

The Arabidopsis *pi4kIIIβ1β2* double mutant is salicylic acid-overaccumulating: a new example of salicylic acid influence on plant stature

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Growth is the best visible sign of plant comfort. If plants are under stress, a difference in growth with control conditions can indicate that something is going wrong (or better). Phytohormones such as auxin, cytokinins, brassinosteroids or gibberellins, are important growth regulators and their role in plant growth was extensively studied. On the other hand the role of salicylic acid (SA), a phytohormone commonly connected with plant defense responses, in plant growth is under-rated. However, studies with SA-overaccumulating mutants directly showed an influence of SA on plant growth. Recently we characterized an Arabidopsis SA-overaccumulating mutant impaired in phosphatidylinositol-4-kinases (*pi4kIIIβ1β2*). This mutant is dwarf. The crossing with mutants impaired in SA signaling revealed that *pi4kIIIβ1β2* stunted rosette is due to high SA, while the short root length is not. This brings into evidence that upper and lower parts of the plants, even though they may share common phenotypes, are differently regulated.

Introduction

The deciphering of how plants regulate their growth and development belongs to the oldest topics in plant biology. In our recent work on *pi4kIIIβ1β2* double mutant we encountered this topic too. Phosphatidylinositol-4-kinases (PI4Ks) catalyze the phosphorylation of the D-4

hydroxyl of the inositol ring of phosphatidylinositol,¹ thus leading to phosphatidylinositol-4-phosphate (PI4P). According to pharmacological sensitivity and primary structure, one can distinguish between type II- and type III-PI4Ks.² PI4P can further be phosphorylated by a PIP-kinase into phosphatidylinositol-4,5-bisphosphate (PI-4,5-P₂). PI4P and PI-4,5-P₂ are phosphoinositides. They have signaling roles as well as structural roles.² For instance, we were able to show that type III-PI4Ks are the ones that provide their phosphoinositide substrates to phosphoinositide-dependent phospholipases C (PI-PLC), in basal conditions or in response to cold.^{3,4} In the *Arabidopsis thaliana* genome, 4 type III-PI4K genes can be found, clustered into 2 α and 2 β subtypes. The *AtPI4KIIIα2* is more likely a pseudogene.⁵ We recently studied a *pi4kIIIβ1β2* double mutant, a kind gift from Prof. E. Nielsen.^{6,7} This mutant has a dwarf phenotype. The rosette is shorter. It is not due to growth retardation, since the kinetics of leaf formation is wild type-like. It really represents a deficiency in the number and size of cells.⁷ The roots are also shorter. When grown on vertical *in vitro* plates, the root length of the *pi4kIIIβ1β2* double mutant was reduced by 72%. Our results indicate that shorter roots are also due to fewer and shorter cells.⁸ We were recently able to show that this double mutant belongs to the family of SA over-accumulating mutants. When grown on soil, 4 week-old plants had

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indeed a SA level 15-fold that of wild-type plants. It is known for more than 10 y that most SA accumulating mutants exhibit stunted growth.^{9,10} Therefore we wondered whether the dwarf phenotypes of the double mutant were due to the high SA level. The *pi4kIIIβ1β2* mutant was therefore crossed with mutants of SA synthesis (*sid2* and *eds1* mutated in isochorismate synthase, and EDS1, an upstream regulator of SA biosynthesis, respectively), of SA accumulation (*nahG* does over express a bacterial SA-hydroxylase) or of SA signaling (*npr1* is deficient in the main downstream component responsible for SA triggered transcriptome changes). The references for these mutants can be found in Janda and Ruelland (2014).¹⁰ Interestingly we could see that in each triple mutant in which SA is no longer over accumulated (*pi4kIIIβ1β2 sid2*, *pi4kIIIβ1β2 eds1* and *pi4kIIIβ1β2 nahG*) or cannot be transduced (*pi4kIIIβ1β2 npr1*), the rosette size was reverted. This clearly shows that the dwarf phenotype observed for rosettes of *pi4kIIIβ1β2* is due to the high level of SA. Interestingly, the root lengths of the triple mutants were also assayed, using the *in vitro* vertical plates. Here, the root length was only very slightly reverted.⁸

Therefore, if it is clear that high SA level is responsible for dwarf phenotype of rosette, it is also clear that it is not responsible for shorter root length of *pi4kIIIβ1β2* double mutant. This arises different questions and remarks. We want to discuss 2 of them in this addendum.

How SA Induces Dwarfism

As already mentioned, the fact that high SA level induces rosette stunted stature is well documented. Most, if not all, SA over-accumulating mutants are dwarf.¹⁰ In contrast, the SA depleted Arabidopsis NahG transgenic plants have a higher growth rate.¹¹ Moreover, it was shown that the shorter size of plants under chilling was due to cold-induced SA accumulation.¹² Yet, how high SA leads to dwarfism is far from being understood. Plant scientists mostly consider that defense against biotic stress has a metabolic cost, and that there should be a

balance trade-off between growth and defense. It is the so-called “fitness cost of resistance.”⁹ It is proposed that constitutively activated resistance exhausts plant because it is a “cost expensive process” and the source of energy is the same for both growth and defense. The fact that high SA induced dwarfism shows that if such a balance trade-off exists, it is a regulated process and that SA has a key role in this trade-off. From our data on *npr1pi4kIIIβ1β2* arises the fact that it is not the high SA *per se* that induces stunted rosette stature, but that the dwarfism is due to a till now un-deciphered phenomenon that depends on SA through the NPR1 signaling pathway. Indeed *npr1pi4kIIIβ1β2* triple mutant has even higher level of SA than *pi4kIIIβ1β2* double mutant, but the expression of *PR-1* was lower.⁸ It is interesting to note that growth alteration in other SA over-accumulating mutants does not necessarily depend on NPR1.¹⁰ So the effects of SA over-accumulation on growth could have distinct causes. What is clear is that it is not only due to energy being used to fight infection and therefore not being any longer available for growth. There is a re-programming, and SA is active in that reprogramming. Yet, how SA leads to short size is not known. Is it due to crosstalk between SA and phytohormones commonly described as growth regulators, such auxin, cytokinins or gibberellic acid? Antagonistic connection between SA and auxin was shown.¹³ Crosstalk could mean that high SA levels prevent other hormone synthesis and/or accumulation. We measured the level of other phytohormones in the *pi4kIIIβ1β2* double mutant. When compared to the WT, the double mutant had less auxin, less trans-zeatin and more cis-zeatin.⁸ The level of jasmonic acid (JA), another phytohormone connected with response to biotic stress,¹⁴ was increased. JA is known to have an inhibiting effect on plant growth.^{15,16} The changes for these hormones in the double mutant, though significant, were not as marked as that measured for SA. Besides, new hormone measurements, in the triple mutants, are necessary to investigate whether the changes in other hormones are downstream SA change, or occur in parallel to SA change as direct

consequences of the PI4K double mutation *per se*. Besides, hormone crosstalk could also mean that high SA will alter the transduction of other hormones. Lipid signaling is a main constituent of hormonal regulation.¹⁷ We showed that SA activates PI4K and phospholipase D.¹⁸⁻²⁰ It is not impossible that such activity might participate in counteracting the effects of the so-considered developmental hormones. It is at least worth investigating this hypothesis. Concerning JA, we can note that the effects of SA and JA in response to pathogens are mostly described as antagonistic.¹⁴ If JA were to be responsible for the stunted stature, downstream of SA, in the double mutant, it would imply that response to JA in that case is not negatively controlled by SA.

Another possible explanation of the action of SA on size is that it would act directly on metabolism, including the photosynthetic apparatus. It was proposed that growth retardation in SA over-accumulating mutants *cpr1*, *cpr5*, *cpr6* and *dnd1* was due to impaired photosynthetic activity. High light intensity probably overcomes this effect.¹ Interestingly, based on microarray analysis of *pi4kIIIβ1β2* seedlings, we show that most of the genes repressed in *pi4kIIIβ1β2* are encoded in chloroplast and participate in chloroplast function.⁸ Although the precise mechanisms are still unknown, SA is suggested to be a key molecule to maintain a proper balance between photosynthesis and growth.²¹

Rosette and Root Statures are Not Necessarily Connected

Another interesting point arising from our data is the fact that shoot size does not necessarily correlate with root length. Such a point has already been documented.²² Plants can have short roots but normal rosette size. Our data elegantly emphasize that what is responsible for stunted stature of rosette is not necessarily responsible for short root. The limiting factors for growth are not the same for different organs. What is the cause of short root length in the *pi4kIIIβ1β2* double mutant? This could be due to a direct effect of PI4K mutations *per se*. This could

be linked to the fact the *pi4kIIIβ1β2* double mutant has altered vesicle trafficking.⁷ Such alteration could lead to a problem in nutrient uptake that would affect root length. PI4Ks could be important to transduce developmental hormones. Interestingly, phosphoinositide gradients were shown to be necessary for a correct polarity of PIN auxin carriers.^{17,23,24} Therefore, the double mutation could lead to an alteration in phosphoinositide gradient formation, thus leading to deficient root tip growth.

Finally, we can conclude this addendum by another question: why does the double mutation in Arabidopsis PI4K β enzymes lead to high SA? Clearly more investigations are necessary to understand *pi4kIIIβ1β2* double mutant size phenotypes and hormone regulation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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