by auxin signalling[13,17]. The in vivo perturbation of auxin biosynthesis or perception, afforded by the mutations discussed above, will be invaluable for this research. However, the recent series of papers already highlights the importance of continually delving into the roles of auxin. To blithely assume, without questioning, that auxin regulates all plant processes does not, on its own, provide an adequate basis for future studies.

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References

1. McAdam, E. L. et al. Evidence that auxin is required for normal seed size and starch synthesis in pea. *N. Phytol.* **216**, 193–204 (2017).
2. Meitzel, T. et al. Trehalose 6-phosphate promotes seed filling by activating auxin biosynthesis. *N. Phytol.* **229**, 1553–1565 (2021).
3. Zhang, Y. et al. Auxin-mediated statolith production for root gravitropism. *N. Phytol.* **224**, 761–774 (2019).
4. Bernardi, J., Battaglia, R., Bagnaresi, P., Lucini, L. & Marocco, A. Transcriptomic and metabolomic analysis of ZmYUC1 mutant reveals the role of auxin during early endosperm formation in maize. *Plant Sci.* **281**, 133–145 (2019).
5. Xu, X. et al. OsYUC11-mediated auxin biosynthesis is essential for endosperm development of rice. *Plant Physiol.* **185**, 934–950 (2021).

6. Zhang, D., Zhang, M. & Liang, J. RGB1 regulates grain development and starch accumulation through its effect on *OsYUC11*-mediated auxin biosynthesis in rice endosperm cells. *Front. Plant Sci.* **12**, 585174 (2021).
7. Amanda, D. et al. Auxin boosts energy generation pathways to fuel pollen maturation in barley. *Curr. Biol.* **25**, 1798–1811 (2022).
8. Wu, D. et al. Auxin receptor OsTIR1 mediates auxin signaling during seed filling in rice. *Plant Physiol.* **194**, 2434–2448 (2024).
9. Abu-Zaitoon, Y. M., Bennett, K., Normanly, J. & Nonhebel, H. M. A large increase in IAA during development of rice grains correlates with the expression of tryptophan aminotransferase *OsTAR1* and a grain-specific *YUCCA*. *Physiol. Plant.* **146**, 487–499 (2012).
10. Kabir, M. R., Nonhebel, H. M., Backhouse, D. & Winter, G. Expression of key auxin biosynthesis genes correlates with auxin and starch content of developing wheat (*Triticum aestivum*) grains. *Funct. Plant Biol.* **48**, 802–814 (2021).
11. Tivendale, N. D. & Millar, A. H. How is auxin linked with cellular energy pathways to promote growth? *N. Phytol.* **233**, 2397–2404 (2022).
12. Darussalam, Cole, M. A. & Patrick, J. W. Auxin control of photoassimilate transport to and within developing grains of wheat. *Aust. J. Plant Physiol.* **25**, 69–77 (1988).
13. Chen, Z. et al. Heat stress responsive Aux/IAA protein, OsIAA29 regulates grain filling through OsARF17 mediated auxin signaling pathway. *Rice* **17**, 16 (2024).
14. Zhao, Z. et al. Auxin regulates source-sink carbohydrate partitioning and reproductive organ development in rice. *Proc. Natl. Acad. Sci.* **119**, e2121671119 (2022).
15. Zhao, Z. A role for a dioxygenase in auxin metabolism and reproductive development in rice. *Dev. Cell* **27**, 113–122 (2013).
16. Gao, C. et al. Full-length transcriptome analysis revealed that 2,4-dichlorophenoxyacetic acid promoted in vitro bulblet initiation in lily by affecting carbohydrate metabolism and auxin signaling. *Front. Plant Sci.* **14**, 1236315 (2023).
17. Panda, D. et al. Light intensity–mediated auxin homeostasis in spikelets links carbohydrate metabolism enzymes with grain filling rate in rice. *Protoplasma* **260**, 1233–1251 (2023).

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Competing interests
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