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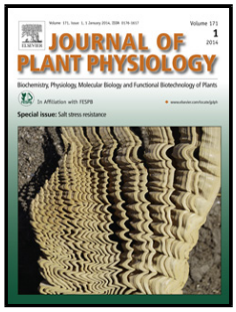


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# Basal stomatal aperture is regulated by GA-DELLAs in Arabidopsis

Nur Afiqah Sukiran[1,2], Patrick Giles Steel[2] and Marc Robert Knight[1*]

1. Department of Biosciences, Durham University, South Road, Durham DH1 3LE, UK.
2. Department of Chemistry, Durham University, South Road, Durham DH1 3LE, UK.

*Author for correspondence

(telephone: +44 191 334 1224; email: m.r.knight@durham.ac.uk)

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## Summary

Stomatal aperture is tightly regulated in order to achieve the best compromise between gas exchange and water conservation. Steady-state (basal) stomatal aperture is therefore understandably a key component in plant fitness. It has been shown previously in tomato that DELLA proteins act as positive regulators of closure of stomata, and their action is enhanced by the hormone ABA, which is itself important in mediating drought stress tolerance. DELLAs are regulated by a variety of signals which promote plant growth, most notably the hormones gibberellins, which have been shown to promote stomatal opening. We have found that DELLA proteins are also used in Arabidopsis for regulating basal stomatal aperture. We also discovered that the perception of endogenous gibberellins via the GID1 receptors is necessary for optimal basal stomatal aperture.



**Keywords**

Stomata, gibberellins, DELLAs, GID1, Arabidopsis

## Introduction

Stomata are pores that form across the epidermal cell layer of plant leaves and stems, serving as the major route for both gaseous exchange and water loss. They are surrounded by guard cells that control the size of the stomatal aperture thus regulating the rate of gas exchange in and out, as well as transpiration (Buckley, 2005; Lawson and Blatt, 2014; Lawson and Vialet-Chabrand, 2019). Stomatal opening and closing is controlled by regulated osmotic swelling and osmotic shrinking of guard cells, respectively (MacRobbie, 1998; Munemasa et al., 2015). This process is regulated by the integration of environmental and endogenous stimuli including light, $CO_2$, abiotic and biotic stress, as well as the action of endogenous plant hormones (Buckley, 2005; Daszkowska-Golec and Szarejko, 2013; Hetherington and Woodward, 2003). The involvement of plant hormones is key to stomatal aperture size (Acharya and Assmann, 2009; Farber et al., 2016). Abscisic acid (ABA) is the best-studied plant hormone involved in this process (Levchenko et al., 2005; Schroeder et al., 2001). ABA is produced to promote stomatal closure when it is important to prevent the plant losing too much water, for example during drought stress (Daszkowska-Golec and Szarejko, 2013; Swamy and Smith, 1999). Other plant hormones such as auxins, cytokinins, ethylene and jasmonic acid also regulate stomatal aperture when plants are exposed to stresses (Daszkowska-Golec and Szarejko, 2013). In tomato, it has been found that DELLA proteins promote ABA-mediated stomata closure (Nir et al., 2017), and it was postulated that this might be through antagonism of gibberellin function. In *Vicia faba* It has been reported that adding exogenous gibberellic acid (GA) causes stomatal opening (Goring et al., 1990). We wished to determine if endogenous gibberellins regulate basal stomatal aperture, adopting a genetic approach, exploiting the genetic model plant *Arabidopsis*. We tested both the steady-state aperture and the GA-induced stomatal opening response in a *della* quintuple mutant, as well as in double gibberellin receptor mutants (*gid1a1b, gid1a1c and gid1b1c*). Taken together our results show that endogenous GA levels control steady-state stomatal aperture in *Arabidopsis* via the GID1 receptors in a mechanism involving DELLA degradation.

## Material and Methods

### Plant material

*Arabidopsis thaliana* wild-type seeds were from laboratory stocks of Columbia (Col-0) and *Landsberg erecta* (Ler-0) accessions. The mutants of *gid1* (Griffiths et al., 2006) were obtained from Dr. Steve Thomas (Rothamsted Research, UK) and were in a Col-0 background, whilst the *della* quintuple mutant (Feng et al., 2008) was in a Ler-0 background and was obtained from the Nottingham Arabidopsis Stock Centre (NASC).

### Plant growth conditions

Sterilised seeds were grown in Percival (CU-36L5D, CLF plant climatics, Emersacker, Germany) with a photoperiod of 16/8 h at a light intensity of 150 µmol $m^2$ $s^{-1}$ and a temperature of 20±1°C. After 7 days, seedlings were transferred onto hydrated 44mm peat plugs (Jiffy Products International AS, Moerdijk, Norway) and grown at 20 °C with a photoperiod of 12/12 h for another 3 weeks.

### Stomatal aperture assay

*Arabidopsis thaliana* at the age of four weeks were used in this assay. Epidermal peels were incubated in the buffer; 10mM KCl and 50mM 2-(N-morpholino)-ethane-sulfonic acid (MES), pH 6.15 at 20°C (Gonzalez-Guzman et al., 2012) for 2 hours before treatment (either adding GA (Sigma, Poole, UK) to a final concentration of 100 µM in 0.1% v/v DMSO or control of 0.1% v/v DMSO). Experiments were conducted under a minimal source of light to reduce the size of the stomatal aperture before treatment. Tissue was incubated for a further 2h before imaging. Stomatal aperture was observed under light microscope (Leica DM 2500, Germany) with 40 times magnification and the images were captured using StreamCatcher software (StarTech, Ontario, Canada), with 10 stomatal images for every peel, to give a total of 30 measurements per sample. The measurements were performed by quantifying the pore width of the stomata using ImageJ software.

## Results

DELLA proteins are highly important negative regulators of growth and endogenous gibberellins are one signal that represses their activity (Dill and Sun, 2001; Sun, 2010). In tomato, it has been found that DELLA proteins promote ABA-mediated stomata closure (Nir et al., 2017). To test the importance of DELLA proteins in regulating basal stomatal aperture, we measured stomatal aperture in a *della* quintuple mutant that lacks all DELLA (GAI, RGA, RGL1, RGL2 and RGL3) function and compared it to wild type (Fig. 1). These data show that the steady-state aperture of untreated *della* quintuple mutants was significantly greater than in wild type. No promotion of stomatal aperture opening was observed in the *della* quintuple mutant after GA application, under the same conditions that the wild type displayed significant opening (Fig. 1). It has been observed that exogenously-applied GA can promote stomatal opening in *Vicia faba* (Goring et al., 1990), however this has, to our knowledge, not be reported before for *Arabidopsis.*

Data in Fig. 1 show that the steady state stomatal aperture is negatively regulated by DELLA proteins in *Arabidopsis* as it is in tomato (Nir et al., 2017). In tomato it was suggested that DELLA activity is enhanced by ABA, working antagonistically to gibberellins, but the role of gibberellins was not investigated specifically (Nir et al., 2017). Therefore, to test if the smaller steady-state aperture in the wild-type compared to the *della* mutant was due to perception of endogenous gibberellin levels, we measured stomatal aperture in mutants of the gibberellin receptors. There are three gibberellin receptors in *Arabidopsis*; GID1A, GID1B and GID1C, which show functional redundancy in growth response and there is no observable phenotype in single mutants (Griffiths et al., 2006; Luchi et al., 2007; Murase et al., 2008). We therefore measured steady-state stomatal aperture in *gid1* double mutants. As can be seen in Fig. 2 the untreated (basal) stomatal aperture was significantly larger in the *gid1a1b* double mutants, compared to the wild type and the *gid1a1c* and *gid1b1c* mutants. Addition of GA, whilst able to significantly increase stomatal aperture in wild type, only lead to a significant increase in *gid1b1c* but not *gid1a1b* or *gid1a1c* mutant stomatal aperture (Fig. 2).

## Discussion

We have shown that basal stomatal aperture in *Arabidopsis* is DELLA-dependent: a loss of function *della* mutant has a larger steady-state aperture than wild type under the same conditions (Fig. 1). Addition of exogenous GA, whilst able to induce opening of stomata in the wild-type (this phenomenon had been previously observed in *Vicia faba* (Goring et al., 1990)), was unable to significantly open the guard cells of the *della* quintuple mutant (Fig. 1). This is most likely due to the lack of DELLA protein in this mutant, therefore GA has no effect as there can be no further DELLA degradation. Therefore, it was possible that the GA-mediated opening of stomata that we observed in the wild type, was due to the degradation of DELLAs mediated by exogenous GA. Taken together, our data suggest that the exogenous GA-mediated opening of stomata in the wild-type occurs through the degradation of DELLAs, thus inhibiting DELLA-mediated promotion of closure. DELLA proteins are key negative regulators of gibberellin signalling (Alvey and Boulton, 2008; Murase et al., 2008) and important in plant environmental responses (Achard et al., 2006). Whilst these negative regulators are more commonly associated with the regulation of gene expression, in tomato, it has been found that DELLA proteins promote ABA-mediated stomata closure (Nir et al., 2017). However, whilst it was postulated that this effect might be due to antagonism to gibberellin signalling, this was not investigated.

The increased steady-state aperture of stomata in the *della* quintuple mutants, along with the observation that the wild type aperture is increased in response to exogenous GA, suggests that endogenous gibberellin regulates steady-state stomatal aperture in wild type. To test this, we examined the basal aperture of the stomata of mutants of the established gibberellin receptors, namely GID1A, GID1B and GID1C (Griffiths et al., 2006; Iuchi et al., 2007; Murase et al., 2008). The steady-state aperture of the *gid1a1b* mutant was significantly larger than for the wild type and the other 2 double *gid1* mutants, suggesting that GID1A and GID1B act redundantly in the wild type to sense endogenous gibberellin levels and so regulate stomatal aperture. The exogenous GA-mediated opening of stomata was lost in *gid1a1b* and *gid1a1c*, suggesting that GID1A is the major contributor to sensing exogenous GA (Fig. 2). These data also suggest that GA perception via the GID1 receptors is necessary for GA-induced stomatal opening.

In summary, our data shows that exogenous GA-mediated opening of stomata is DELLA-dependent, and taken together with data on GID1 receptor mutants that steady-state apertures are mediated by endogenous gibberellin levels in wild type. This highlights an important role for GA/GID1/DELLA in fine-tuning basal stomatal aperture. We speculate that endogenous gibberellin levels might convey information relating to growth rate/status of the plant, this signal being integrated along with environmental information to make a decision on optimal aperture.

**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Figure legends

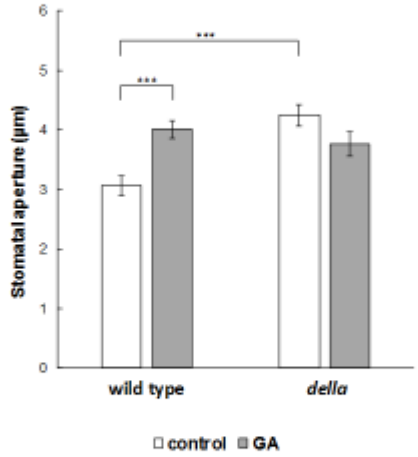


Fig. 1

**Fig. 1** Basal stomatal aperture of *della* quintuple mutant are increased. Assays were conducted using three epidermal layers for each treatment with 10 stomatal measures for each layer. Error bars represent standard errors for the 30 stomata per treatment (n=30). GA concentration for this experiment was 100µM. Asterisks indicate statistically significant differences (independent t-test, *** P<0.001 between wild type control and wild type GA treated and between wild type control and *della* control). This experiment was replicated 3 times independently.

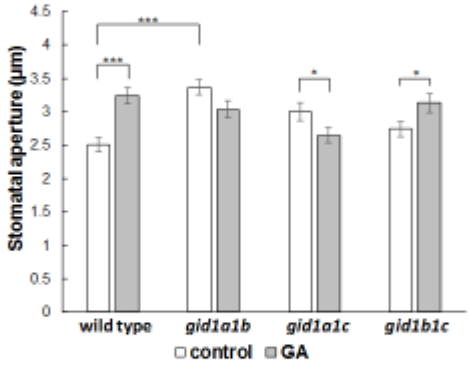


Fig. 2

**Fig. 2** Basal stomatal aperture of *gid1a1b* mutants is increased and insensitive to addition of GA. Assays were conducted as described for Fig. 1. using wild type, *gid1a1b*, *gid1a1c* and *gid1b1c* mutants. Asterisks indicate statistically significant differences (independent t-test, * P<0.05 between control and GA-treated *gid1a1c* and between control and GA-treated *gid1b1c*; *** P<0.001 between wild type control and wild type GA treated; and between wild type control and *gid1a1b* control). This experiment was replicated 3 times independently.