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HORMONE SIGNALLING

A novel gibberellin promotes seedling establishment

A previously unknown biologically active gibberellin present in seeds of *Arabidopsis thaliana* is formed by hydration of the gibberellin precursor GA_{12} through the action of GAS2, a 2-oxoglutarate-dependent dioxygenase that decreases sensitivity to abscisic acid and promotes seed germination and seedling establishment.

Peter Hedden

The gibberellin (GA) group of plant hormones promote growth and other developmental processes, including seed germination. While there are currently 136 naturally occurring compounds that are classified as GAs on the basis of their structure, only a relatively small number of these possess biological activity and are involved in the regulation of plant development. For a GA to be biologically active it was thought to require certain structural characteristics[1–3], as exemplified by GA_4, which is the main biologically active GA in *Arabidopsis* (Fig. 1). All bioactive GAs possess a C_{19} skeleton (containing 19 carbon atoms), which is formed biosynthetically from C_{20} precursors, including GA_{12} (Fig. 1), that were thought to possess no intrinsic biological activity. Now in a paper published in *Nature Communications*, Liu et al.[4] show that a simple derivative of GA_{12}, formed by hydration of its double bond and with seemingly little structural similarity to known biologically active GAs, has GA-like activity. The compound, which was detected in *Arabidopsis* and maize seeds, was chemically characterized as 16,17-dihydroGA_{12} 16α-ol ($DHGA_{12}$) (Fig. 1). It stimulated hypocotyl elongation when applied to *Arabidopsis* seedlings grown in far-red light and promoted cotyledon greening in the presence of the hormone abscisic acid (ABA), although in both cases with lower activity than GA_4. Furthermore, the authors used microscale thermophoresis to show that $DHGA_{12}$ binds to a GA receptor (GA INSENSITIVE DWARF1C) and molecular docking with a receptor model to suggest how this interaction occurs. The authors propose a role for $DHGA_{12}$ in promoting seed germination and early seedling growth.

Seed germination is regulated by a balance between GA and ABA, which act antagonistically[5]. The authors used a chemical activation screen for genes that overcome the inhibitory effect of ABA on *Arabidopsis* seed germination. The screen for promotion of germination in the presence of ABA utilized transgenic *Arabidopsis* plants with transfer DNA (T-DNA) insertions containing an estradiol-inducible promoter, such that expression of genes with insertion of the T-DNA in their promoters is activated by application of the chemical[6]. Expression of one of the induced genes, namely *GAIN-OF-FUNCTION IN ABA-MODULATED SEED GERMINATION 2* (*GAS2*), resulted in reduced sensitivity to ABA. The gene is expressed in roots and leaves, induced by light and ABA, and suppressed by treatment with GA_4. Overexpression of *GAS2* promoted seed germination and early seedling development in the presence of ABA, while germination was delayed in non-chemically induced plants — in which *GAS2* is not expressed — or plants containing mutations in *GAS2* produced by genome editing. *GAS2* was found to encode a 2-oxoglutarate-dependent dioxygenase (2ODD) of previously unknown function. Due to the antagonistic action of GAs with respect to ABA and the known involvement of 2ODDs in GA biosynthesis[7], the authors suspected that GAS2 may have a role in GA metabolism, which they tested using enzyme assays with GAS2 protein produced in *Escherichia coli*. They found that GAS2 acted on GA_{12}, but surprisingly, it did not catalyse the expected oxidation but instead hydrated the C-16,17 double bond, a reaction not previously associated with 2ODD activity. Consistent with this activity, plants in which *GAS2* was overexpressed contained higher amounts of $DHGA_{12}$ in imbibed seeds compared with wild-type seeds, whereas it was not detectable in seeds from non-induced T-DNA plants. Compared with wild-type seeds, those from the overexpression line contained lower amounts of GA_{12} and also of GA_4, a later product of the biosynthetic pathway. While the reduction of GA_4 can be partly explained by diversion of its precursor from the main biosynthetic pathway, another cause appears to be reduced expression of a GA 20-oxidase gene, which is required for GA_4 synthesis, in the *GAS2* overexpression line.

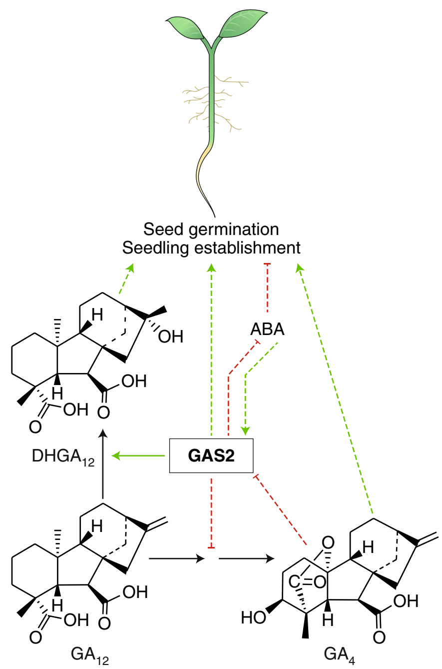

Fig. 1 | GAS2 reduces sensitivity to ABA, promoting seed germination and seedling establishment. GAS2 encodes a 2-oxoglutarate-dependent dioxygenase which was shown in vitro to hydrate the double bond of the GA precursor GA_{12} to form the previously unidentified $DHGA_{12}$. Application of $DHGA_{12}$ promoted seed germination and seedling establishment in the presence of ABA, although less strongly than GA_4. Imbibed seeds of plants overexpressing GAS2 accumulated $DHGA_{12}$, but contained reduced levels of GA_{12}, GA_4 and ABA. Expression of GAS2, which was strongest in the roots and leaves, was promoted by exogenous ABA and repressed by GA_4. Green arrows indicate promotive activities, while red bar-headed lines indicate repression. Continuous lines indicate enzymatically catalysed reactions, while dashed lines indicate regulatory activities.

These are interesting and novel findings, showing a clear involvement of GAS2 in promoting seed germination. However, they may not tell the whole story. Of particular note is the strong reduction in the content of the highly active GA_4 in seeds of the overexpression line, while there is a smaller increase in the level of the less active $DHGA_{12}$. This is inconsistent with the ability of GAS2 to promote seed germination, for which the observed reduction in ABA levels in the *GAS2* overexpression line must be relevant. Furthermore, cotyledon greening and hypocotyl elongation could be only partially recovered in the *gas2* mutant lines by application of $DHGA_{12}$ or GA_4, indicating the involvement of other factors. The altered expression of genes involved in ABA and GA metabolism in the *GAS2* overexpression and genesilenced lines suggests that GAS2 may have a regulatory role, perhaps through formation of an unidentified product. The mechanism by which GAS2 catalyses the hydration of GA_{12} is unclear and this may not be its only activity. Could it also have *bona fide* oxygenase activity against an unknown substrate? Detailed comparison of metabolites in the lines with altered *GAS2* expression could prove helpful in identifying potential substrates and products. Nevertheless, the demonstration that $DHGA_{12}$ has GA-like activity, despite its apparent dissimilarity from the canonical structure thought to be essential for activity, is a surprising and unexpected finding that will be of considerable interest to researchers in the plant hormone field. ❒

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Competing interests

The author declares no competing interests.