ORIGINAL RESEARCH

Plant growth promotion by the interaction of a novel synthetic small molecule with GA-DELLA function

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Abstract

Synthesized small molecules are useful as tools to investigate hormonal signaling involved in plant growth and development. They are also important as agrochemicals to promote beneficial properties of crops in the field. We describe here the synthesis and mode of action of a novel growth-promoting chemical, A1. A1 stimulates enhanced growth in both shoot and root tissues of plants, acting by increasing both dry and fresh weight. This suggests that A1 not only promotes uptake of water but also increases production of cellular material. A1 treatment of Arabidopsis leads to the degradation of DELLA growth-inhibitory proteins suggesting that A1-mediated growth promotion is dependent upon this mechanism. We performed genetic analysis to confirm this and further dissect the mechanism of A1 action upon growth in Arabidopsis. A quintuple del1 mutant was insensitive to A1, confirming that the mode of action was indeed via a DELLA-dependent mechanism. The ga1-5 gibberellin synthesis mutant was similarly insensitive, suggesting that to promote growth in Arabidopsis A1 requires the presence of endogenous gibberellins. This was further suggested by the observation that double mutants of GID1 gibberellin receptor genes were insensitive to A1. Taken together, our data suggest that A1 acts to enhance sensitivity to endogenous gibberellins thus leading to observed enhanced growth via DELLA degradation. A1 and related compounds will be useful to identify novel signaling components involved in plant growth and development, and as agrochemicals suitable for a wide range of crop species.

1 | INTRODUCTION

Plant growth and development involves the integration of many environmental signals and plant hormones (Gray, 2004). Plant hormones including gibberellic acid (GA), abscisic acid (ABA), cytokinin, ethylene, and brassinosteroids regulate many aspects of plant growth and development at relatively low concentrations (Gray, 2004; Rigal et al., 2014). Cytokinin, auxin, GA, and brassinosteroids are considered essential for plant growth, as gauged by the phenotype of mutants with disrupted hormone biosynthesis or perception (Depuydt & Hardtke, 2011). GA specifically promotes important processes in plant growth and development such as seed germination, cell elongation, cell division, as well as floral transition (Richards et al., 2001).

One sentence summary: identification of a novel synthetic small molecule capable of enhancing plant growth through the enhancement of response to endogenous gibberellins.
Bioactive gibberellic acids (GAs) are diterpene phytohormones that modulate plant growth and development throughout the plant life cycle (Sun, 2010). The major function of GAs is to stimulate organ growth through the enhancement of cell elongation and cell division (Gupta & Chakrabarty, 2013; Hedden & Phillips, 2000). The GA receptor was first identified in rice where OsGID1 gene encodes a protein possessing GA-binding activity, and its mutation results in a severe dwarf phenotype that does not respond to GA in either stem elongation or seed germination (Stepanova, 2008; Stepanova et al., 2005; Tao, 2008). In Arabidopsis, there are three homologs of the GA receptor, AtGID1a, AtGID1b and AtGID1c (Nakajima et al., 2006). Single mutation of GID1a, GID1b and GID1c results in the same phenotype as wild type in terms of stem elongation and root length. This suggests that the receptors have a redundant function in Arabidopsis; however, the specificity of GID1 homologs function can be observed from double mutants (luchi et al., 2007; Suzuki et al., 2009).

In GA signaling, the key mechanism is GA repression of DELLA protein function. DELLA proteins are negative regulators of plant growth that belong to the GRAS protein superfamily of transcriptional regulators. The controlled degradation of these proteins is a major event in plant growth (Hauvermale et al., 2012). There are five DELLA repressor proteins in Arabidopsis: REPRESSOR OF GIBBERELLIC ACID (RGA), GA-INSENSITIVE (GAI), RGA-Like Proteins 1,2 and 4 (RGA1, RGA2 and RGA3). Activation of the GA signaling pathway is initiated by the interaction between bioactive GAs and GID1 that promotes a conformational change in the receptor. The formation of a GA-GID1-DELLA complex enables a protein–protein interaction between the DELLA and the F-box protein SLY1 resulting in ubiquitination and degradation of the DELLA protein (Griffiths et al., 2006; Hirano et al., 2010). The degradation of DELLA protein allows for the activation of transcription factors downstream of them to affect the required growth responses.

Growth in etiolated seedlings is regulated by phytochrome interacting factors (PIFs), a subset of basic helix–loop–helix (bHLH) transcription factors (Li et al., 2016). PIFs mediate hypocotyl elongation, and their activity is negatively regulated by the red light photoreceptor PHYB and by DELLA proteins that act to repress the GA signaling pathway (de Lucas et al., 2008). The activation of PHYB by light leads to destabilization of PIFs whilst the accumulation of DELLA proteins block PIF activity by binding the DNA-recognition domain of this factor. In contrast, PIF proteins accumulate and directly regulate genes to maintain skotomorphogenesis in the dark (Li et al., 2016), leading to elongated hypocotyls. For this reason, hypocotyl growth is an often used growth assay to monitor GA-DELLA signaling.

Here, we describe a chemical study of growth in Arabidopsis seedlings, which has led to the identification of a compound that promotes growth via the GA-DELLA pathway. Our findings suggest that this chemical acts by enhancing the potency of endogenous GAs, and therefore, this compound and its derivatives have significant potential as probes for plant growth and also as use as agrochemicals.

## 2 | RESULTS

### 2.1 | Treatment with A1 results in promotion of root growth in Arabidopsis

In our earlier work, a small-scale chemical genetics study was undertaken to explore the role of the known calmodulin inhibitors, N-(6-aminohexyl)-1-naphthalenesulfonamide (W5) and N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W7) in plants. W5 and W7 are both naphthalene sulfonamide compounds, with the only difference between them being the C-5 chlorine substituent in W7. Despite their high degree of structural similarity, the activity of these antagonists is significantly different with W5 showing reduced activity as compared with W7 (Gilroy et al., 1987; Kaplan et al., 2006; Sinclair et al., 1996). To attempt to better understand this difference, a small set of structurally related analogues of W5 and W7, chlorinated (AC1, AC2, AC3, AC4) and nonchlorinated (A1, A2, A3, A4), were prepared and monitored for their biological activity (Figure 1a). As expected for calmodulin inhibitors, most compounds inhibited root growth compared with control (DMSO). However, surprisingly, one compound A1 led to enhanced root growth (Figure 2), an observation that merited further study.

### 2.2 | A1 promotes both fresh and dry weight accumulation in Arabidopsis

Having discovered the root growth-promoting properties of A1, we wished to establish if the effects were limited to the roots, or whether A1 could also promote growth of shoot tissue. To investigate this, plants were treated with A1 and then divided into shoot and root material and the fresh and dry weight recorded. As can be seen in Figure 3, the fresh weight of both roots and shoots increased in response to A1. Also in both roots and shoots the dry:fresh weight ratio also increased, demonstrating that the effect of A1 was to enhance true biomass (Figure 3) in all plant tissue, and not just uptake of water.

### 2.3 | A1 stimulates DELLA degradation in Arabidopsis

DELLA proteins are the key negative regulators of plant growth (Hauvermale et al., 2012; Yoshida et al., 2014). DELLA degradation in response to growth signals (such as gibberellins) leads to enhanced growth of plant tissues, including roots (Ubeda-Tomas et al., 2008). The observed promotion of root and shoot growth by A1 (Figures 2 and 3) lead to the hypothesis that A1 might therefore mediate DELLA degradation. To investigate this suggestion, the stability of DELLA proteins upon treatment with A1 was investigated (Figure 4). An assay which allows the visualization of DELLA degradation is to image fluorescent protein fusions to DELLA proteins (Silverstone et al., 2001). Exploiting this technology, *Arabidopsis thaliana* seedlings expressing...
2.4 | *Arabidopsis deltal* loss of function mutant is insensitive to A1

The data presented in Figure 4 suggested that A1 acts via DELLA, and we thus hypothesized that A1 action might be mediated through the GA-DELLA signaling pathway. A1 was also observed to have an effect on other GA-dependent processes such as partially reversing ABA-mediated germination inhibition (Figure S1). To determine the mechanism of A1 action via the GA/DELLA signaling pathway, we tested the response of A1 to DELLA and GA synthesis/signaling mutants. For these assays, we focused on hypocotyl growth, because it is well established that DELLA can inhibit the binding of PIFs to their target promoter leading to a reduction in GA-regulated hypocotyl growth (Castillon et al., 2007; de Lucas et al., 2008; Feng et al., 2008). In this way, the hypocotyl assay is more diagnostic for GA than root assays. To establish the use of A1 in this assay, we first tested hypocotyl growth by growing the seedlings on agar plates containing A1, with GA and PAC used as positive and negative controls, respectively, for 3 days in reduced light conditions to achieve a balance between maximum and minimum hypocotyl growth. From these data, it was clear that A1 stimulated hypocotyl growth in a manner very similar to GA (Figure S2). Having established the hypocotyl assay, we then confirmed genetically that the A1 effect upon growth was indeed DELLA-dependent. To test this, a mutant line that lacks all DELLA (GAI, RGA, RGL1, RGL2 and RGL3) function was used. Due to the loss of all DELLA function, this mutant displays a longer hypocotyl phenotype as compared with wild type without treatment (Figure 5). Unlike wild type, no promotion of hypocotyl growth was observed after A1 treatment of this mutant (Figure 5), supporting the suggestion that A1 requires DELLA for its growth-promoting effect.
auxins and gibberellins. We discounted a role for auxins as A1 had no effect upon hypocotyl growth in pif4-101 and pif4-2 mutants (Figure S3) nor did A1 stimulate the expression of auxin-inducible genes such as IAA2 and IAA4 (Figure S3). Therefore, we focused our attention upon gibberellins. As A1 stimulated hypocotyl growth in a very similar fashion to GA, it was possible that A1 was acting as an artificial GA. To determine whether this was the case, hypocotyl growth assays were performed using GA biosynthesis mutants, in order to test if A1 could restore growth. The ga1-5 mutant contains low levels of bioactive GA, which leads to an increase in DELLA protein and consequent growth inhibition, and hence displays a dwarfed phenotype (Fridborg et al., 1999). As expected, in hypocotyl growth assays, ga1-5 mutants had shorter hypocotyls than wild type in untreated conditions. The treatment of the seedlings with A1 could not recover hypocotyl elongation (Figure 6). These data suggest that A1 is not simply acting like GA, but it is also possible that A1 requires endogenous GA to obtain its effect on growth promotion, as ga1-3 with low levels of gibberellins does not respond to A1.

### DISCUSSION

The chemical genetic approach employs small molecule compounds to interrogate biological processes due to their ability to selectively modulate protein function (Stockwell, 2000a, 2000b). When compared with classical genetic studies, it can offer a number of advantages. This includes a rapid time scale for activity, the ability to titrate effects, regulated activity (as opposed to constitutive), and in particular an ability to reduce the problem of genetic redundancy which can complicate standard genetic knock out experiments. This is due to the ability of a small molecule to specifically interact with a single protein and act as either an antagonist or agonist, subsequently allowing identification of protein function through a biochemical approach (Toth & van der Hoorn, 2010). Because of these beneficial features of using the chemical genetic approach, we created a series of chemicals based on the calmodulin inhibitors W5 and W7 (Gilroy et al., 1987; Kaplan et al., 2006) in order to investigate calcium signaling in plants. As inhibition of calmodulin has been reported to arrest plant growth (Sinclair et al., 1996), we tested these for efficacy in plants using a simple root growth assay for our screen. Whilst most compounds either inhibited growth or had no effect as expected, surprisingly, one compound A1 actually promoted root growth (Figure 2). Further analyses revealed that A1 promotes not only root growth, but also shoot growth and this effect was associated with increases in both dry and fresh weight (Figure 3). This suggests that A1 stimulates bona fide increases in growth including the production of new cellular material, and not just uptake of water.

Central to the regulation of all plant growth are the DELLA proteins (Dill et al., 2001; Hauvermale et al., 2012). These are key negative regulators of plant growth, which limit growth under conditions whereby maximal plant growth is not appropriate, for example, under stress conditions (Achard et al., 2003; Rowe et al., 2016). When growth rate needs to be increased, controlled DELLA protein
degradation is induced (Dill & Sun, 2001; Sun, 2010). We therefore hypothesized that the enhancement of growth mediated by A1 (Figures 2 and 3) might be occurring through the direct or indirect regulation of DELLA protein degradation. In support of this hypothesis, we found that A1 was as capable as GA at reducing fluorescence in an RGA-GFP fusion line (Figure 4). Moreover, using a hypocotyl growth assay as a more specific marker of GA-DELLA-based signaling, a DELLA quintuple mutant was, in contrast to wild type plants, insensitive to A1 confirming a direct link between A1 and the DELLA proteins (Figure 5).

As we had evidence that A1 was promoting growth through effects on DELLA proteins, we investigated whether the promotion of growth by A1 might involve gibberellins specifically. We therefore tested the effect of A1 upon a ga1-5 mutant, which is reduced in gibberellin biosynthesis and has lower levels of gibberellins (Fridborg et al., 1999). The ga1-5 mutant displayed shorter hypocotyls in the absence of any treatment (Figure 6), which is due to reduced levels of endogenous gibberellins as described before (Koomneef & van der Veen, 1980; Fridborg et al., 1999; Sun, 2010). Whilst A1 was capable of stimulating increased hypocotyl growth in wild-type, under the same conditions, it had no effect upon the hypocotyls of ga1-5 (Figure 6). This finding suggests that the mechanism by which A1 stimulates hypocotyl growth is not through A1 acting as a simple gibberellin substitute. The fact that A1 does not mimic gibberellins is consistent with the literature as whilst there are in excess of one hundred natural GA analogues reported only a very few exhibit significant bioactivity. In contrast to other phytohormones, simple modulators of the GA signaling pathway, such as A1, are relatively rare. Helminthosporal (Figure 1B1) and related derivatives, first identified by Coombe, promote hypocotyl growth and seed germination, similar to GA (Coombe et al., 1974; Miyazaki et al., 2017; Miyazaki et al., 2018). However, this has an equally complex structure to GA. The thiophenyl sulphone (Figure 1B2) (Yoon et al., 2013) exhibits GA antagonism whilst succinimide (Figure 1B3) (Jiang, Shimotakahara et al., 2017) and AC94377 (Figure 1B4) (Jiang, Otani, et al., 2017) appear to function as GA mimics. The last two are proposed to function as selective agonists of GID1 leading to DELLA degradation and downregulation of the expression of GA20ox genes and the up-regulation of GA2ox genes. In contrast, A1 had no effect on any of the three possible GID1 double mutants (Figure 7), as determined in the hypocotyl growth assay, nor on the expression of these genes (Figure S4), strongly suggesting a different mode of action. Whilst A1...
was capable of stimulating increased hypocotyl growth in wild-type, under the same conditions, it had no effect upon the hypocotyls of a ga1-5 mutant which is reduced in gibberellins biosynthesis and has lower levels of gibberellins suggesting that the mechanism by which A1 stimulates hypocotyl growth, requires endogenous gibberellins and is not a simple GA receptor agonist.

In summary, we have identified a simple, easy to prepare, small molecule A1 that acts to increase plant growth through the degradation of DELLA proteins in a mechanism that requires the presence, and perception, of endogenous gibberellins. Future work to identify the molecular basis (and target) for potential A1 sensitization of GA perception will provide new insights into GA/DELLA signaling and pave the way to use A1 and related compounds as growth-promoting agrochemicals.

4 | MATERIALS AND METHODS

4.1 | Synthesis of chemicals

Naphthalene sulfonyl chloride (1.00 g, 4.41 mmol) was dissolved in 15 ml of dry DCM and added dropwise to a solution of ethylene diamine (5.9 ml, 88.2 mmol, 20 equiv) in 10 ml of dry DCM. After stirring at room temperature for 1 h, the reaction was quenched by addition of 10 ml of H2O. The mixture was extracted with DCM (3 × 10 ml) and the combined organic layers were dried over MgSO4. The mixture was concentrated to afford a crude product as a light yellow oil (38.8 g, 80%). Without further purification, this product (38.8 g, 3.5 mmol) was dissolved in 10 ml of dry DCM and added to a solution of di-tert-butyl dicarbonate (1.08 g, 4.94 mmol, 1.4 equiv) in 10 ml of dry DCM. The mixture was stirred at room temperature for 16 h when TLC analysis confirmed complete consumption of the amine. The reaction was then quenched with 10 ml of H2O and the reaction mixture extracted with DCM (3 × 10 ml). The combined organic layers were dried over MgSO4, concentrated to afford a light yellow oil (3.5 mmol) which was dissolved in 10 ml of dry DCM and HCl...
(1 ml of a 4.0 M solution in dioxane [excess]) added. The mixture was then stirred at room temperature for 16 h when TLC analysis (hexane:ethyl ether, 2:1) showed complete consumption of starting material. After concentrating under vacuum, the solid obtained was washed with diethyl ether, filtered and dried under vacuum overnight to afford the title salt as a white solid (0.92 g, 65%). M.p: 178.8–179.3, 90% yield. The product was subjected to 5 days chemical treatment as described above, were measured. Dry weights were recorded after placing the plant material in oven at 65°C for 3 days. The measurements were performed on 15 seedlings for each treatment.

4.4 | Fresh and dry weight measurements

Leaves and roots fresh weights of 12 days old seedlings, which were subjected to 5 days chemical treatment as described above, were measured. Dry weights were recorded after placing the plant material in oven at 65°C for 3 days. The measurements were performed on 15 seedlings for each treatment.

4.5 | Confocal laser scanning microscopy techniques

Confocal microscopy was performed using a Leica SP5 CLSM FLIM FCCS (Leica Microsystems, Wetzlar, Germany). GFP:RGA seeds were germinated and grown on 1.2% MS vertically for 7 days and then incubated in chemical solution (at the final concentration of 100 μM) for 2 and 24 h before being analyzed. At least five roots were imaged for each time point. The excitation wavelength of the argon laser was 488 nm, and the emission was detected using a bypass filter of 495–550 nm. The fluorescence intensity of the images (1024 × 1024 pixel size) was measured using Leica software, LAS AF Lite.

4.6 | Statistical analysis

Data are shown as means ± standard errors (SEs). A t-test was performed to compare the hypocotyl and root length within each genotype between chemical treatment and control conditions (*p value < .05; **p value < .01). The normality of data distribution was tested using the Shapiro–Wilk normality test. Significant differences were analyzed using a one-way analysis of variance (ANOVA). SigmaPlot was used for the analysis (Systat Software Inc., San Jose, USA).

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CONFLICT OF INTEREST
The authors declare no competing interests.

AUTHOR CONTRIBUTION
M.R.K. and P.G.S. conceived the project and original research plans; M.R.K. and P.G.S. supervised the experiments; M.R.K., N.A.S., P.G.S., and S.P. designed the experiments. N.A.S. performed most of the experiments, and S.P. performed experiments presented in Figure 3: M.R.K. and N.A.S. wrote the article with contributions from all the authors; M.R.K. agrees to serve as the author responsible for contact and ensures communication.

DATA AVAILABILITY STATEMENT
The data and other finding of this study are available from the corresponding author upon reasonable request.

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REFERENCES
Achard, P., Vriezen, W. H., Van Der Straeten, D., & Harberd, N. P. (2003). Ethylene regulates Arabidopsis development via the modulation of DELLA protein growth repressor function. Plant Cell, 15(12), 2816–2825. https://doi.org/10.1105/tpc.015685
Castillon, A., Shen, H., & Huq, E. (2007). Phytochrome interacting factors: Central players in phytochrome-mediated light signaling network. Trends in Plant Science, 12(11), 514–521. https://doi.org/10.1016/j.tpls.2007.10.001
Coome, B. G., Mander, L. N., Paleg, L. G., & Turner, J. V. (1974). Gibberellin-like activity of helminthosporic acid analogues. Functional Plant Biology, 1, 473–481. https://doi.org/10.1071/PP9740473
de Lucas, M., Daviere, J. M., Rodriguez-Falcon, M., Pontin, M., Iglesias-Pedraz, J. M., Lorrain, S., Fankausher, C., Blazquez, M. A., Titareno, E., & Prat, S. (2008). A molecular framework for light and gibberellin control of cell elongation. Nature, 451(7177), 480–484. https://doi.org/10.1038/nature06520
Depuydt, S., & Hardtke, C. S. (2011). Hormone signalling crosstalk in plant growth regulation. Current Biology, 21(9), 365–373. https://doi.org/10.1016/j.cub.2011.03.013
Dill, A., Jung, H. S., & Sun, T. P. (2001). The DELLA motif is essential for gibberellin-induced degradation of RGA. Proceedings of the National Academy of Sciences of the United States of America, 98(24), 14162–14167. https://doi.org/10.1073/pnas.251534098
Dill, A., & Sun, T. P. (2001). Synergistic derepression of gibberellin signaling by removing RGA and GAI function in Arabidopsis thaliana. Genetics, 159, 777–785. https://doi.org/10.1093/genetics/159.2.777
Dürr, J., Lolas, I. B., Sarensen, B. B., Schubert, V., Houben, A., Melzer, M., Deutzmann, R., Grasser, M., & Grasser, K. D. (2014). The transcript elongation factor SPT4/SPT5 involved in auxin-related gene expression in Arabidopsis. Nucleic Acids Research, 42(7), 4332–4347. https://doi.org/10.1093/nar/gku096
Feng, S. H., Martinez, C., Gusmaroli, G., Wang, Y., Zhou, J. L., Wang, F., Chen, L. Y., Yu, L., Iglesias-Pedraz, J. M., Kircher, S., Schafer, E., Fu, X. D., Fan, L. M., & Deng, X. W. (2008). Coordinated regulation of Arabidopsis thaliana development by light and gibberellins. Nature, 451(7177), 475–479. https://doi.org/10.1038/nature06448
Franklin, K. A., Lee, S. H., Patel, D., Kumar, S. V., Spartz, A. K., Gu, C., Yee, S., Yu, P., Breen, G., Cohen, J. D., Wigge, P. A., & Gray, W. M. (2011). PHYTOCHROME-INTERACTING FACTOR 4 (PIF4) regulates auxin biosynthesis at high temperature. Pnas, 108(50), 20231–20235. https://doi.org/10.1073/pnas.110682108
Fridborg, I., Kuusk, S., Moritz, T., & Sundberg, E. (1999). The Arabidopsis dwarf mutant schl exhibits reduced gibberellin responses conferred by overexpression of a new putative zinc finger protein. The Plant Cell, 11(6), 1019–1031. https://doi.org/10.1105/tpc.11.6.1019
Gilroy, S., Hughes, W. A., & Trewavas, A. J. (1987). Calmodulin antagonists increase free cytosolic calcium levels in plant protoplasts in vivo. FEBS Letters, 212, 133–137. https://doi.org/10.1016/0014-5793(87)81571-5
Gray, W. M. (2004). Hormonal regulation of plant growth and development. PLoS Biology, 2(9), 1270–1273. https://doi.org/10.1371/journal.pbio.0020311
Griffiths, J., Murase, K., Rieu, I., Zentella, R., Zhang, Z. L., Powers, S. J., Gong, F., Phillips, A. L., Hedden, P., Sun, T. P., & Thomas, S. G. (2006). Genetic characterization and functional analysis of the GID1 gibberellin receptors in Arabidopsis. Plant Cell, 18(12), 3399–3414. https://doi.org/10.1105/tpc.106.047415
Gupta, R., & Chakraborty, S. K. (2013). Gibberellic acid in plant: Still a mystery unresolved. Plant Signaling & Behavior, 8(9), 1–5. https://doi.org/10.4161/psb.25504
Hauvermale, A. L., Arizumi, T., & Steber, C. M. (2012). Gibberellin signaling: A theme and variations on DELLA repression. Plant Physiology, 160, 83–92. https://doi.org/10.1104/pp.112.200956
Hedden, P., & Phillips, A. L. (2000). Manipulation of hormone biosynthetic genes in transgenic plants. Current Opinion in Biotechnology, 11, 130–137. https://doi.org/10.1016/S0958-1669(00)00071-9
Hirano, K., Asano, K., Tsuji, H., Kawamura, M., Mori, H., Kitano, H., Ueguchi-Tanaka, M., & Matsuoka, M. (2010). Characterization of the molecular mechanism underlying gibberellin perception complex formation in rice. Plant Cell, 22(8), 2680–2696. https://doi.org/10.1105/tpc.110.075549
Iuchi, S., Suzuki, H., & Kim, Y.-C. (2007). Multiple loss-of-function of Arabidopsis gibberellin receptor AtGID1 completely shuts down a gibberelin signal. The Plant Journal, 50(6), 958–966. https://doi.org/10.1111/j.1365-313X.2007.03098.x
Jiang, K., Otani, M., Shimotakahara, H., Yoon, J. M., Park, S. H., Tomoko, M., Takeshi, N., Hidemitsu, N., Masatoshi, N., & Tadao, A. (2017). Substituted phthalaldehyde ACA94377 is a selective agonist of the gibberellin receptor GID1. Plant Physiology, 173, 825–835. https://doi.org/10.1104/pp.16.00937
Jiang, K., Shimotakahara, H., Luo, M., Otani, M., Nakamura, H., Moselhy, S. S., Abualnaja, K. O., Al-Malki, A. L., Koomsani, T. A., Kitahata, N., Nakano, T., Nakajima, Y., & Asami, T. (2017). Chemical screening and development of novel gibberelin mimics. Bioorganic & Medicinal Chemistry Letters, 27(16), 3678–3682. https://doi.org/10.1016/j.bmcl.2017.07.012
Kaplan, B., Davydov, O., Knight, H., Galon, Y., Knight, M. R., Fluhar, R., & Fromm, H. (2006). Rapid transcriptome changes induced by cytosolic Ca2+ transients reveal ABR-related sequences as Ca2+–responsive cis elements in Arabidopsis. Plant Cell, 18(10), 2733–2748. https://doi.org/10.1105/tpc.106.042713
Koornneef, M., & van der Veen, J. H. (1980). Induction and analysis of gibberellin sensitive mutants in Arabidopsis thaliana (L.) heynh. Theoretical and Applied Genetics, 58(6), 257–263. https://doi.org/10.1007/BF00265176
Li, K., Yu, R., Fan, L. M., Wei, N., Chen, H., & Deng, X. W. (2016). DELLA-mediated PIF degradation contribute to coordination of light and gibberellin signalling in Arabidopsis. Nature Communications, 7, 11868. https://doi.org/10.1038/ncomms11868
Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2–ΔΔCT method. Methods, 25, 402–408. https://doi.org/10.1006/meth.2001.1262
Miyazaki, S., Jiang, K., Kobayashi, M., Asami, T., & Nakajima, M. (2017). Helminthosporic acid functions as an agonist for gibberellin receptor. *Bioscience Biotechnology and Biochemistry, 81*(11), 2152–2159. https://doi.org/10.1080/09168451.2017.1381018

Miyazaki, S., Tomita, K., Yamane, H., Kobayashi, M., Asami, T., & Nakajima, M. (2018). Characterization of a helminthosporic acid analog that is a selective agonist of gibberellin receptor. *Bioorganic & Medicinal Chemistry Letters, 28*(14), 2465–2470. https://doi.org/10.1016/j.bmcl.2018.06.005

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum, 15*(3), 473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Nakajima, M., Shimada, A., Takashi, Y., Kim, Y. C., Park, S. H., Ueguchi-Tanaka, M., Suzuki, H., Katoh, E., Iuchi, S., Kobayashi, M., Maeda, T., Matsuoka, M., & Yamaguchi, I. (2006). Identification and characterization of Arabidopsis gibberellin receptors. *Plant Journal, 46*(5), 880–889. https://doi.org/10.1111/j.1365-313X.2006.02748.x

Press, M. O., Lancot, A., & Queitsch, C. (2016). PIF4 and ELF3 act independently in Arabidopsis thaliana thermo-responsive flowering. *PLoS ONE, 11*(8), e0161791. https://doi.org/10.1371/journal.pone.0161791

Richards, D. E., King, K. E., Ait-ali, T., & Harberd, N. P. (2001). How gibberellin regulates plant growth and development: A molecular genetic analysis of gibberellin signaling. *Annual Review of Plant Physiology and Plant Molecular Biology, 52*(1), 67–88. https://doi.org/10.1146/annurev.arplant.52.1.67

Rigal, A., Ma, Q., & Robert, S. (2014). Unraveling plant hormone signaling through the use of small molecules. *Frontiers in Plant Science, 5*, 373. https://doi.org/10.3389/fpls.2014.00373

Rowe, J. H., Topping, J. F., Liu, J. L., & Lindsey, K. (2016). Abscisic acid regulates root growth under osmotic stress conditions via an interacting hormonal network with cytokinin, ethylene and auxin. *New Phytologist, 211*(1), 225–239. https://doi.org/10.1111/nph.13882

Silverstone, A. L., Jung, H.-S., Dill, A., Kawaide, H., Kamiya, Y., & Sun, T. (2001). Repressing a repressor: Gibberellin-induced rapid reduction of the RGA protein in Arabidopsis. *The Plant Cell, 13*(7), 1555–1565. https://doi.org/10.2307/3871386

Sinclair, W., Oliver, I., Maher, P., & Tretheway, A. (1996). The role of calmodulin in the gravitropic response of Arabidopsis thaliana agr-3 mutant. *Planta, 199*, 343–351. https://doi.org/10.1007/BF00195725

Stepanova, A. N. (2008). TAA1-mediated auxin biosynthesis is essential for hormone crosstalk and plant development. *Cell, 133*, 177–191. https://doi.org/10.1016/j.cell.2008.01.047

Stepanova, A. N., Hoyt, J. M., Hamilton, A. A., & Alonso, J. M. (2005). A link between ethylene and auxin uncovered by the characterization of two root-specific ethylene-insensitive mutants in Arabidopsis. *Plant Cell, 17*(8), 2230–2242. https://doi.org/10.1105/tpc.105.033365

Stockwell, B. R. (2000a). Chemical genetics: Ligand-based discovery of gene function. *Nature Reviews. Genetics, 1*, 116–125. https://doi.org/10.1038/35038557

Stockwell, B. R. (2000b). Frontiers in chemical genetics. *Trends in Biotechnology, 18*(11), 449–455. https://doi.org/10.1016/S0167-7799(00)01499-2

Sun, T. (2010). Gibberellin-GID1-DELLA: A pivotal regulatory module for plant growth and development. *Plant Physiology, 154*, 567–570. https://doi.org/10.1104/pp.110.161554

Suzuki, H., Park, S.-H., & Okubo, K. (2009). Differential expression and affinities of Arabidopsis gibberellin receptors can explain variation in phenotypes of multiple knock-out mutants. *The Plant Journal, 60*(1), 48–55. https://doi.org/10.1111/j.1365-313X.2009.03936.x

Taniguchi, M., Nakamura, M., Tazaka, M., & Morita, M. T. (2014). Identification of gravitropic response indicator genes in Arabidopsis inflorescence. *Plant Signaling & Behavior, 9*, 1–6. https://doi.org/10.4161/psb.29570

Tao, Y. (2008). Rapid synthesis of auxin via a new tryptophan-dependent pathway is required for shade avoidance in plants. *Cell, 133*, 164–176. https://doi.org/10.1016/j.cell.2008.01.049

Toth, R., & van der Hoorn, R. A. (2010). Emerging principles in plant chemical genetics. *Trends in Plant Science, 15*, 81–88. https://doi.org/10.1016/j.tplants.2009.11.005

Ubeda-Tomas, S., Swarup, R., Coates, J., Swarup, K., Laplaze, L., Beemster, G. T. S., Hedden, P., Bhalerao, R., & Bennett, M. J. (2008). Root growth in Arabidopsis requires gibberellin/DELLA signalling in the endodermis. *Nature Cell Biology, 10*(5), 625–628. https://doi.org/10.1038/ncb1726

Yoon, J.-M., Nakajima, M., Mashiguchi, K., Park, S. H., Otani, M., & Asami, T. (2013). Chemical screening of an inhibitor for gibberellin receptors based on a yeast two-hybrid system. *Bioorganic & Medicinal Chemistry Letters, 23*(4), 1096–1098. https://doi.org/10.1016/j.bmcl.2012.12.007

Yoshida, H., Hirano, K., Sato, T., Mitsuda, N., Nomoto, M., Maeo, K., Koketsu, E., Mitani, R., Kawamura, M., Ishiguro, S., Tada, Y., Ohme-Takagi, M., Matsuoka, M., & Ueguchi-Tanaka, M. (2014). DELLA protein functions as a transcriptional activator through the DNA binding of the INTERMEDIATE DOMAIN family proteins. *Pnas, 111*(21), 7861–7866. https://doi.org/10.1073/pnas.1321669111

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