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Strigolactones: The First Members of a New Family of "Shoot Branching Hormones" in Plants?

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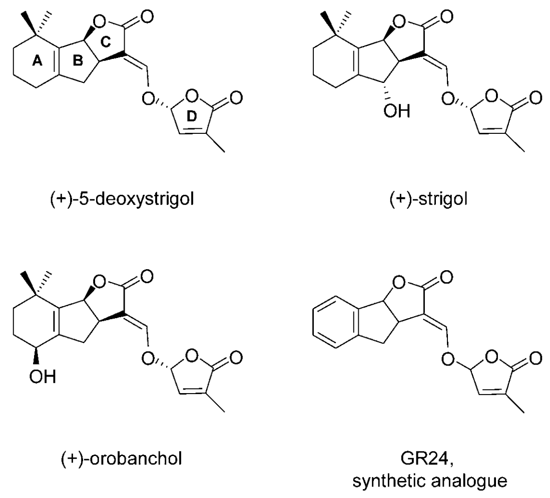
Plants show an incredible wealth of habits and statures ranging from less ramified to highly branched, from small bushes to big trees. Although the architecture is largely determined by genetic factors, an individual plant is also able to adapt its growth in response to the local environment and biotic effects. Thus, shoot branching depends on local nutrients, especially phosphate, but also on biotic factors such as herbivory and infection. An endogenous factor is the growth hormone auxin, which is released by the apical bud and suppresses the growth of subapical lateral shoots, causing apical dominance.[1] Lower concentrations of auxin or removal of the apical bud lead to multiple shoot tips, and the plant becomes highly branched. The effect is impressively documented by newly developing shoots on a recently cut tree. Accordingly, branching and growth of lateral stems is a process that is highly regulated by genetic, hormonal, and environmental factors. Previously only auxins, abscisic acid, and cytokinins have been assumed to control shoot branching.[2,3] However, recent studies on a series of mutants of *Arabidopsis thaliana*, rice (*Oryza sativa*), and pea (*Pisum sativum*) with enhanced shoot branching suggested the existence of a novel type of hormone(s) that might be derived from carotenoids. These mutants with similar phenotypes include *ramosus* (*rms*) in pea, *more axillary growth* (*max*) in *Arabidopsis*, *dwarf* (*d*) or *high-tillering dwarf* (*hdt*) in rice, and *decreased apical dominance* (*dad*) in petunia. In particular, the genetically well characterized *Arabidopsis* mutants show substantial branching variation, thus providing an ideal platform for determining the genetic and molecular factors of the shoot-branching regulatory network. Two recently published papers by Gomez-Roldan et al.[4] and Umehara et al.[5] have now presented the first evidence that the long-known strigolactones and related compounds (Scheme 1) might be the first members of a family of apocarotenoid signals that are involved in the regulation of the branching process.[4,5]

Strigolactones were previously known as plant-derived isoprenoids occurring in the rhizosphere. They stimulate the seed germination of obligate parasitic plants like *Striga* or *Orobanche*[6–8] and enhance the seed germination of certain nonparasitic plants. Moreover, upon phosphate depletion, they function as root-derived signals causing elevated hyphal branching and spore germination of symbiotic mycorrhizal fungi, to enhance the uptake of soil nutrients.[9] They are present in the root exudates of mono- and dicotyledonous plants (for example, rice and pea)[6] and have additionally been detected in their shoots.[10,11] Since they are also found in the nonmycotrophic plant *A. thaliana*, a pivotal role for these apocarotenoids in coordinating plant growth below as well as above ground has been suggested.[12,13]

In particular, work with mutants of pea, petunia, rice, and, especially, *Arabidopsis* combining enhanced shoot branching with a reduced stature, the *max1* to *max4* mutants,[14] with defects in carotene degradation and signal transduction (vide infra), revealed the importance of carotene-dioxygenases (CCDs) for the generation of an unknown end product that affects branching. Nine CCDs have been identified in *Arabidopsis*, of which five are involved in abscisic acid (ABA) synthesis, the others (MAX) have a different target complex. In the mutants *rms5*/*max3*/*htd1*/*d17-1*[15] and *rms1*/*max4*/*d10-1* from pea, *Arabidopsis*, and rice,[16,17] the two dioxygenases, namely *CCD7* and *CCD8*, encode for enzymes that degrade carotenoids into a variety of products (Scheme 2).[18] *CCD8* can cleave the product of the *CCD7*-catalyzed reaction,[19] and *MAX1*, which encodes a cytochrome P450,[20] is assumed



(+)-5-deoxystrigol

(+)-strigol

(+)-orobanchol

GR24, synthetic analogue

Scheme 1. Representative strigolactone structures.

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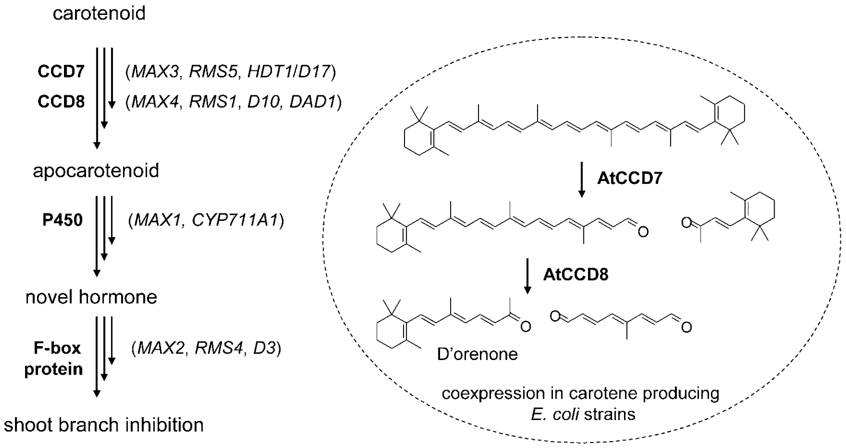

Scheme 2. Postulated pathway for the generation of the novel branch-inhibiting hormone.

to act downstream of the two CCDs. *MAX2* encodes an F-box-protein and acts locally in the shoot, being responsible for signal transduction.[21] Overall, the *MAX* genes or the corresponding genes from the other mutants (Scheme 2) were suspected to constitute a degradative pathway leading from carotenoids to a novel family of "branching hormones". The first compound has now been identified as a member of the family of strigolactones, since LC-MS/MS revealed clearly reduced levels of these compounds in the branched mutants.[4,5] This finding was strengthened further by external addition of the synthetic strigolactone analogue GR24 (Scheme 1) directly to axillary buds of *ccd8* pea plants; this restored the wild-type shoot architecture by inhibiting bud outgrowth. Application of GR24 did not affect the *d3/rms4/max2* mutant architecture, since their defects are downstream of the biosynthetic pathway (Scheme 2), most likely involved in signal transduction. The effect of GR24 on axillary bud outgrowth of *Arabidopsis* was comparably impressive (Figure 1). Again, the number of axillary shoots remained unaffected by GR24 in the *max2* mutants, but the *max3* and *max4* plants displayed an almost complete suppression of axillary bud outgrowth.

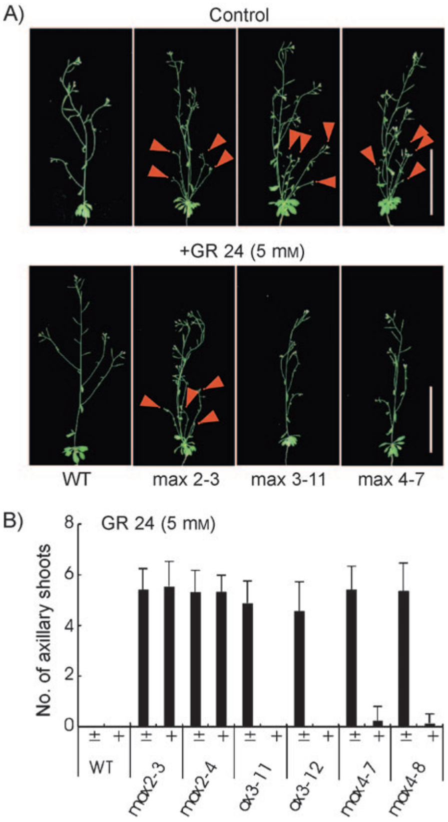

Figure 1. Effect of synthetic GR24 on the axillary bud outgrowth of Arabidopsis. A) 30-day-old wild-type (WT) and max mutants. Red arrowheads indicate the outgrowth of axillary buds. Scale bars = 10 cm. B) The number of axillary shoots (over 5 mm) is shown as mean ± s.d., $n = 12$-–16.

Detailed knowledge on the cleavage products of the different CCDs came from heterologous expression of the enzymes in *E. coli* and the systematic analysis of their product spectrum.[19,22] One of the resulting or postulated cleavage products (D'orenone) has been recently found to inhibit root-hair formation in various plant species; a process formally related to the inhibition of the outgrowth of shoot buds.[23]

D'orenone suppresses root-hair growth by interfering with auxin transport and signaling (D'orenone affects PIN2).[23] A similar mode of interaction has been proposed for the novel hormone. Auxin is actively transported downwards from the apical bud in the shoots and inhibits bud outgrowth,[24] while the cytokinins move upwards and activate bud outgrowth. There is strong evidence that the RMS/MAX/D pathway affects this hormonal network by downregulating auxin transporters thereby limiting the transport capacity for auxin in the stem. In consequence, developing buds are not able to export their auxin to the stem and the accumulating hormone suppresses outgrowth.[24] In the *max* mutants there is a higher auxin transport capacity in the stem and, hence, buds can export auxin and grow. However, this model remains to be established experimentally.[24] It also remains to be clarified whether only the strigolactones or other downstream metabolites can act as the hormones in the RMS/MAX/D pathway. The interesting idea has been put forward that the strigolactones might have a principal role in mediating the detection of nutrient availability by roots and the resulting alterations in shoot architecture. Since their biosynthesis is promoted by phosphate starvation, for example, in tomato,[25] this is probably an adaptive strategy of plants to synthesize strigolactones to minimize shoot branching and maximize the symbiotic interaction with arbuscular mycorrhizal fungi that facilitate the uptake of mineral nutrients. The seeds of root parasitic plants abuse these plant-derived chemical signals to find their potential hosts in the soil.[6–8] Considering the massive crop losses of cereals caused by *Striga* and *Orobanche* in developing countries, it would be of outstanding importance to develop cheap but highly active synthetic strigolactone analogues (cf. GR24) or to find a plant variety that can be used to cause premature germination of the parasitic plants by exuding high levels strigolactones.[26,27]

With respect to the tremendous structural differences between the simple but bioactive apocarotenoid D'orenone and the much more elaborated family of strigolactones, there seems to exist a large playground for synthetic chemists to develop new analogues with 1) longer persistence in the field, 2) more selective windows of activity, or 3) more polar conjugates that might facilitate transport. Moreover a careful analytical approach with root exudates and tissues of the mutant plants will be another promising strategy to find novel members of the new family of plant hormones. Since several *RMS/MAX/D* loci for strigolactone biosynthesis have already been discovered, genetic approaches in molecular breeding could also be used to design new plant varieties with a reduced risk of parasite infection. At least under experimental conditions the rice *d10-1* mutant is much less infected by *S. hermonthica* than the wild type, and, hence, confirms the feasibility of this concept in principal.[5] One could even think of applications in horticulture of using compounds that alter plant architecture by producing novel plant habits without the need for genetic tools and molecular breeding; however, the latter techniques promise to be more effective.[28]

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[1] V. Ongaro, K. Bainbridge, L. Williamson, O. Leyser, *Mol. Plant* **2008**, *1*, 388–400.
[2] M. G. Cline, *Bot. Rev.* **1991**, *57*, 318–358.
[3] A. Nordstrom, P. Tarkowski, D. Tarkowska, R. Norbaek, C. Astot, K. Dolezal, G. Sandberg, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 8039–8044.
[4] V. Gomez-Roldan, S. Fermas, P. B. Brewer, V. Puech-Pages, E. A. Dun, J. P. Pillot, F. Letisse, R. Matusova, S. Danoun, J. C. Portais, H. Bouwmeester, G. Becard, C. A. Beveridge, C. Rameau, S. F. Rochange, *Nature* **2008**, *455*, 189–194.
[5] M. Umehara, A. Hanada, S. Yoshida, K. Akiyama, T. Arite, N. Takeda-Kamiya, H. Magome, Y. Kamiya, K. Shirasu, K. Yoneyama, J. Kyozuka, S. Yamaguchi, *Nature* **2008**, *455*, 195–200.
[6] H. J. Bouwmeester, C. Roux, J. A. Lopez-Raez, G. Becard, *Trends Plant Sci.* **2007**, *12*, 224–230.
[7] C. E. Cook, P. Coggon, A. T. McPhail, M. E. Wall, L. P. Whichard, G. H. Egley, P. A. Luhan, *J. Am. Chem. Soc.* **1972**, *94*, 6198–6199.
[8] A. J. Humphrey, M. H. Beale, *Phytochemistry* **2006**, *67*, 636–640.
[9] K. Akiyama, K. Matsuzaki, H. Hayashi, *Nature* **2005**, *435*, 824–827.
[10] K. Yoneyama, X. N. Xie, D. Kusumoto, H. Sekimoto, Y. Sugimoto, Y. Takeuchi, K. Yoneyama, *Planta* **2007**, *227*, 125–132.
[11] K. Yoneyama, K. Yoneyama, Y. Takeuchi, H. Sekimoto, *Planta* **2007**, *225*, 1031–1038.
[12] J. M. Bradow, W. J. Connick, A. B. Pepperman, *J. Plant Growth Regul.* **1988**, *7*, 227–239.
[13] J. M. Bradow, W. J. Connick, A. B. Pepperman, L. H. Wartelle, *J. Plant Growth Regul.* **1990**, *9*, 35–41.
[14] M. E. Auldridge, A. Block, J. T. Vogel, C. Dabney-Smith, I. Mila, M. Bouzayen, M. Magallanes-Lundback, D. DellaPenna, D. R. McCarty, H. J. Klee, *Plant J.* **2006**, *45*, 982–993.
[15] J. H. Zou, S. Y. Zhang, W. P. Zhang, G. Li, Z. X. Chen, W. X. Zhai, X. F. Zhao, X. B. Pan, Q. Xie, L. H. Zhu, *Plant J.* **2006**, *48*, 687–696.
[16] T. Arite, H. Iwata, K. Ohshima, M. Maekawa, M. Nakajima, M. Kojima, H. Sakakibara, J. Kyozuka, *Plant J.* **2007**, *51*, 1019–1029.
[17] K. Sorefan, J. Booker, K. Haurogne, M. Goussot, K. Bainbridge, E. Foo, S. Chatfield, S. Ward, C. Beveridge, C. Rameau, O. Leyser, *Genes Dev.* **2003**, *17*, 1469–1474.
[18] J. Booker, M. Auldridge, S. Wills, D. McCarty, H. Klee, O. Leyser, *Curr. Biol.* **2004**, *14*, 1232–1238.
[19] S. H. Schwartz, X. Q. Qin, M. C. Loewen, *J. Biol. Chem.* **2004**, *279*, 46940–46945.
[20] J. Booker, T. Sieberer, W. Wright, L. Williamson, B. Willett, P. Stirnberg, C. Turnbull, M. Srinivasan, P. Goddard, O. Leyser, *Develop. Cell* **2005**, *8*, 443–449.
[21] P. Stirnberg, K. van de Sande, H. M. Ottoline Leyser, *Development* **2002**, *129*, 1131–1141.
[22] S. H. Schwartz, B. C. Tan, D. A. Gage, J. A. D. Zeevaart, D. R. McCarty, *Science* **1997**, *276*, 1872–1874.
[23] M. Schlicht, O. Šamajová, D. Schachtschabel, S. Mancuso, D. Menzel, W. Boland, F. Baluška, *Plant J.* **2008**, *55*, 709–717.
[24] V. Ongaro, O. Leyser, *J. Exp. Bot.* **2008**, *59*, 67–74.
[25] J. A. López-Ráez, T. Charnikhova, V. Gómez-Roldán, R. Matusova, W. Kohlen, R. de Vos, F. Verstappen, V. Puech-Pages, G. Bécard, P. Mulder, H. Bouwmeester, *New Phytol.* **2008**, *178*, 863–874.
[26] D. M. Joel, *Crop Prot.* **2000**, *19*, 753–758.
[27] M. C. Press, J. D. Scholes, C. R. Riches, *World's Worst Weeds*, *Proc. Int. Symp.* **2001**, 71 – 88.
[28] H. Klee, *Nature* **2008**, *455*, 176–177.

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