Spatial analysis of brassinosteroid signaling in the stem cell niche of Arabidopsis primary root

Caracterització molecular de la funció de BRI1 en les cèl·lules mare de l'arrel d'Arabidopsis

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Tesi Doctoral:

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Per a tu,

ABSTRACT

The present PhD thesis work reports the molecular and genetic dissection of the mechanisms by which plant steroid hormones Brassinosteroids (BRs) control root growth and development in *Arabidopsis thaliana* (Arabidopsis). A genetic, physiological and cellular analysis of existing BR mutants, together with the discovery of a novel pathway for the control of BR mediated stem cell divisions in the root of Arabidopsis is reported.

The results presented here demonstrate a role for BRs in stem cell homeostasis of the Arabidopsis primary root. Specifically, our analysis show that BRs promote columella stem cell differentiation and the division of a set of low mitotic cells called quiescent center (QC) that maintain the surrounding stem cells. Using a microgenomic approach, a novel BR signaling component, BRAVO (Brassinosteroids at Vascular and Organizing Centre) has been identified that is specifically expressed in the stem cells. BRAVO encodes a R2-R3 MYB transcription factor (MYB56) that acts as a negative regulator of BR-mediated QC divisions. This study uncovers a fine example of negative regulation model; BRAVO is directly repressed and interacts with BES1 creating a molecular switch that controls QC divisions.

Overall, the present PhD thesis advance a new role for brassinosteroid hormones in the regulation of stem cells is proposed. BRAVO provides plasticity to the stem cells to response to DNA damage, and at the same time robustness to ensure QC function upon damage. Considering the importance of plant stem cell homeostasis in plant adaptation to environmental changing conditions, the BRAVO pathway uncovers new mechanisms to understand plants life span.

RESUM

Aquesta tesi doctoral té com objectiu principal investigar els efectes de les hormones vegetals esteroides, Brassinosteroids (BRs), durant el desenvolupament de l'arrel primària d'*Arabidopsis thaliana* (Arabidopsis). Per tal d'assolir aquest objectiu hem realitzat una caracterització genètica, fisiològica i anàlisis cel·lular de mutants de BRs. Així mateix, hem descobert una nova ruta de senyalització que controla les divisions de les cèl·lules mare mitjançades per BRs.

Els nostres resultats experimentals mostren com els BRs controlen la homeòstasi de les cèl·lules mare de l'arrel. En concret, els BRs promouen la diferenciació de les cèl·lules mare de la columel·la i la divisió d'un grup de cèl·lules mitòticament inactives que actuen en el manteniment de les cèl·lules mare, el centre quiescent (QC). Mitjançant un enfocament microgenòmic hem identificat un nou element de la ruta de senyalització dels BRs específic de les cèl·lules mare, i l'hem anomenat BRAVO (Brassinosteroids at Vascular and Organizing Centre). BRAVO és un factor de transcripció R2R3 de la família MYB (MYB56), que actua com a regulador negatiu de les divisions de QC. Els nostres resultats mostren un model de regulació negativa, on BES1 reprimeix directament i interacciona amb BRAVO, creant un interruptor molecular que controla les divisions del QC.

El treball realitzat durant aquesta tesis doctoral permet proposar una nova funció dels BRs en el control de les cèl·lules mare. BRAVO dona plasticitat a les cèl·lules mare per a poder respondre al dany sobre l'ADN, així com els hi atorga robustesa per evitar-lo. El control de la homeòstasi de les cèl·lules mare en plantes és vital per entendre l'adaptació d'aquests organismes sèssils i la longevitat que presenten algunes especies.

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ABBREVIATIONS

BR: BRASSINOSTEROID

BRI1: BRASSINOSTEROIDS INSENSITIVE 1

SERK; SOMATIC EMBRYOGENESIS RECEPTOR KINASE

LRR-RLK: LEUCINE-RICH-REPEAT RECEPTOR-LIKE-KINASE

BL: BRASSINOLIDE

BKI1: BRI1 KINASE INHIBITOR 1

BAK1: BRI1 ASSOCIATED KINASE 1

BIN2: BRASSINOSTEROID INSENSITIVE 2

BSK: BR-SIGNALING KINASE

BSU1: BRI1 SUPRESSORS-1

BZR1: BRASSINOZOLE RESISTANT 1

BES1: BRI1 EMS SUPRESSOR 1

BRRE: BRASSINOSTEROID RESPONSE ELEMENT

CPD: CONSTITUTIVE PHOTOMORPHOGENESIS AND DWAFISM

YDA: YODA

TMM: TOO MANY MOUTHS

SPCH: SPEECHLESS

LOB: LATERAL ORGAN BOUNDARIES

SAM; SHOOT APICAL MERISTEM

BRX: BREVIS RADIX

MP: MONOPTEROS

SHY2: SHORT HYPOCOTYL 2

WER: WEREWOLF

GL2: GLABRA 2

RBR: RETINOBLASTOMA

SCR: SCARECROW

PLT: PLETHORA

CSC: COLUMELLA STEM CELL

QC: QUIESCENT CENTER

SHR: SHORT ROOT

KIP: KINASE-INTERACTING PROTEIN

WOX5: WUSCHEL-RELATED HOMEOBOX 5

WT: WILD-TYPE

DEX: DEXAMETHASONE

BRZ: BRASSINAZOLE

NAA: 1-NAPHTHALENEACETIC ACID

NPA: N-1-NAPHTHYLPHTHALAMIC ACID

GA: GIBBERELLIN

BRAVO: BRASSINOSTEROID AT VASCULAR AND ORGANIZING CENTER

WOL: WOODEN LEG

GO: GENE ONTHOLOGY

PI: PROPIDIUM IODIDE

CHIP: CHROMATIN INMUNOPRECIPITATION

FRET-FLIM: FLUORESCENCE RESONANCE ENERGY TRANSFER-FLUORESCENCE LIFETIME IMAGING MICROSCOPY

LCM: LASER CAPTURE MICRODISSECTION

FACS: FLUORESCENT ACTIVATED CELL SORTING

CHAPTER 1

GENERAL INTRODUCTION

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Brassinosteroid signaling in root development.
Josep Vilarrasa-Blasi, Mary-Paz González-García and Ana I. Caño-Delgado*
“Plant Roots: The hidden half”. Fourth edition, Chapter 17.

SUMMARY

Brassinosteroids (BRs) are plant steroid hormones that regulate multiple aspects of plant growth and development. Genetic and biochemical approaches in *Arabidopsis thaliana* (Arabidopsis) have identified BR receptors and major signaling components in the signaling pathway. In aerial plant organs, the growth-promoting properties of BRs are exemplified by their positive effect on cell elongation. The comprehensive characterization of BR contribution to root growth has shown that in addition to cell elongation, BR-mediated cell cycle progression is central for growth and meristem maintenance in the primary root. This chapter summarizes the state of knowledge on BR signaling in Arabidopsis and the current understanding of BR action in root development.

INTRODUCTION

Brassinosteriods (BRs) are polyhydroxylated triterpenoids essential for plant growth. BRs participate in a myriad of developmental processes, such as seed germination, pollen tube growth, male fertility, vascular development, flowering time and senescence (Kim and Wang, 2010). During the plant life cycle, BRs modulate the plant response to environmental factors such as light, temperature, salt and pathogens among others (Bajguz and Hayat, 2009). BRs were originally discovered in *Brassica napus* pollen (Grove M D, 1979). In the past two decades, genetic and biochemical analyses have identified the main BR signaling and synthesis components in the plant model species Arabidopsis and rice (Kim and Wang, 2010; Vert et al., 2005). The BR pathway is currently among the best studied signal transduction pathways in plants (Clouse, 2011; Kim and Wang, 2010; Vert et al., 2005) (Figure 1). Despite the amount of efforts involved in its study, fundamental aspects concerning the cell/organ specific contribution of BR signaling to plant development, the connections with other signaling pathways participating in related processes are just starting to draw a complete picture of BR action during development (Bai et al., 2012; Bell et al., 2012; Gendron et al., 2012; Gudesblat et al., 2012; Kim et al., 2012; Oh et al., 2012).

The characterization of existing BR mutants at the cellular level combined with the identification of novel cell-type specific signaling components operating in BR-mediated responses will advance our understanding of BR action in plant biology in the coming years.

Brassinosteroid signaling pathway

Plant and animal steroids share high structural similarity although the cellular mechanisms for BR signaling in plants differs from nuclear-localized receptors found in animals (Thummel and Chory, 2002). BRs are perceived by a plasma membrane localized leucine-rich-repeat receptor-like-kinase (LRR-

RLK) protein, BRASSINOSTEROID INSENSITIVE 1 (BRI1), that belongs to a family of protein kinases that contain more than 200 members (ShiuBleecker, AB, 2001). The *BRI1* gene was identified as a putative BR receptor through a genetic screen for BR-insensitive mutants in Arabidopsis (Li and Chory, 1997) and was reported to be ubiquitously expressed in the plant (Friedrichsen et al., 2000). An intensive study of its structure has indicated that BRI1 has an extracellular domain consisting of 24 LRR domains interrupted by a 70-amino-acid island domain (ID) placed between 20th and 21st LRR, a surface pocket for brassinolide (the most active BR compound, BL) binding, a transmembrane domain, a cytoplasmic serine/threonine kinase domain (KD), a juxtamembrane domain and a short C-Terminal domain (Friedrichsen et al., 2000; Hothorn et al., 2011; Li and Chory, 1997; Wang et al., 2005). In the presence of BL, homodimerized BRI1 receptors interact with co-receptors of the SOMATIC EMBRYOGENESIS RECEPTOR KINASE (SERK) family that are required for signaling, of which SERK3/BAK1 is the most prominent (Li et al., 2002; Nam and Li, 2002) (Figure 1). In this scenario, BR binds to the extracellular domain of BRI1 (Kinoshita et al., 2005) and the kinase of BRI1 phosphorylates tyrosine residues of the negative regulator BRI1 KINASE INHIBITOR1 (BKI1) (Jaillais et al., 2011b). This promotes BRI1 heterodimerization with its co-receptor BRI1 ASSOCIATED KINASE-1 (BAK1), activating the signaling complex that inactivates BRASSINOSTEROID INSENSITIVE 2 (BIN2) (a homolog to mammalian GSK3 kinases) through BR-SIGNALING KINASES (BSKs) and BRI1 SUPPRESSORS 1 (BSU1) (Kim and Wang, 2010; Mora-García et al., 2004; Peng et al., 2008; Tang et al., 2008). A dynamic and transient interplay between the main ligand-binding receptors and the co-receptors has been proposed (Jaillais et al., 2011a).

BR ligand perception at the plasma membrane triggers distinct transcriptomic responses in the plant nucleus that enables the plant to adapt to internal cues and major environmental conditions (Goda, 2002; Nemhauser et al., 2006). This differential gene expression involves the stabilization of at least two transcription factors specific to plants BZR1 (BRASSINAZOLE RESISTANT 1) and BES1 (BRI1 EMS SUPRESSOR 1, also designated BZR2,

BRASSINAZOLE RESISTANT 2) (Wang et al., 2002; Yin et al., 2002). Both can be phosphorylated by BIN2 kinase (He et al., 2002). The phosphorylated forms of BES1/BZR1 interact with 14-3-3 phosphoproteins promoting proteasome-mediated degradation, thus attenuating the signal (Gampala et al., 2007; Ryu et al., 2007). BIN2 kinase activity is negatively regulated by BSU1-mediated dephosphorylation and subsequent degradation in a proteasome dependent manner (Mora-García et al., 2004). BIN2 inactivation leads to dephosphorylation of BZR1/BES1 by protein phosphatase 2A (PP2A) and promotes its translocation into the nucleus. Upon nuclear translocation BZR1/BES1 can regulate BR-responsive genes, which represent approximately the 6% of the Arabidopsis genome (Gudesblat and Russinova, 2011; Sun et al., 2010; Yu et al., 2011).

BES1 and BZR1 transcription factors of Arabidopsis share 88% amino acid sequence identity. Genome-wide identification of BZR1 and BES1 target genes led to the identification of complex regulatory networks that integrate with hormonal and light signaling pathways during plant growth. BZR1 and BES1 proteins can bind to both E-boxes (CANNTG) and BRRE (BR response element (CGTGT/CG)), and function as transcriptional activators or repressors depending on their specific target (Sun et al., 2010; Yu et al., 2011). The large number of non-overlapping target genes for BZR1 and BES1 further evidence the tissue-specific nature of these responses (Gudesblat and Russinova, 2011). Future studies should uncover whether BRs can modulate specific cellular responses biasing BES1/BZR1 action.

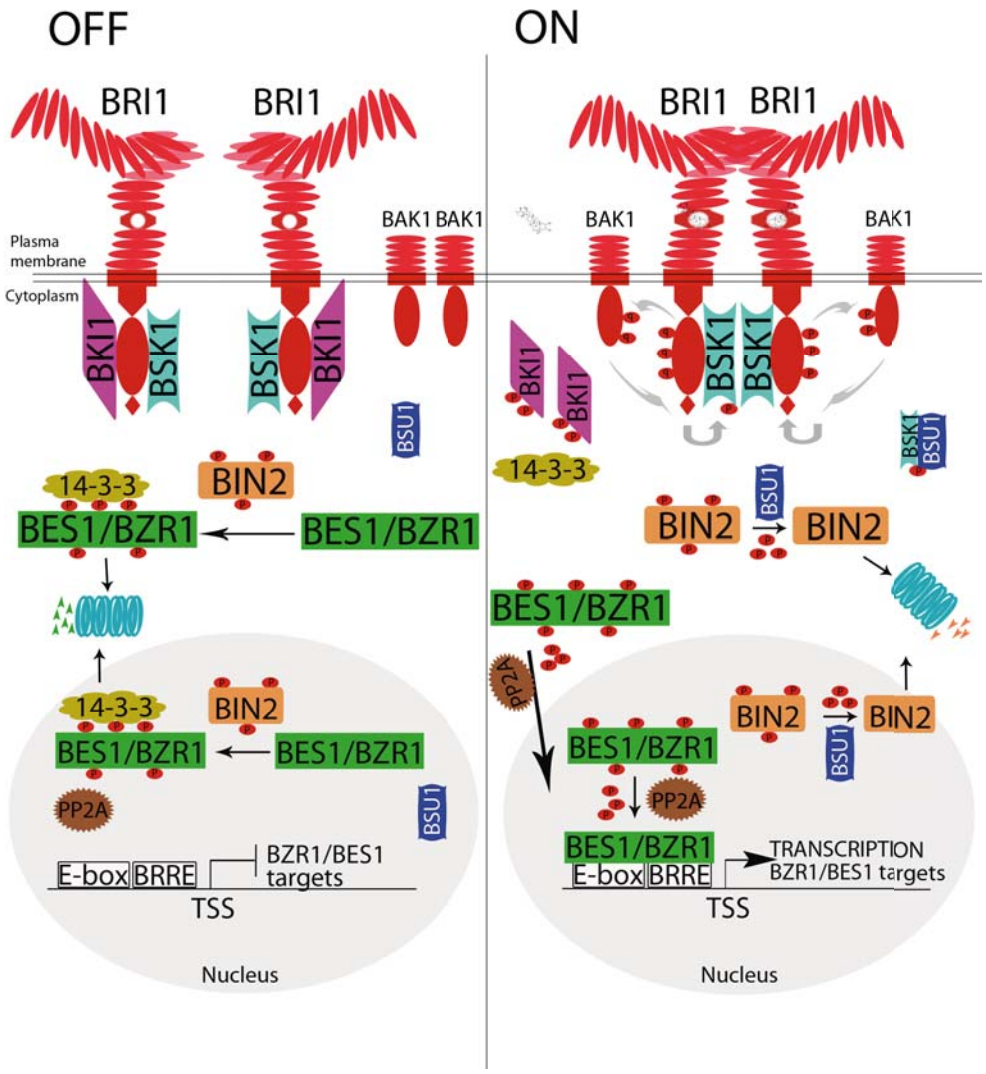

Figure 1. Brassinosteroids signaling pathway. (left) in the absence of BRs, BKI1 binds to the inactive form of BRI1 together with BSKs, therefore BSU remains inactive. In the cytoplasm, BIN2 kinase phosphorylates BZR1/2 transcription factors, promoting their cytoplasm retention thought the binding of 14-3-3 phosphoproteins and the subsequent proteasome mediated degradation. In consequence no changes in gene expression are observed. (right) After the perception of active BRs in the island domain of BRI1 receptor, BRI1 kinase phosphorylates BKI promoting its release to the cytoplasm and allowing heterodimerization between BRI1 and BAK1. Transphosphorylation between BRI1 and BAK1 activates the signaling complex, mediating phosphorylation of BSKs. BSK phosphorylation will bind to BSU, that in turn mediates BIN2 proteasome degradation by dephosphorylation of a conserved Tyr residue. The negative regulation of BIN2 upon BR perception, together with the PP2A

phosphatase activity mediates dephosphorylation of BES1/BZR1 transcription factors. Dephosphorylated BES1/BZR1 transcription factors move to the nucleus and bind to regulatory regions of the BR-regulated genes and promote or repress its expression.

The modulation of BRs signaling events in the cell is generally accomplished by BRI1 vesicle trafficking (Geldner et al., 2007; Viotti et al., 2010). The hypothesis of BRI1 complex endocytosis upon BRI1 receptor activation was originally proposed. These studies were based on the use of green fluorescent protein (GFP)-tagged BRI1 and different pharmacological inhibitors to describe the subcellular dynamics of the receptor in Arabidopsis roots. Upon blocking the transition between early and late-endosome mediated by Brefeldin A, BRI1 was accumulated, resulting in enhanced signaling responses (Geldner et al., 2007; Russinova et al., 2004; Viotti et al., 2010). An elegant study that implemented the use of a fluorescently labeled BR, Alexa Fluor 647 castasterone (AFCS) has facilitated the visualization of BRI1-AFCS endocytosis in living cells. This innovative study demonstrates that BRI1 receptor is active at the plasma membrane, and addresses a role for BRI1-mediated endocytosis in BR signal attenuation (Irani et al., 2012).

Based on the BRI1 ligand binding-domain, three additional BRI1-like receptor homologues have been identified in Arabidopsis, (BRI1-like genes) (Caño-Delgado et al., 2004; Ceserani et al., 2009) and rice (Yamamuro et al., 2000). Of those, the BRL1 and BRL3 have true receptor functions and play specific roles in vascular development, where their transcriptional expression is enriched (Caño-Delgado et al., 2004). A proteomic approach aimed to identify BRL3 interactors, unveiled BAK1 among other known BRI1 interactors as part of specific BR-signalosome (Fàbregas et al., 2013). The fact that BRL3 shares interactors with BRI1 receptor, but not BRI1 *per se*, opens a new avenue for specific signalosomes orchestrating cell-type specific responses (Fabregas et al., 2013). Future studies dissecting specific signaling events will shed light in the intricate coordination of BR-responses.

Brassinosteroids contribute to important aspects of plant postembryonic development

Plants use BRs to translate environmental stimuli into developmental responses. BR-deficient mutants and plants treated exogenously with BL or BR-synthesis inhibitors are dramatically affected in seed germination (Steber and McCourt, 2001), photomorphogenesis (Li et al., 1996), organ size and architecture (Clouse et al., 1996), apical dominance (Clouse et al., 1996), senescence (Li and Chory, 1997), vascular development (Caño-Delgado et al., 2004; Szekeres et al., 1996), male fertility (Ye et al., 2010), pollen tube growth (Grove M D, 1979) and flowering time (Domagalska et al., 2007; Li and Chory, 1997) (Figure 2). At the cellular level, BRs mediate cell growth by controlling cell division, elongation and differentiation activities. The ability of BR hormones to promote cell growth have been described in a variety of plant species (Fujioka and Sakurai, 1997). The most classical example is the elongation of the pollen tube, a fundamental step toward reproductive success in flowering plants (Grove M D, 1979). In Arabidopsis, genetic analysis has shown that strong BR mutants are male sterile, and the mutant defects in pollen tube elongation were found to correlate with a reduction in the expression of genes required for microspore mother cell development (*SPL/NZZ*), microspore development (*TDF1*, *AMS* and *MYB103*) and were required for tapetal development and pollen wall formation (*MS1/MS2*). Those genes are direct targets of BES1 (Ye et al., 2010).

Decades of physiological studies in several plant species have shown that BL application promotes cell elongation regulating the expression of primary cell-wall-loosening enzymes such as xyloglucan endotransglycosylases (XETs) (Zurek et al., 1994). Upon germination BR-deficient mutants display severe growth defects in the size of hypocotyls in seedlings that can be reversed by exogenous application of BL (Azpiroz et al., 1998; Szekeres et al., 1996). Recently, a mechanism for BR-mediated promotion of cell elongation has been proposed through the targeted expression of cell wall biosynthesis enzymes by BES1 and BZR1 detected in two parallel ChIP-chip experiments

(Sun et al., 2010; Yu et al., 2011). One further example is the demonstration by chromatin immunoprecipitation experiments that the BR-activated BES1 can bind to the promoters of a group of Arabidopsis cellulose synthase genes (*AtCESA*) that control primary cell wall elongation (Xie et al., 2011).

BRs mutants exhibit an array of cellular defects in leaf growth and development. BRs loss-of-function mutants have small and round-shaped leaves with short petioles (Choe et al., 1998; Li and Chory, 1997; Szekeres et al., 1996), whereas BR gain-of-function mutants exhibit enlarged leaf size with elongated petioles (Choe et al., 2001; Oh et al., 2011; Wang et al., 2002; Yin et al., 2002) (Figure 2). The dwarfed leaf phenotype of the BR loss-of-function mutants have been attributed to impaired cell growth (Chory et al., 1991; Clouse et al., 1996; Kauschmann et al., 1996; Szekeres et al., 1996).

In addition, several *ucu1/bin2* mutant alleles were shown to display leaves rolling spirally downwards concomitant with a reduced cell expansion of abaxial epidermal cells (Perez-Perez et al., 2004). In the same direction, increased BRI1 levels or modification of Tyr-phosphorylation properties of BRI1 receptor produces plants with increased leaf size (Gonzalez et al., 2010; Oh et al., 2011). The analysis of these transgenic plants indicated that BRs contribute to leaf growth by promoting cell division. Despite the results described above, a reduction in cell division could be compensated by an increase in cell area not necessarily resulting in larger leaves (Aguirrezabal et al., 2006), which has hampered a clear dissection between primary and secondary BR-mediated growth defects in this organ. Analysis of the BR loss-of-funtion mutant (CONSTITUTIVE PHOTOMORPHOGENESIS AND DWARFISM) (CPD), reveals that the reduced cell number in the leaf epidermis is due to cell cycle and a delayed exit from cell division, due to a prolonged M-phase (Zhiponova et al., 2013). The authors elegantly uncoupled cell division and cell elongation defects mediated by BRs in leafs, however the molecular mechanisms involved in this developmental process remains unknown.

In general, because BR mutants generally show alterations in organ size, it has been difficult to establish if the observed phenotypes are directly caused by overall cellular defects (cell division and cell elongation), by deregulation of a

cell-type and/or stage-specific signaling programs (cell differentiation) or a combination of both effects. A merge of quantitative phenotypic analyses with the identification of cell-specific BR responses will advance our understanding of tissue-specific BR signaling.

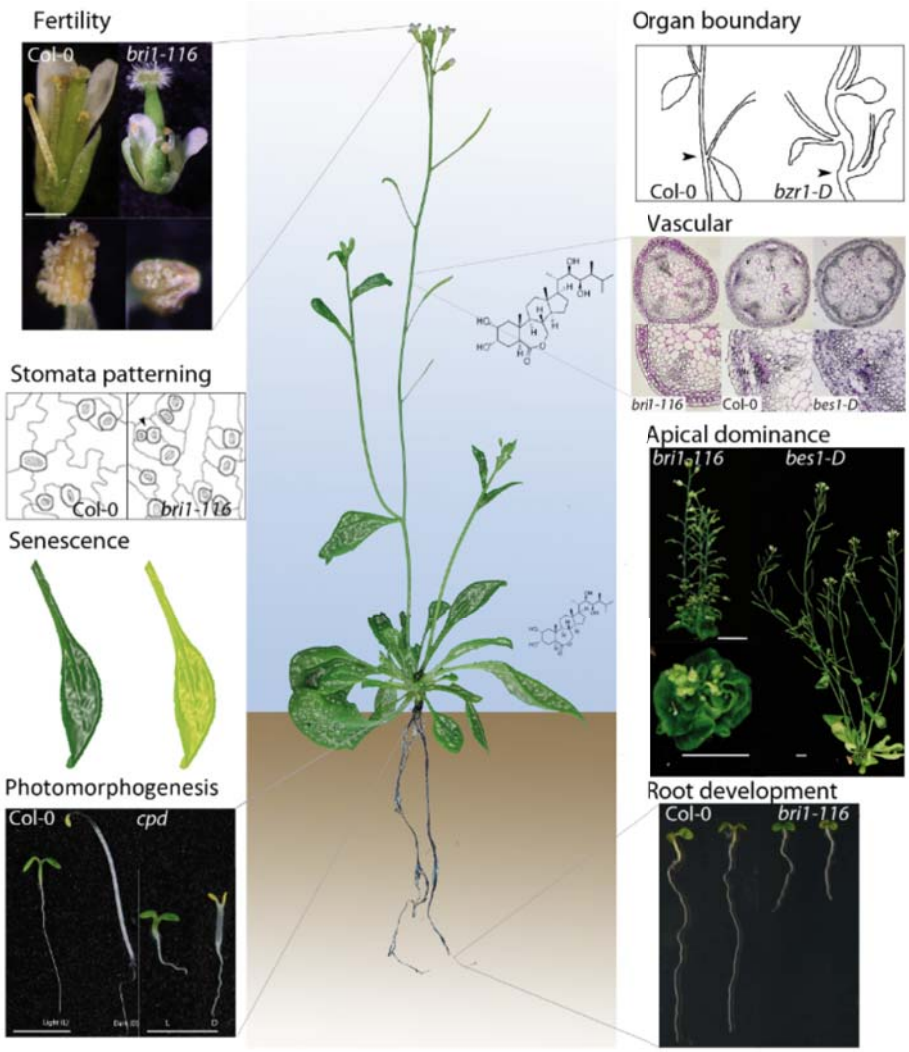

Figure 2. Roles of Brassinosteroids in plant development. Fertility; BR mutants show defects in the elongation of the stamens and in pollen grain production, producing sterile plants. Stomata patterning; BR control stomata development by interacting with the stomata patterning canonical pathway. Vascular; BRs control vascular bundle (vb) number in the shoot by promoting early procambial divisions (note differences between number of vb). Senescence; exogenous application of active BRs promotes senescence and BRs mutants show impaired senescence programs. Photomorphogenesis;

Arabidopsis thaliana seedlings grow in dark typically show elongated hypocotyl and close cotyledons, in contrast mutants defective in BRs biosynthesis show typical photomorphogenic responses with open cotyledons and short hypocotyl in dark. Organ boundary; BRs control organ boundary development in the shoot apex. Apical dominance; BR mutants loosed the apical dominance. Root development; BR mutants show short roots, due to expansion and cell cycle defects.

The role of BRs in vascular development in Arabidopsis was initially characterized in the BR-deficient mutants *cpd* and *dwf7* (Choe et al., 2001; Szekeres et al., 1996) and BR perception mutants (Caño-Delgado et al., 2004). The specificity of the BRI1 homolog receptors BRL1 and BRL3 expressed in the vascular tissues and the mutant phenotypes at the inflorescence stems have allowed to propose a role for BRs in modulating the xylem/phloem differentiation ratio (Caño-Delgado et al., 2004). Analysis of BR mutants has identified a role for BRs in promoting vascular bundle formation (Caño-Delgado et al., 2010; Ibañes et al., 2009). Mutants with reduced BRI1 receptor activity, signaling or BR levels were found to have a reduced number of vascular bundles, whereas mutants with increased BR signaling or BR levels exhibited an increased number of vascular bundles (Ibañes et al., 2009). While the mechanisms for BR-mediated provascular divisions and cell differentiation are unknown, the available transcriptomic studies in *Zinnia* and Arabidopsis cell cultures point to members of class III homeodomain transcription factors as candidate regulators of this process (Ohashi-Ito and Fukuda, 2003; Ohashi-Ito et al., 2002). The combination of computational approaches with molecular analyses will help to clarify the role of BR signaling in provascular divisions during vascular development (Fàbregas and Caño-Delgado, 2014).

Stomata are small pores present in leaves and in stem epidermis that function in gas exchange (Bergmann and Sack, 2007). Increasing levels of BRs in both gain-of-function mutants and exogenous application of BRs led to reduced number of stomata, while BR loss-of-function mutants showed stomata clustering and increased stomata number (Kim et al., 2012). Yeast two hybrid, pull-down, kinase assays and *in vivo* co-immunoprecipitation demonstrated that

BIN2 interacts with mitogen-activated protein (MAPK) kinase YODA (YDA) inhibiting YDA activity by phosphorylation (Kim and Wang, 2010). Additionally, MAPK kinases act downstream of the TOO MANY MOUTHS (TMM) receptor and ERECTA family to regulate SPEECHLESS (SPCH), MUTE and FAMA. Upon activation, MAPK kinases phosphorylate SPCH which results in the its inactivation to regulate stomata divisions (Bergmann and Sack, 2007). An additional layer for BR-regulated stomata development comes from biochemical studies showing that BIN2 can phosphorylate SPCH, and limit epidermal cell proliferation in hypocotyls (Gudesblat et al., 2012). Thus, BRs regulate stomata development though the modulation of different canonical stomata patterning genes, in a cell-type specific manner.

To date, the most extensive study on the spatial BR-regulation was achieved thought the analysis of BR defects in the shoot apical meristem (SAM). SAM is limited by boundary regions, formed by quiescent cells that rarely divide compared to the surrounding cell types (Fletcher, 2002). LATERAL ORGAN BOUNDARIES (LOB) transcription factor is specifically expressed in the organ boundaries, and loss-of-function mutants have fused organs (Bell et al., 2012). Complementation of the *lob* phenotype with *brz1-D* gain-of-function mutant, together with the regulation and interaction of LOB and BAS1 suggest that BR-signaling can direct specific developmental programs by regulating cell-type specific morphogenetic programs (Bell et al., 2012). A parallel study showed that BZR1 differential accumulation in SAM cell-types control organ boundaries directly though the regulation of boundary identity genes (Gendron et al., 2012). Thus, both a cell-type specific regulation of LOB and a differential accumulation of BR-signaling components may ensure organ boundary formation and morphogenesis in the SAM.

The coordination of different signaling pathways to integrate environmental inputs and provide the best fitness is a major question that should cope the increasing demand of food resources and climate change.

Brassinosteroid contribution to root growth and development

The simple and stereotyped organization of the Arabidopsis primary root has motivated the research of fundamental biological questions in plant organogenesis and development (Dolan et al., 1993). The *bri1* mutants were originally described by showing a short root phenotype in the presence of BL (Clouse et al., 1996). Since then, the analyses of root phenotypes in several BR-deficient and insensitive mutants have assigned a role for BRs in distinct aspects of root growth and development, such as gravitropism (Kim et al., 2000), lateral root formation (Bao et al., 2004), root-hair cell fate (Cheng et al., 2014; Kuppusamy et al., 2009), meristem size and distal stem cell differentiation (González-García et al., 2011; Hacham et al., 2011). Physiological studies have proven valuable to understand how BRs modulate root developmental processes in concerted action with other plant hormones, such as auxin and cytokinin (Scacchi et al., 2010). Physiological studies using exogenous hormone application have shown that BRs can stimulate root gravitropism independently of auxin (Kim et al., 2000). In contrast, the characterization of *bri1* and BR-biosynthesis mutants *dwarf4* have proposed that at low levels both hormones synergistically promote lateral root emergence (Bao et al., 2004), where BR action is subordinated to that of auxin (Yoshimitsu et al., 2011). During root growth, the functional analysis of BREVIS RADIX (BRX) has connected BRs synthesis and auxin signaling (Mouchel et al., 2006). At the protophloem cells of the root apical meristem, the concerted action of BRX, MONOPTEROS (MP) and SHORT HYPOCOTYL 2 (SHY2) establish a feedback loop that regulates PIN3 in the control of root meristem size (Scacchi et al., 2010). Contrary to that, BR signaling in the root was reported not to have a significant effect on auxin efflux carriers PIN1, 3 and 7 (Hacham et al., 2011). Certainly, the role of auxin gradients in BR-mediated cell cycle progression and differentiation deserves further analysis.

BRs at the root epidermis

The characterization of *bri1* epidermal patterning defects together with the expression of hair-fate reporters assigned a role for BR signaling in providing positional information to root epidermal cells (Cheng et al., 2014; Kuppusamy et al., 2009). BRs control the expression of key regulators of epidermal patterning, such as WEREWOLF (WER) and its downstream target GLABLA2 (GL2). Exogenous BL application increase the transcript levels of these two genes (Nemhauser et al., 2006). In addition, the hair epidermis specific GL2:GUS transcriptional reporter appeared to be miss expressed in the *bri1* mutant roots, which exhibited radial patterning defects in the epidermis. Although nor *GL2* neither *WER* genes were among the list of BZR1/BES1 high fidelity targets, genetic studies demonstrate that BR-regulated root epidermal cell patterning is dependent on the WER-GL3/EGL3-TTG1 complex (Cheng et al., 2014). Furthermore, BIN2 phosphorylates both EGL3, promoting its nuclear trans localization, and TTG1 to inhibit WER-GL3/EGL3-TTG1 activity (Cheng et al., 2014).

The role of the BRI1 receptor at the epidermis has been shown sufficient to promote the growth of both shoots and roots (Hacham et al., 2011; Savaldi-Goldstein et al., 2007). In the root, driving the expression of BRI1 receptor under GL2 promoter recovers the root meristem defects of *bri1* null mutants, whereas the specific expression of BRI1 in inner cell layers failed (Hacham et al., 2011). In addition, BRI1 expression in the epidermal non-hair cells inhibits root growth, thought the deposition of crystalline callose (Fridman et al., 2014).

Based on these results, a signaling mechanism from the epidermis to the inner-meristematic cells has been suggested, although the molecular nature of the moving signal remains to be identified.

BRs promote cell elongation during primary root growth

The role of BRs in promoting cell elongation in the primary root has been demonstrated at both physiological and genetic levels (Mouchel et al., 2006; Müssig et al., 2003; Szekeres et al., 1996). However, despite the positive effects

of BRs on root cell elongation, the roots of plants with increased BRs were shorter than those of wild-type ones (González-García et al., 2011). Notably, the short root phenotypes at 6-day-old seedlings of all BR mutants studied also showed a reduction with the number of cells in the meristem, indicating that optimal BR signaling is required to maintain meristem size (González-García et al., 2011). These findings prompted the study of BR-mediated root growth from a different perspective.

Root analysis for the study of BRs in cell-cycle progression and differentiation

The role of BRs in cell cycle progression has remained controversial for decades (Clouse et al., 1996; Miyazawa et al., 2003). The cellular analysis of *bri1* roots has uncovered that short *bri1* roots were caused by both defects in cell expansion and in the normal progression of cell cycle resulting in short meristem size (González-García et al., 2011; Hacham et al., 2011). Intriguingly, plants treated with BL or mutants with enhanced BR signaling such as *bes1-D* displayed a reduced root meristem, suggesting that balanced levels of the BRI1 receptor and downstream signaling are needed to maintain normal cell division activities in the root meristem (González-García et al., 2011).

The effects of BRs in meristem division have been analyzed using different cell cycle reporter lines. The analysis was done using a D-box *pCYCB1;1:GUS* and *pCYCB1;1:GFP* that visualize cells at the G2-M phase of the cell cycle (Colón-Carmona et al., 1999), a reporter of the plant specific cell cycle inhibitor ICK2/KRP2 (De Veylder et al., 2001), and immunofluorescence of KNOLLE to label the cell plates of dividing cells (Volker et al., 2001).

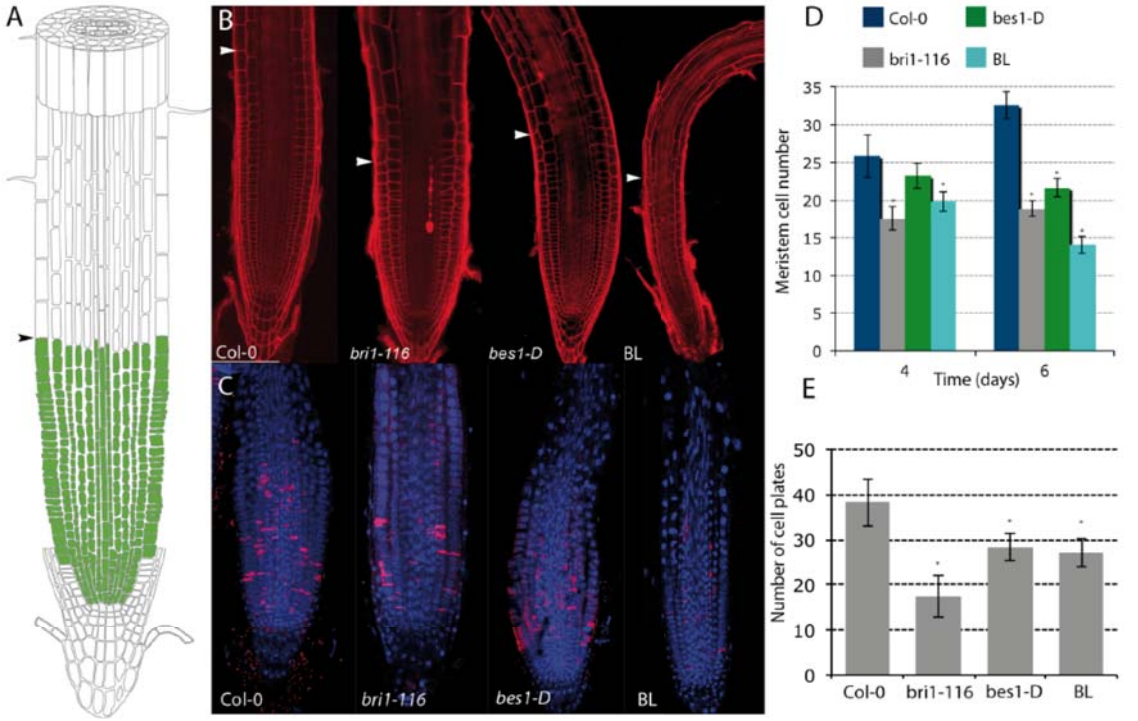

Figure 3. Cell cycle defects in Brassinosteroid mutants. (**A**) Root cartoon of the *Arabidopsis thaliana* root tip, green cells represent the meristematic region (dividing cells). (**B**) Root meristems of Col-0, *bri1-116 (*loss-of-function), *bes1-D (*gain-of-function) and BL stained counterstained PI (red), white arrow point to the end of the meristematic region. (**C**) Inmunolocalization of KNOLLE protein (pink) and cell nucleus stained with DAPI (blue) in different BR mutant background and BL treatment, right graph represents the number of cell plates (in pink) in each condition. (**D**) Graphic representation of the number of cells within the meristematic region. (**E**) Graphic representation of the number of cell plates. Note that both, number of meristematic cells and KNOLLE cell plates are impaired in the different BR mutants and BRs exogenous application compared to Col-0 (wild-type).

Different cell cycle reporters were analyzed in BR mutant backgrounds and BL treated plants. In the *bri1* mutants, the miss expression of CYB1;1, ICK2/KRP2 and immunofluorescence analysis of KNOLLE (Volker et al., 2001) revealed a reduced number of cell plates in the root meristem pointing to an arrested or slower cell cycle. These results together with the premature

appearance of epidermal root hairs in *bri1* mutants indicated that BRI1 signaling is required to control, not only cell elongation, but also the balance between cell division and differentiation in the primary root.

Conversely, several lines of experimental evidence support that increased BR signaling levels can lead to an acceleration of cell cycle progression during primary root growth: (i) a reduction of CYCB1;1 expression, (ii) a reduction in the number of KNOLLE-labeled cell plates, (iii) the reduced ICK2/KRP2 levels, (iv) an accelerated cellular differentiation revealed by the differentiation of root hairs in the proximal side of the meristem.

Quantification of BRI1 receptor *in planta* revealed that BRI1 density, rather than total number is constant along the root meristem and is reduced in expanding and mature cells (van Esse et al., 2011). Interestingly, receptor density is also reduced in the direct target cells of BL signaling, the stem cell niche, including the quiescent centre (QC) cells (González-García et al., 2011; van Esse et al., 2011). Moreover, mathematical modeling suggest that ligand availability rather than receptor concentration determines BRI1-mediated activities in roots (van Esse et al., 2012). Identification of BRL3 interactors *in planta* have shed light in cell-specific signalosomes fine-tuning BR-signaling (Fàbregas et al., 2013). Indeed, genetic analysis of *brl1brl3bak1-3* triple mutants revealed that this specific signaling module regulates root growth and development by contributing to the cellular activities of provascular and quiescent center cells, where its expression is enriched (Fàbregas et al., 2013).

Together, these root analyses open a new window to study how do cells behave in response to BRs. Root growth dynamic studies using a combination of mathematical modeling with a spatial/temporal analysis of BRs action in root growth will be very helpful to understand how BRs balance cell division, elongation and differentiation in the plant.

Concluding remarks

The genetic and biochemical studies carried in Arabidopsis during the last two decades have uncovered how plants perceive and transduce the BRs signal from the plasma membrane receptors to the transcriptional regulators in the nuclei that control of plant growth. It is currently accepted that BRs can modulate distinct developmental processes in the plant by simultaneously acting on cell division, elongation and differentiation. The root analysis of BR mutants provides a superior model to investigate how BRs control distinct cellular functions. The action of BRI1-like members and the signal specificity of BR-mediated responses have emerged as an alternative to integrate signaling cues into developmental programs. Identification of specific signaling modules operating at cell-type specific resolution are challenges for the next years.

OBJECTIVES

The general objective of this PhD Thesis was to investigate the mechanistic bases for Brassinosteroid action in the primary root of Arabidopsis thaliana.

To this end, the following specific objectives have been accomplished:

1. Investigate the contribution of Brassinosteroids to root growth and development by genetic and physiological analysis.

2. To dissect the spatial contribution of BRI1-mediated signaling in the primary root growth and development of Arabidopsis.

3. To identify and functional characterize a novel stem-cell specific regulator of the BR signaling in the root.

CHAPTER 2

RESULTS

GENETIC AND CELLULAR ANALYSIS OF BRASSINOSTEROID ACTION IN THE PRIMARY ROOT OF ARABIDOPSIS

Part of the results reported in this chapter were published at:
Brassinosteroid control meristem size by promoting cell cycle progression in Arabidopsis roots.
Mary-Paz González-García, Josep Vilarrasa-Blasi, Miroslava Zhiponova, Fanchon Divol, Santiago Mora-García, Eugenia Russinova and Ana I. Caño-Delgado.
Development 138, 849-859 (2011) doi:10.1242/dev.057331.

SUMMARY

Brassinosteroids (BRs) play crucial roles in plant growth and development. Previous studies have shown that BRs promote cell elongation in vegetative organs in several plant species, but their contribution to meristem and stem cell homeostasis remains unexplored. Our analyses report that both loss- and gain-of-function BR-related mutants in *Arabidopsis thaliana* have reduced meristem size, indicating that a balanced BR signaling is needed for the optimal root growth. In the BR-insensitive *bri1-116* mutant, root cell cycle defects can be counteracted by enhancing the mitotic activity, by either *CYD3;1* overexpression or *RETINOBLASTOMA* knockdown. We report that BRI1/BES1 signaling module promotes QC self-renewal and the differentiation of columella stem cell (CSC) upstream of *SCARECROW* and *PLETHORA* mediated signaling. Spatial analysis revealed; (i) BRI1 signaling acts non-cell autonomously from the meristematic dividing cells to control root growth, and (ii) BRI1 signaling in the QC cells controls stem cell differentiation non-cell autonomously. Moreover, local expression of BES1-D in the QC cells promotes QC divisions. Together, our results provide evidence that BRs play a regulatory role in the control of cell division and differentiation in the Arabidopsis root stem cell niche.

INTRODUCTION

Apical growth in both shoots and roots is a defining feature of vascular plants (Graham et al., 2000). Generative apices, established early in embryonic development, sustain polar growth and generate radial patterns of tissues along the body axes. Production of new cells is ensured by groups of stem cells located in the meristems. These cells have a low proliferative activity and divide asymmetrically. While one of the daughter cells takes over the role of the stem cell, the other sets off into a proliferation and differentiation program that progresses as its lineage leaves the meristematic region. Thus, net growth is the outcome of a balance between cell proliferation, differentiation and elongation.

The primary root of Arabidopsis is a superior developmental model because of its simple and stereotyped organization of cell types (Dolan et al., 1993; Ioio et al., 2007; van den Berg et al., 1998). Root tips provide the most easily accessible group of stem cells in the plant body and have been extensively used to visualize the dynamics of cell division and elongation (Beemster and Baskin, 1998; Casamitjana-Martinez et al., 2003). Cell division occurs at the meristem; progressively aged cells begin to differentiate in the elongation and differentiation zones reaching their final size/fate. At the basal part of the meristem, the stem cells surround the quiescent centre (QC), a group of three to four cells with very low proliferative activity that have been proposed to maintain the stem cell identity by means of short-range signals of a still unknown nature (Dolan et al., 1993; van den Berg et al., 1997). The position of the QC is defined by the overlap between domains of high auxin levels at the root tip (Friml et al., 2003), highly expressed auxin-responsive members of the *PLETHORA* (*PLT*) family of transcription factors (Aida et al., 2004; Galinha et al., 2007) and the radial expression of the transcription factors *SCARECROW* (*SCR*) and *SHORT ROOT* (*SHR*) (Di Laurenzio et al., 1996; Sabatini et al., 2003). The maintenance of QC cells by phytohormones and transcription factors ensures root growth.

Root growth is affected by most if not all plant hormones, indicating disturbances in meristem size. A series of comprehensive studies revealed that cytokinin (CK), auxins and gibberellin (GA) control meristem size (Garay-Arroyo

et al., 2012). Spatiotemporal control of the AUX/IAA protein SHORT HYPOCOTYL 1 (SHY2) by both GAs and CKs balance cell differentiation and cell divisions, establishing meristem size, thought the regulation of auxin flow mediated by PIN gene expression (Ioio et al., 2008; Moubayidin et al., 2010). Additionally, GA regulates meristem size by degradation of DELLA growth repressors and enhancing cell division (Achard et al., 2009). Notably, the endodermis drives the GA-response in the root meristem, as expression a non-degradable DELLA protein in the endodermis blocked root meristem growth (Ubeda-Tomas et al., 2009).

BRs were originally discovered by their ability to promote cell elongation. Indeed, BR-related mutants exhibit short-root phenotype and exogenous application of BRs promote root growth at low concentrations, but inhibit it at high concentrations (Clouse et al., 1996; Mouchel et al., 2006; Müssig et al., 2003). Moreover, expression of BR-biosynthetic enzymes or the BRI1 receptor in the epidermis of the shoot apical meristem was sufficient to recover the dwarfism caused by reduced BR signaling in *bri1* mutants (Savaldi-Goldstein et al., 2007). However, the impact of the BR-signaling pathway in specific spatial developmental processes remains poorly understood.

Here, we investigated the role of BRs on root growth and root stem cell maintenance. Loss-of-function *bri1-116* null mutants and gain-of-function *bes1-D* mutants showed that a balance in BR signaling is required to maintain meristem size and overall root growth. The cell division defects of *bri1-116* are complemented by overexpression of *CYCD3;*1 or RBR silencing revealing that BR signaling is needed for a normal cell cycle progression. Importantly, these alterations can be traced back to the root stem cell niche, where BR-dependent signaling is necessary for normal QC identity and promotion of stem cell differentiation. Importantly, BRs appeared to act upstream or independently of PLETHORA (PLT) and SCARECROW (SCR) pathways in the meristem maintenance and columella stem cell differentiation. Moreover, BRI1-mediated signaling promotes root growth cell-autonomously from the meristematic dividing cells, while it promotes columella stem cell differentiation non-cell autonomously from the QC cells. Activation of BR-signaling thought BES1 promotes cell division

of QC cells cell-autonomously. These results uncover the importance of BRs in controlling the activity of the stem cell niches in plants.

PREVIOUS RESULTS OBTAINED AT OUR LABORATORY

To assess the role of BRs in root growth, the root length of mutants with altered BR-signaling, wild-type and plants treated with BL were analysed. A general trend was observed for all seedlings that displayed shorter roots than those of the wild-type plants. The contribution of BRs to cell divisions was assessed by measuring both meristem cell number and cell-cycle markers in plants with impaired BR-signaling. The reduced number of meristematic cells in both loss- gain-of-function mutants and plants treated exogenously with BRs reveals the importance of a balanced BR signaling in the control of the meristem size. The reduction of meristem cell number was caused by alterations in cell cycle progression, as reported by missexpression of cell division markers CyclinB1,1, KNOLLE and the plant-specific cell cycle inhibitor KIP (kinase-interacting protein)-related protein 2 (KRP2)/ICK1. Cell cycle defects were restored by increasing the mitotic activity with overexpression of CYCD3;1 (Figure 1 A, B, D, E). Interestingly, cell cycle defects were traced back to the stem cell niche where the expression of several QC specific markers was modified by BRs.

BR control the normal cell cycle progression in the root meristem

The accumulation of pCYCB1;1:GUS and pICK2/KRP2:GUS in *bri1-116* roots could reflect an arrest in cell division, thus we investigated whether an increase in the mitotic activity could reverse these cell cycle defects. We used mutant plants overexpressing *CyclinD3;1* (*CYCD3;1OE*) (Riou-Khamlichi et al., 1999) and post-embryonic meristem-specific silencing of RETINOBLASTOMA-RELATED (*Rbi*) (Wildwater et al., 2005), both shown to stimulate mitotic activities. The number of meristematic cells in *bri1-116;CYCD3;1OE* plants was indeed higher than in the *bri1-116* mutants and similar to that of wild-type or *CYCD3;1OE* plants (Figure 1 A, B, D, E). In the same direction, *bri1-116;Rbi* double mutant plants showed a similar number of meristematic cells compared to *Rbi* mutants and wild-type plants (Figure 3 C-D, F-G).

Stimulating cell divisions can thus restore cell-cycle progression defects observed in a BRI1-deficient background. These results indicate that a balanced BR-signaling is required for a normal progression of the cell cycle in the root meristem, contributing to the regulation of meristem size and root growth.

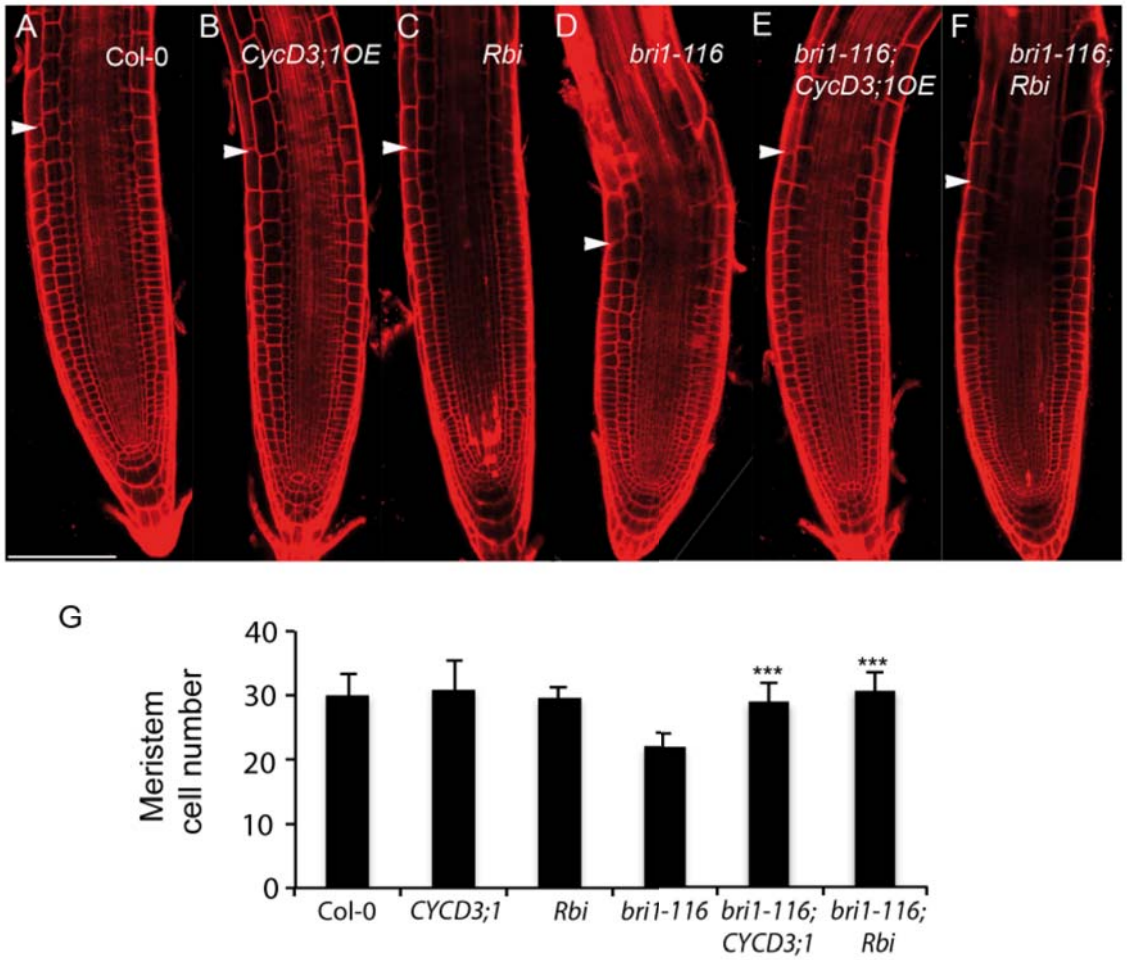

Figure 1. Rescue of *bri1-116* mutant phenotype in the root meristem.

(A-F) Confocal images **of s**ix-day-old primary roots counterstained with propidium iodide. (**A**) Col-0 (wild-type), (**B**) *CYCD3;1OE*, (**C**) *Rbi*, (**D**) *bri1-116*, (**E**) *bri1-116;CYCD3;1*, (**F**) *bri1-116;Rbi*. Scale bar represents 100 µM, white arrows indicate the boundary between the meristematic and elongation zones of the root. **(G)** Quantification of meristematic cell number. *** Indicate significant difference compared to *bri1-116* (p-value<0.01).

BR signaling controls QC identity

Root cell proliferation is initiated within a population of stem cells surrounding the QC (Scheres, 2007). Thus, we investigated whether the reduction in root meristem size observed in BR-related mutants could derive from defects in the stem cell niche. Significant increased occurred in the expression of the QC-localized marker *WOX5* (Sarkar et al., 2007) after exogenous treatment with BL (Figure 2 A, B) . In contrast, no changes in *pWOX5:GFP* expression were detected in the *bri1-116* mutant background after BL application, demonstrating that BRI1-mediated signaling promotes BL responses in the QC cells (Figure 2 C-F). .

To investigate the identity of the new cells expressing the QC marker we analysed the expression of *SCR*, a marker for QC and endodermis identity (Sabatini et al., 2003). Both *bes1-D* mutants and BL-treated plants expressed *pSCR:GFP* in additional cells compared to the wild-type (Figure 2 I, K, M). Additionally, expression of SHORT ROOT (SHR), an activator of SCR (Nakajima et al., 2001), showed additional QC cell layers after BL treatment (Figure 2 G, H). In contrast, *bzr1*-D gain-of-function mutants (Wang et al., 2002) did now showed additional QC cells labelled with SCR (Figure 2 L). Thus, BRs regulate QC self-renewal though BRI1-signaling and downstream of BES1. However, the *WOX5* expression domain was larger than that of *SCR*, suggesting that BRs are able to promote the expression of *WOX5* independently of SCR. These results address a role for the BRI1-dependent signaling pathway in the control of the QC identity. Most importantly, these results indicate cells specific functions for the role of BES1 and BRZ1 transcription factors in the control of root development.

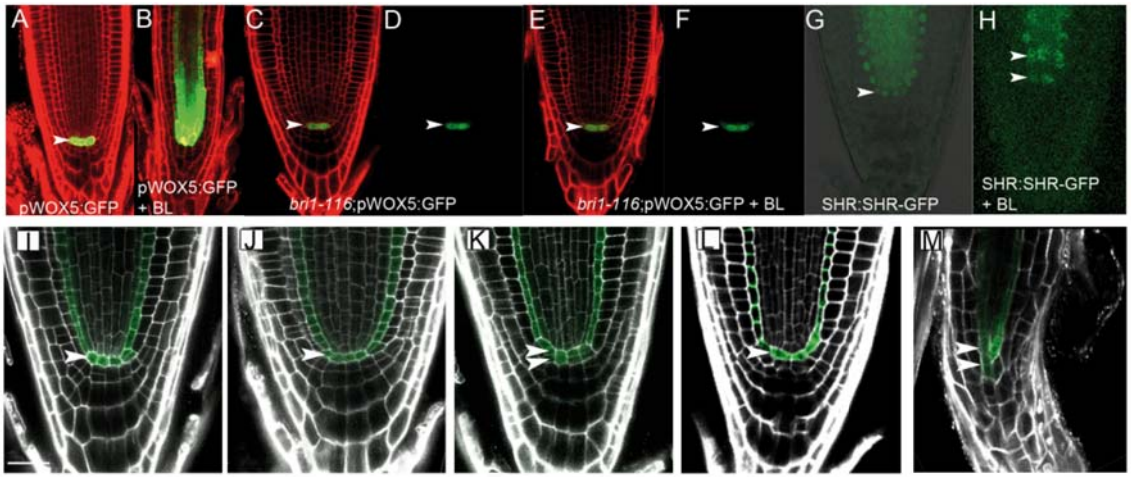

Figure 2. BRs control QC identity in the root stem cell niche.

(**A-M**) Confocal images of six-day-old primary roots counterstained with propidium iodide. (**A**) pWOX5:GFP untreated and (**B**) treated with 4 nM BL continuously. (**C, D**) bri1-116;pWOX5:GFP untreated and (**E-F**) treated with 4 nM BL continuously. (**G**) pSHR:SHR-GFP untreated and (**H**) treated with 4 nM BL continuously. (**I-M**) Expression of SCR in different BR-mutant backgrounds. (**I**) pSCR:GFP, (**J**) *bri1-116*;pSCR:GFP, (**K**) *bes1-D*;pSCR:GFP, (**L**) *bzr1-D*;pSCR:GFP, (**M**) pSCR:GFP treated with 4 nM BL continuously. White arrows point QC cells. Scale bar: 25μM.

BRs controls meristematic activities independent or upstream of PLETHORA and SCARECROW

The proper maintenance of the stem cells by the low proliferating QC cells is essential to maintain stem cell activities (Shishkova et al., 2008), and unsure indeterminate root growth. Two parallel pathways specify the QC identity; the PLT pathway and the SCR pathway (Aida et al., 2004; Sabatini et al., 2003). To further characterize BRs action in the stem cell niche, a physiological and genetic analysis of both PLT and SCR pathways in response to BRs was carried. Exogenous treatment of *plt1plt2* double mutants with BL, showed a hypersensitive response (Figure 3 A, B). Wild-type plants displayed a dose-dependent response to BRs leading to a progressive root shortening, from 10%

root growth reduction at 0,004 nM BL to 50% root reduction at 4nM BL (Figure 3 A, B). In contrast, *plt1plt2* double mutants were hyper-responsive to BL at 0,004 nM BL leading to a 50% root reduction, and 80% root decrease at 4nM of BL (Figure 3 A, B). Analysis of *bri1-116plt1plt2* triple mutants exhibited a synergic effect in root growth compared to the parental lines (Figure 3 C, D). The meristem length of *bri1-116plt1plt2* mutants was similar to that of *plt1plt2* mutants, while meristem cell number was increased in *bri1-116plt1plt2* compared to that of *plt1plt2* mutants (Figure 3 E-G). PLETHORA proteins are expressed in a dose dependent manner along the root (Galinha et al., 2007), with higher expression values on the root tip that progressively shut down until the differentiation zone. Analysis of PLETHORA protein expression in response to BRs did not show any significant difference comparing to control plants (Figure 4). These results suggest that BRs control root growth either independently or upstream of PLETHORA, while meristem cell number is primary controlled by BRI1.

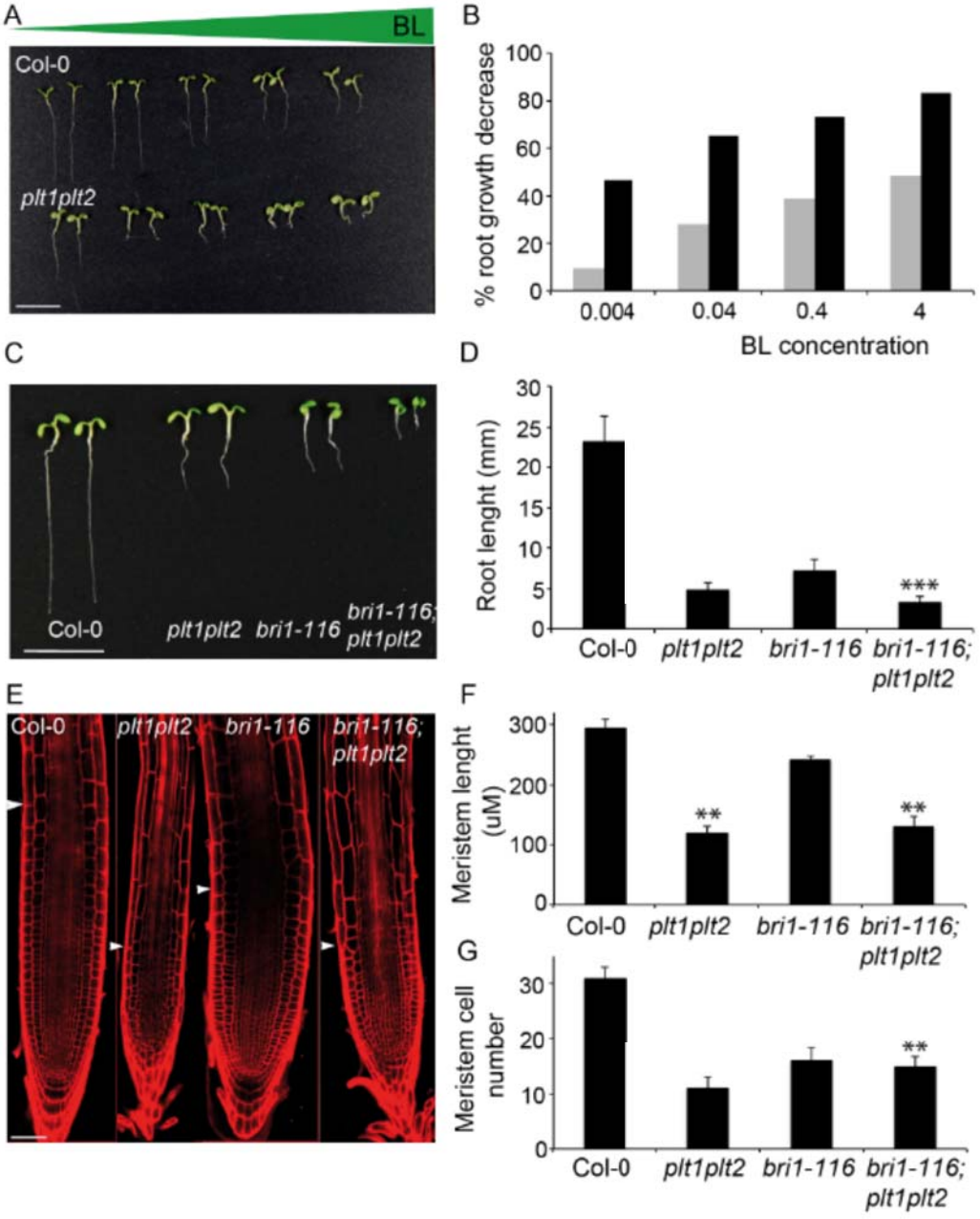

Figure 3. Genetic and physiological responses of *plt1plt2* to BRs.

(A) Six-day-old seedlings of Col-0 and *plt1plt2*, from left to right increasing concentrations of BL; 0.004, 0.04, 0.4, and 4 nM BL, respectively. (**B**) Root growth decrease (RGD) in Col-0 (grey bars) and *plt1plt2* (black bars), RGD was calculated comparing roots of treated plants with untreated six-day-old seedlings. (**C**) Six-day old seedling of the indicated genotypes. (**D**) Root length quantification of six-day-old seedlings, asterisk indicates (p-value<0.01 between *bri1-116plt1plt2* and *plt1plt2*). (**E**) Confocal images of six-day-old root meristems counterstained with PI of the indicated genotypes, white arrows

indicate the boundary between the meristematic and elongation zones of the root (**F**) Quantification of meristem length. (**G**) Quantification of meristem cell number, ***, ** indicates (p-value<0.01, p-value<0.05) respectively. Statistic analysis in D between *bri1-116plt1plt2* and *plt1plt2,* F between *plt1plt2, bri1-116plt1plt2* and *bri1-116;* G between *plt1plt2* and *bri1-116plt1plt2.* Scale bar A-C, E represents 1cm, 50 µM respectively.

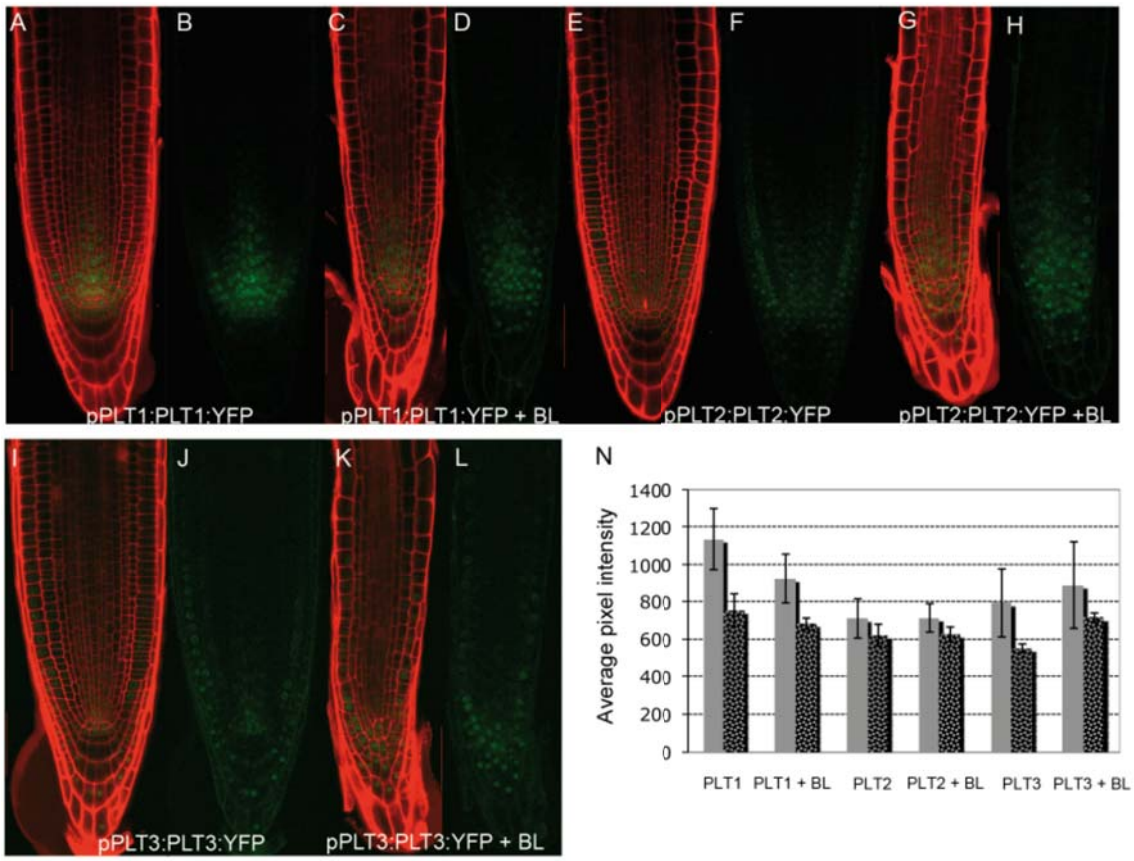

Figure 4. Exogenous BL application does not significantly modifies PLETHORA protein content nor localization.

(A-L) Six-day-old seedlings, (A, C, E, G, I, K), six-day-old seedlings counterstained with PI, (**A-D**) pPLT1:PLT1-YFP, (**E-H**) pPLT2:PLT2-YFP, (**I-L**) pPLT3:PLT3-YFP; without (**A, B, E, F, I, J**) and with (**C, D, G, H, K, L**) continuous 4nM BL treatment. (**N**) Quantification of GFP fluorescent signal. Grey bars represent quantification of the same 3 individual cells, QC cells; while black bars represents quantification of the same area in the stem cell niche.

Exogenous application of BL to *scr-4* mutants led to root growth decrease similar to *plt1plt2* mutants (Figure 5 A, B). Moreover *bri1-116;scr-4* double

mutants display a synergistic effect in root growth. Analysis of meristem length in *bri1-116;scr-4* double mutants and meristem cell number showed an epistatic relationships between BRI1 and SCR pathways in the maintenance of the meristem (Figure 5 C, D, E).

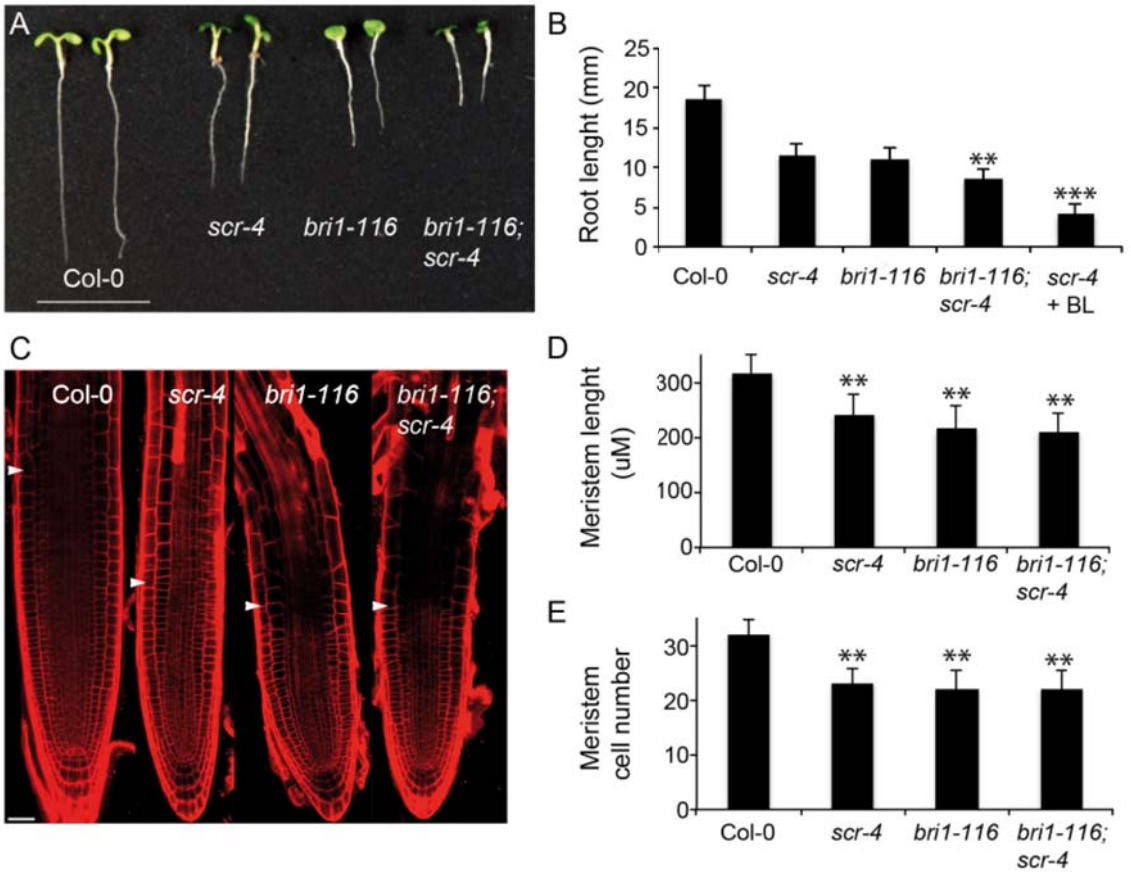

Figure 5. Genetic and physiological response of *scr-4* to BRs.

(A) Six-day-old seedlings of the indicated genotypes. Scale bar: 1 cm. (**B**) Quantification of root length of six-day-old seedlings. (**C**) Six-day-old meristems counterstained with PI, white arrows indicate the boundary between the meristematic and elongation zones of the root. Scale bar 25 µm (**D**) Quantification of meristem length in six-day-old seedlings. (**E**) Quantification of meristem cell number in six-day-old seedlings. ** and *** indicates p-value<0.05 and p-value<0.01 respectively. Statistic analysis in B performed between *bri1-116* and *bri1-116;scr-4*, *scr-4* and *scr-4* + BL. Statistic analysis in D-E all genotypes compared to WT (Col-0).

BRs promote the differentiation of distal columella stem cells

To study the functional relevance of the misexpression of QC-associated genes in BR-related mutants, we investigated whether columella stem cells (CSCs) undergo abnormal differentiation visualizing starch granules accumulation (Truernit et al., 2008). In agreement with previous results (Stahl et al., 2009), 80% of the wild-type plants had a single layer of CSCs and 18% two layers adjacent to the QC (Figure 6 A, F), whereas 100% of the *bri1-116* roots had a single layer of CSCs (Figure 6 B, F). While starch granules are absent from wild-type CSCs and only appear in differentiating columella cells, starch granules occurred in cells at the position of CSCs in *bes1-D* roots (Figure 6 C, F) and in plants treated with 4 nM BL (Figure 6 E, F). Conversely, no significant changes in columella stem cells differentiation where observed in *bzr1-D* mutants (Figure 6 D, F). These results show that BRs promote the differentiation of distal stem cells at the root apex downstream of BES1, revealing the importance of BRs in the control of root stem cell dynamics.

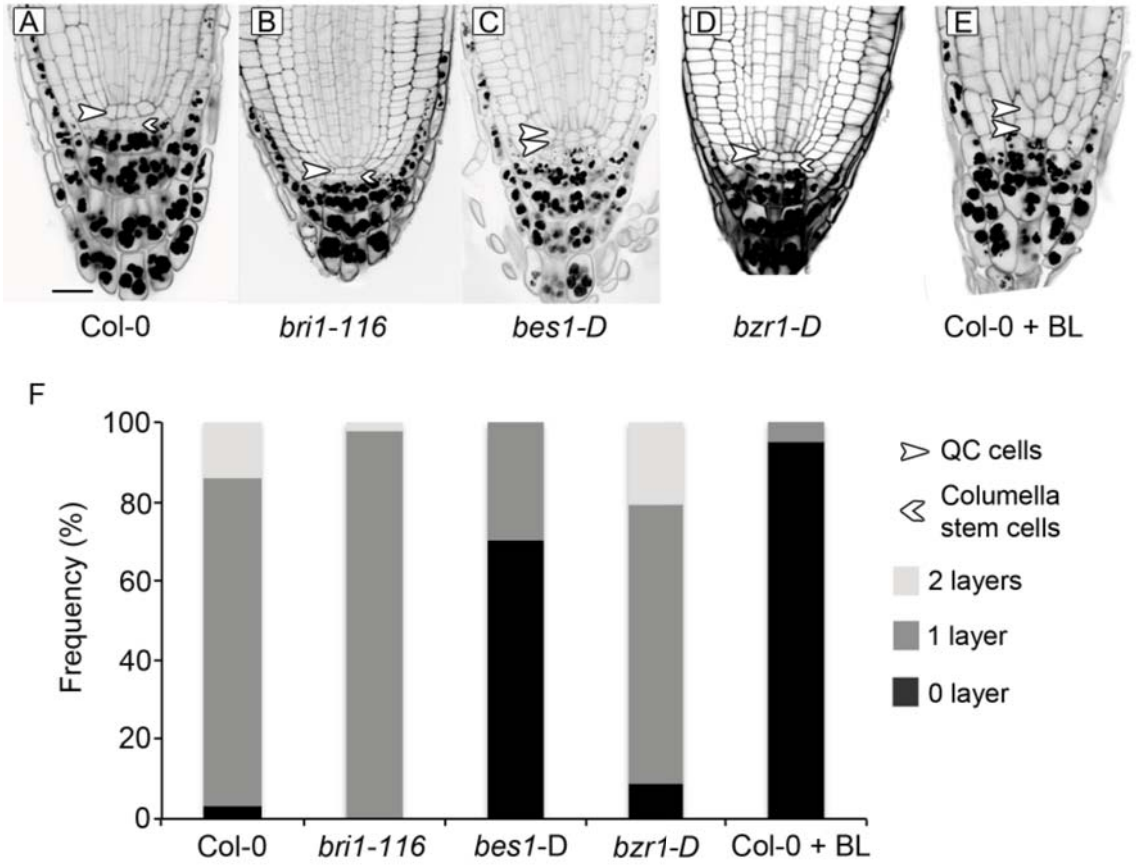

Figure 6. Brassinosteroids promote differentiation of distal stem cells.
(A-E) Confocal images of **s**ix-day-old primary root tips mPS-PI stained**, (A)** Col-0 (wild-type), **(B)** *bri1-116*, **(C)** *bes1*-D, **(D)** *bzr1*-D and **(E**) Col-0 treated continuously with 4nM BL. **(F)** Quantitative analysis of the effects of BRs in CSC differentiation. Frequency distribution of the number of cell layers is given between the QC and the first differentiated columella cells that contain starch granules (n>50 in each genotype). Scale bar represents 20μM.

To unveil the genetic relationship between BR signaling and key components of the root stem cell niche maintenance, a genetic and physiological analysis of mutants with impaired maintenance of columella stem cells was done. The null *bri1-116* mutants were crossed to the null *scr-4* mutants (Fukaki et al., 1998), the *wox5-1* (Sarkar et al., 2007), the double *plt1plt2* mutants (Aida et al., 2004), and the RBR knock down lines (*Rbi*) (Wildwater et al., 2005). Six-day-old double *bri1-116;scr-4*, *bri1-116;wox5-1, bri1-*

116;plt1plt2 mutant roots showed CSC phenotypes identical to those of the *scr-4, wox5-1, plt1plt2* single mutants (Figure 7 A, B, D, J, K, M, U). Similarly, double mutants of *bes1-D;scr-4, bes1-D;wox5*-1 phenotypes resembles *scr-4* and *wox5-1* single mutants, respectively (Figure 7 A, B, N, O, U). Exogenous treatment with BL triggered root exhaustion in *scr-4* and *plt1plt2* mutants, while *wox5-1* mutants showed a total lack of columella stem cells (Figure 7 E, F, H, I, U). Furthermore, overexpression of WOX5 led to supernumerary stem cell production (Sarkar et al., 2007), which persists after exogenous treatment of BL (Figure 9 Q, R, S, U), suggesting that WOX5 acts downstream of BRs in the control of CSC differentiation.

Downstream of the SCR patterning gene, the RBR canonical pathway controls the amount of stem cells (Wildwater et al., 2005). In one hand, supernumerary stem cells in *Rbi* knock down seedlings were abolished by exogenous BL application, displaying a number of layers similar to wild-type but with increase number of double layers (Figure 7 C, G, U). Similarly, *bes1-D;Rbi* double mutants had a decrease in the number of supernumerary stem cells(Figure 7 P, U). In the other hand, *bri1-116;Rbi* double mutants showed an increased in the number of CSC comparing to *bri1-116* single mutant (Figure L, U).

The genetic and physiological analyses suggest that BRs control the CSC differentiation upstream of SCR, PLT and WOX5 pathways. In contrast, BRs act in part independently or downstream of RBR.

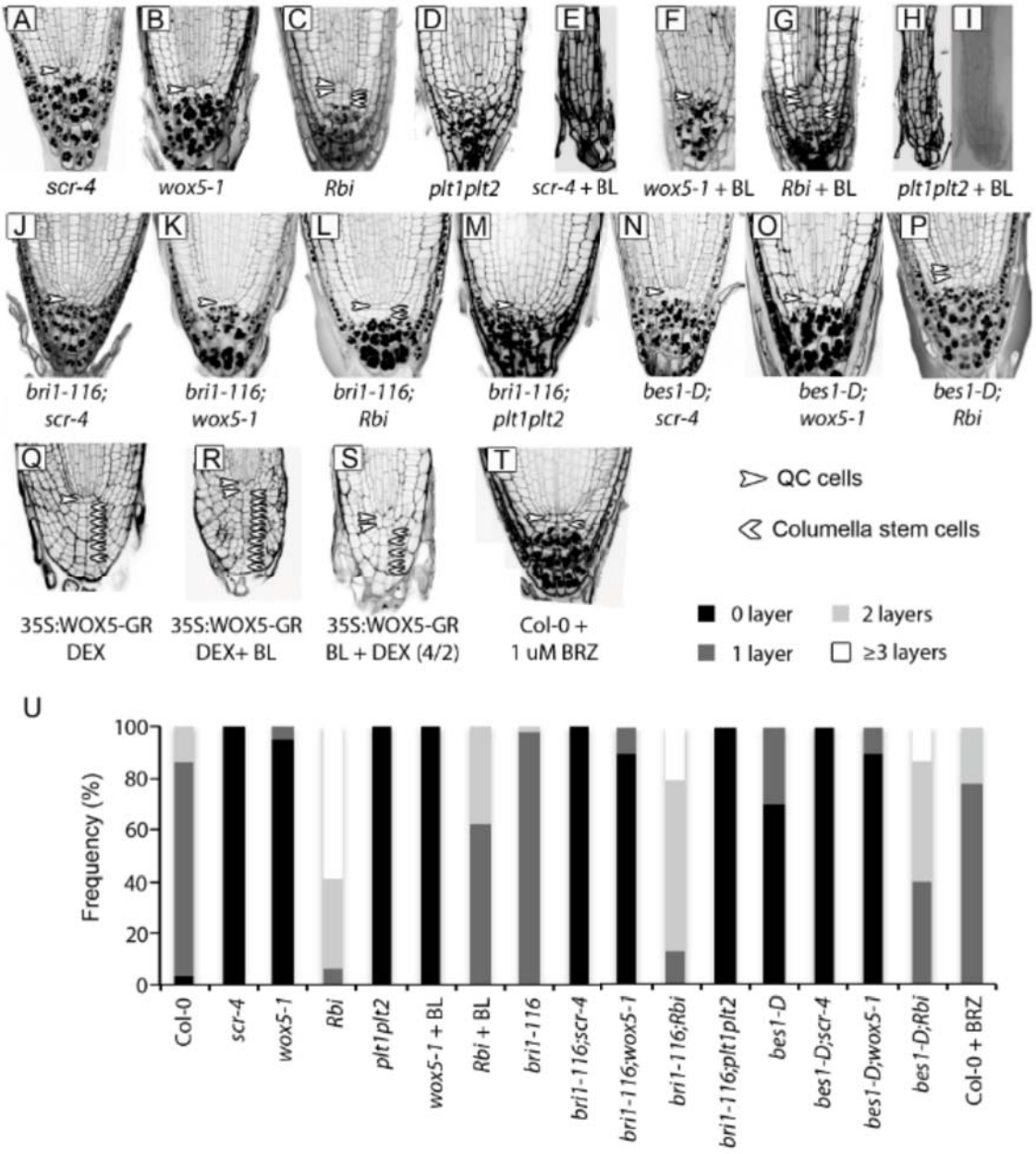

Figure 7. Genetic and physiological analysis of stem cell/patterning components with BR-signaling mutants.

(A-T) Confocal images of mPS-PI six-day-old primary roots stained. (**A**) *scr-4*, (**B**) *wox5-1*, (**C**) *Rbi*, (**D**) *plt1plt2*, (**E**) *scr-4* BL treated, (**F**) *wox5-1* BL treated, (**G**) *Rbi* BL treated, (**H, I**) *plt1plt2* BL treated, (**J**) *bri1-116;scr-4*, (**K**) *bri1-116;wox5-1*, (**L**) *bri1-116;Rbi*, (**M**) *bri1-116;plt1plt2*, (**N**) *bes1-D;scr-4*, (**O**) *bes1-D;wox5-1*, (**P**) *bes1-D;Rbi*, (**Q**) 35S:WOX5-GR continuously treated with 1 µM of dexamethasone (DEX). (**R**) 35S:WOX5-GR treated with 1 µM of DEX and 4 nM BL continuously, (**S**) 35S:WOX5-GR treated 4 days with 4 nM BL and 2 days with both DEX and BL. (**T**) Col-0 plants treated with 1 µM of brassinazole (BRZ220).

(**U**) Frequency distribution of the number of cell layers given between the QC and the first differentiated columella cells that contain starch granules.

BRI1-mediated signaling appears to control stem cell homeostasis downstream or independently of auxin

Auxins control multiple developmental processes, including root patterning and root cell division and elongation (Overvoorde et al., 2010). Local auxin levels mediated by both transport and biosynthesis play critical roles in CSC maintenance by the signaling circuit auxin-WOX5-PLT1(Ding and Friml, 2010). Moreover, pharmacological treatment with auxin (1-naphthaleneacetic acid (NAA)) or auxin transport inhibitor (*N*-1-naphthylphthalamic acid (NPA)), promotes an increased auxin level in the root tip and promoted CSC differentiation (Ding and Friml, 2010; Sabatini et al., 1999). To uncover whether BRs effects in the CSC differentiation were mediated by auxin we block auxin transport in *bri-116* plants with NPA. NPA treatment of Col-0 plants promotes CSC differentiation concomitantly to the increase levels of DR5:GUS (Figure 8 A-D). Interestingly, NPA treatment of *bri1-116* roots promote expansion of the DR5:GUS domain but did not promote CSC differentiation (Figure 8 E-H). Thus, the effect of BRs in the columella stem cell differentiation may be independent or downstream of auxin.

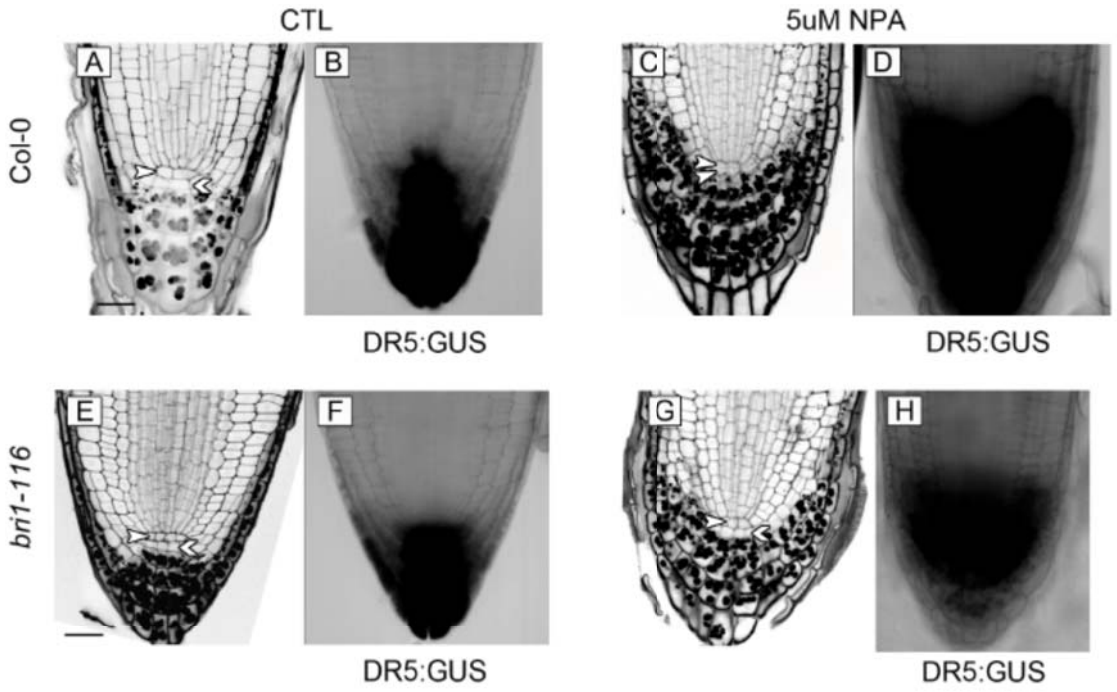

Figura 8. BRs maintain columella stem cell identity independently or upstream of auxins.

(A-H) Confocal images from six-day old seedlings mPS-PI stained. (**A**, **B**) DR5:GUS seedlings grown in control media. (**C**, **D**) DR5:GUS seedlings treated with 5µM NPA continuously. (**E**, **F**) *bri1-116*;DR5:GUS seedlings grown in control media. (**G**, **H**) *bri1-116*;DR5:GUS seedlings treated with 5µM NPA continuously. (A, C, E, G) Confocal images signal from the laser channel, while (B, D, F, H) images from the transmission channel. Right arrows indicate QC cells, left arrows indicates CSC. Scale bars represent 20µM.

BRI1 signaling controls root growth cell-autonomously from the dividing cells

Plant growth is achieved by integration of multiple signals, hormone-mediated growth have been shown to act in specific cell layers to control final organ size (Ubeda-Tomas et al., 2012). As an example, local expression of *gai* (non-degradable DELLA protein) in the endodermis have been shown to control gibberellin (GA) mediated growth (Ubeda-Tomas et al., 2009). Local expression of both BRI1 and the BR-biosynthetic enzyme CONSTITUTIVE PHOTOMORPHOGENESIS AND DWARFISM (CPD) in the epidermis is sufficient to recover dwarfism of BR loss-of-function mutants (Savaldi-Goldstein et al., 2007). In light of our results, we tested the contribution of BRI1-mediated signaling in the meristematic cells and to the slowly dividing QC cells by local expression of the BRI1 receptor under the RP5a promoter, which is specifically expressed in the meristematic dividing cells (Weijers et al., 2001). Expression of BRI1 in the meristematic cells of wild-type Col-0 plants, pRP5a:BRI1-YFP;Col-0, show not apparent effects on root growth, neither in meristem lenght nor cell number (Figure 9 A, C-D, J, K, L). In contrast, pRP5a:BRI1-YFP expression in the *bri1-116* recovered root growth to WT levels by increasing the meristem activity (length, cell number) (Figure 9 A-B, E-F, J, K, L). Thus, BRI1-mediated signaling controls root growth non-cell autonomously from the meristematic dividing cells. Moreover, the dwarf phenotype of the shoot-apical meristem of *bri1-116* mutants was also recovered (Figure 9 I). The recovery of the aerial part in pRP5a:BRI1-YFP;*bri1-116* was not comparable to Col-0 plants (Figure 9 I), suggesting that BRI1-mediated signaling in other cell-types is also necessary. Notably the expression of RP5a was confined to the basal part root meristem, suggesting that not all meristematic cells are dividing.

Expression of BRI1 receptor under the QC specific promoter WOX5 (Sarkar et al., 2007), did not have any effect on Col-0 plants nor *bri1-116* (Figure 9 A-B, G-H). Interestingly, exogenous treatment of pWOX5:BRI1-YFP;*bri1-116* with BL promoted meristem length shortening and meristem cell number reduction

(Figure 10). Thus, local effect of the BRI1 receptor in the QC cells may be attenuated, while an excess of ligand (BL application) may overcome this attenuation exerting a non-cell-autonomous control of the meristematic activities.

BRI1-mediated signaling undergoes continuous endocytosis (Geldner et al., 2007), and endocytosis impairment enhances BR-signaling (Irani et al., 2012). All endocytic studies have been carried out in the epidermis root cells due to its size and easy accessibility. In an attend to test whether similar processes are occurring in the QC cells we visualized BRI1 endocytosis in pWOX5:BRI1-YFP;*bri1-116*. QC cells expressing the BRI1 receptor under the WOX5 promoter undergo internalization and colocalizes with FM4-64 (Figure 11), suggesting that in the QC cells BRI1 receptor undergo endocytosis.

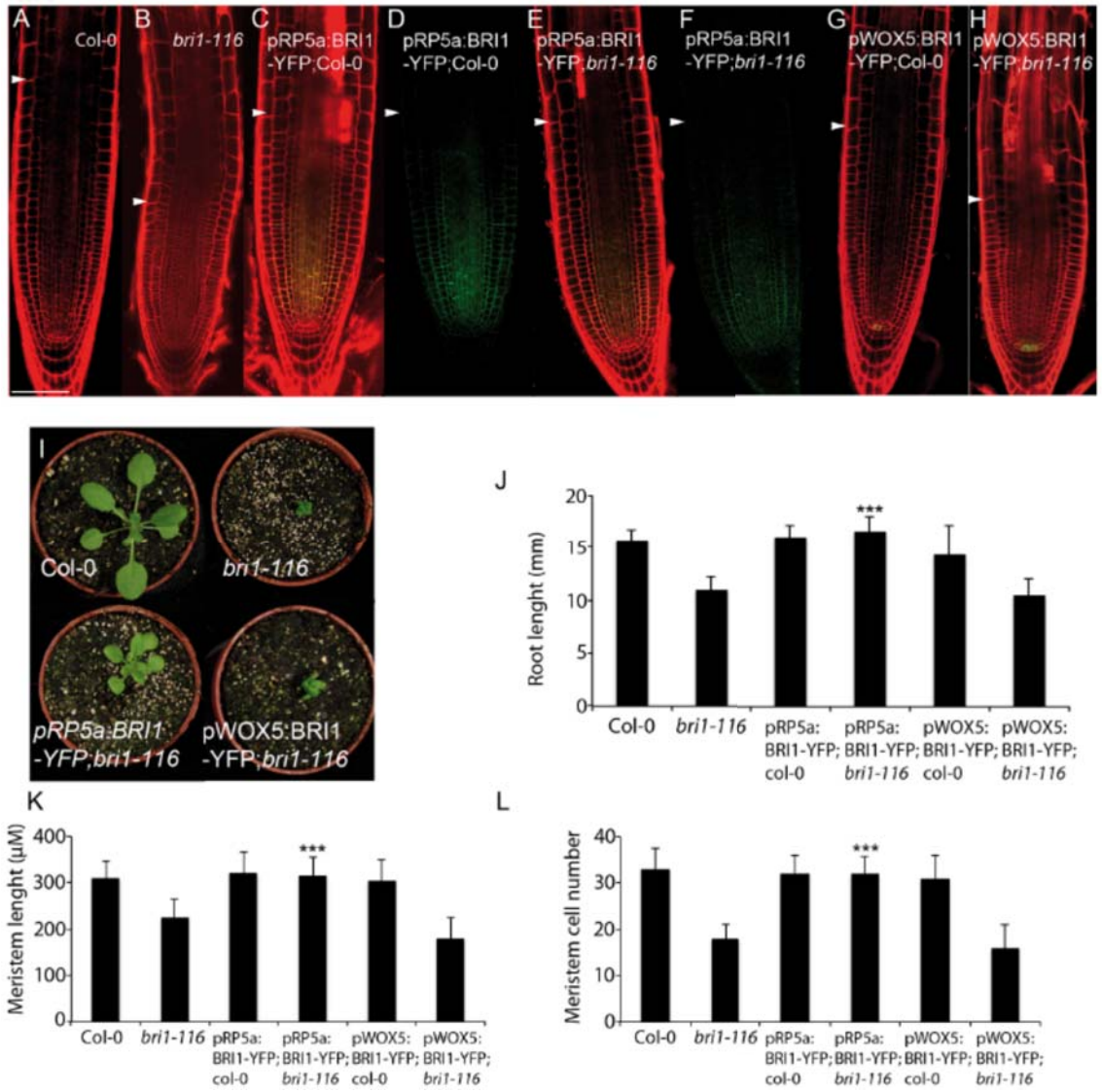

Figure 9. BRI1-mediated signaling control growth non-cell autonomously from the dividing cells.

(**A-H**) Six-day-old seedlings counterstained with PI. (**A**) Col-0, (**B**) *bri1-116*, (**C-D**) pRP5a:BRI1-YFP;Col-0, (**E-F**) pRP5a:BRI1-YFP;*bri1-116*, (**G**) pWOX5:BRI1-YFP;Col-0, (**H**) pWOX5BRI1-YFP;*bri1-116*. white arrows indicate the boundary between the meristematic and elongation zones of the root, scale bar :50 µM. (**I**) 20 day-old seedlings of the indicated genotypes. (**J**) Root length quantification of six-day-old seedlings. (**K**) Meristem length quantification of six-day-old seedlings. (**L**) Meristem cell number quantification of six-day-old seedlings. *** indicates p-value<0.01 between *bri1-116* and pRP5a:BRI1-YFP;*bri1-116*.

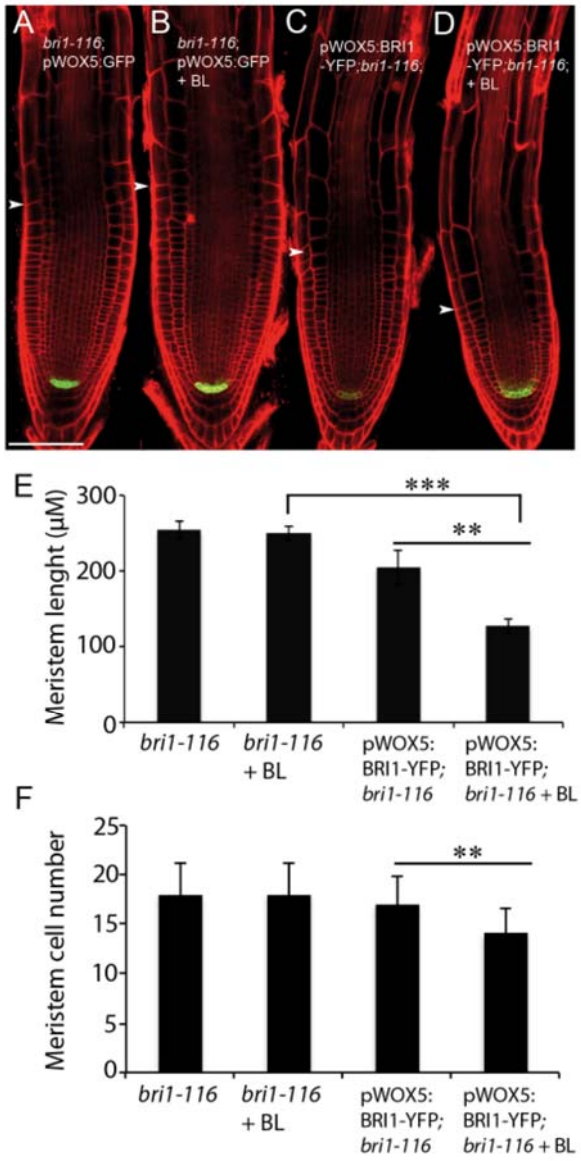

Figure 10. Local activation of BR-signaling in the QC cells controls meristem size non-cell autonomously.

(A-D) Six-day-old seedlings counterstained with PI. (**A**) *bri1-116*;pWOX5:GFP, (**B**) *bri1-116*;pWOX5:GFP continuously treated with 4nM BL, (**C**) pWOX5:BRI1-YFP;*bri1-116,* (**D**) pWOX5:BRI1-YFP;*bri1-116* continuously treated with BL. White arrows indicate the end of the meristematic zone. (**E**) Quantification of the meristem cell length of six-day-old seedlings. (**F**) Quantification of the meristem cell number of six-day-old seedlings. ** or *** indicate p-value<0.05 and p-value<0.01 respectively. Scale bar indicates 50µM.

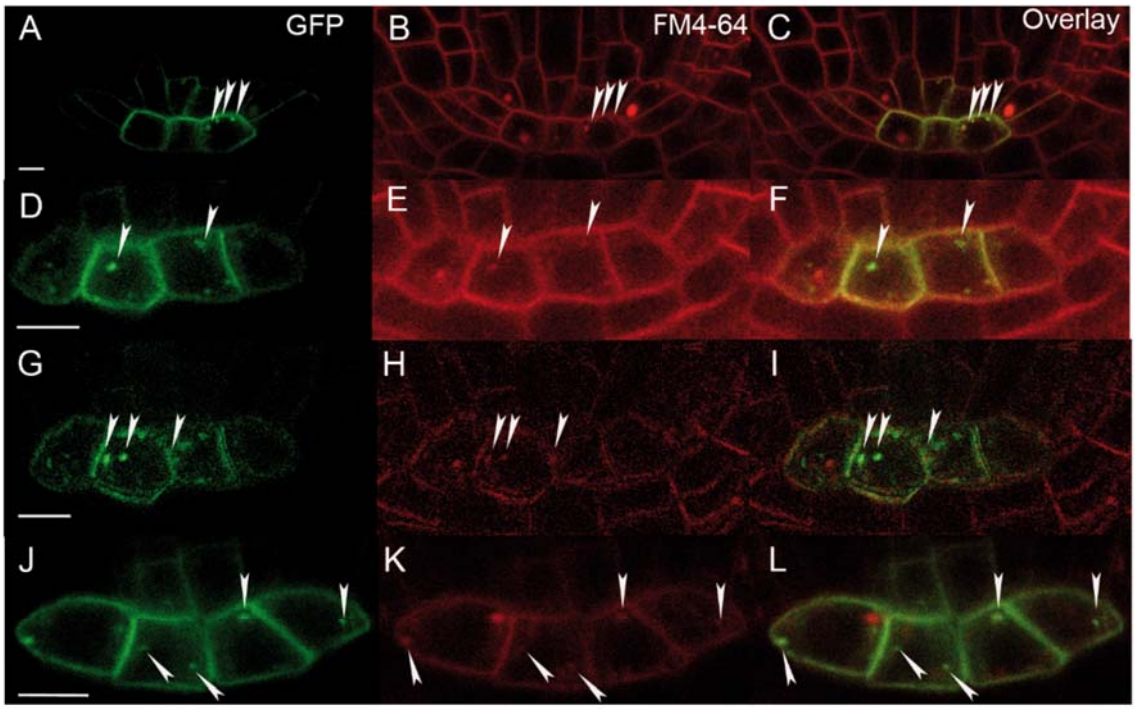

Figure 11. Endocytosis of BRI1 receptor in QC cells.

(A-L) Confocal images of QC cells from pWOX5:BRI1-YFP;*bri1-116* six-day-old primary roots. (**A, D, G, J**) GFP channel, (**B, E, H, K**) FM4-64 channel, (**C, F, I, L**) Overlay of GFP channel and FM4-64 channel. Scale bars: 5 µM.

BRI1-mediated signaling controls columella stem cell differentiation non-cell autonomously from the QC cells

Maintenance of the stem cell niche lies on proper signaling from the rarely dividing QC cells to the surrounding stem cells (van den Berg et al., 1997). Our results showed that BRs promote CSC differentiation. To further understand the spatial requirement of BRs to promote stem cell differentiation we used our local expression lines pWOX5:BRI1-YFP;bri1-116, in combination with published lines that express BRI1 in the epidermis (pGL2:BRI1-YFP;*bri1-116*), the endodermis and QC cells (pSCR:BRI1-YFP;*bri1-116*) and in the stele (pSHR:BRI1-YFP;*bri1-116*) (Hacham et al., 2011).

Exogenous treatment of Col-0 seedlings with BL promotes both QC divisions and CSC differentiation, while *bri1-116* mutants remain insensitive (Figure 12 A-D, M). Expression of BRI1 in the GL2, SCR, SHR and WOX5 domain in *bri1-116* mutant background, did not change neither the QC cells nor the columella stem cell differentiation that remain similar to *bri1-116* (Figure 12 E-H, M). BL application to *bri-116* mutant plants harboring functional BRI1 in; the QC cells (pWOX5:BRI1-YFP;*bri1-116)* or the endodermis and QC cells (pSCR:BRI1-YFP;*bri1-116*) promote CSC cell differentiation (Figure 12 K, L, M). In contrast, plants expressing BRI1 in the epidermis (pGL2:BRI1-YFP;*bri1-116*) or in the stele (pSHR:BRI1-YFP;*bri1-116*) did not undergo CSC differentiation after BL treatment (Figure 12 I, J, M). Surprisingly, no QC divisions appeared in none of the treated plants, except for wild-type plants, suggesting that BRI1 signaling does not control QC divisions cell autonomously. Altogether, these results indicate that local activation of BRI1 signaling in the QC cells promotes CSC differentiation.

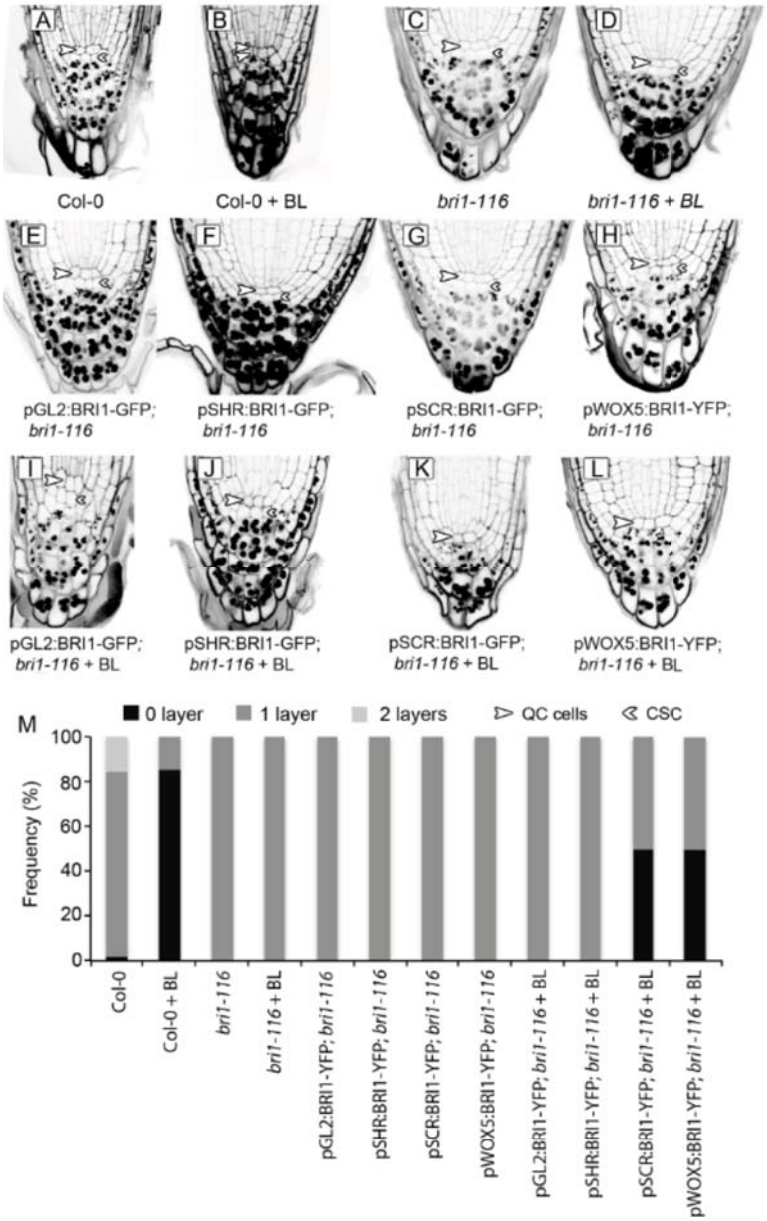

Figure 12 BRI1- mediated signaling control columella stem cells differentiation from the QC cells non-cell autonomously.

(**A-L**) Confocal images of six-day-old primary roots mPS-PI stained. (**A**) Col-0 (wild-type) untreated, (**B**) treated with 4 nM BL continuously. (C) *bri1-116* untreated and (**D**) treated with 4 nM BL continuously. (**E**) pGL2:BRI1-YFP;*bri1-116* untreated and (**I**) treated with 4nM BL continuously, (**F**) pSHR:BRI1-YFP;*bri1-116* untreated and (**J**) treated with 4 nM Bl continuously. (**G**) pSCR:BRI1-YFP;*bri1-116* untreated and (**K**) treated with 4 nM BL continuously. (**H**) pWOX5:BRI1-YFP;*bri1-116* untreated and (**L**) treated with 4 nM BL continuously. (**M**) Frequency distribution of the number of cell layers given

between the QC and the first differentiated columella cells that contain starch granules.

Local expression of BES1-D in the QC promotes QC divisions

Cell-cell communication is an essential feature that integrates stimuli to further process of developmental responses. In a single cell resolution, signaling pathways can be attenuated at multiple levels, ie. receptor level, repression of downstream responding factors. Our results showed that BRs promote QC divisions, but local expression of BRI1 in the QC cells fail to promote QC divisions. Thus, we reasoned that the absence of QC divisions can be due to (i) the attenuation of BRI1-signaling at the receptor level, or (ii) the requirement of a non-cell autonomous signal from other cell types. To discard the first possibility we express the neomorphic BES1-D mutant protein, constitutively active, under the control of WOX5, a QC specific promoter. Analysis of pWOX5:BES1-D-YFP;Col-0 revealed no changes in root length nor meristem length (Figure 13 A-C). In contrast, QC cells from pWOX5BES1-D-YFP;Col-0 lines were divided, compared to wild-type plants (Figure 13 D-G). In addition, short BL treatment (22h) did not altered pWOX5:GFP expression, while it activates hyper-proliferation of QC cells in pWOX5:BES1-D-YFP;Col-0 (Figure 13 H-K). Together, local activation of BR-signaling in the QC cells activates cell divisions, suggesting that QC cells lack BR signaling.

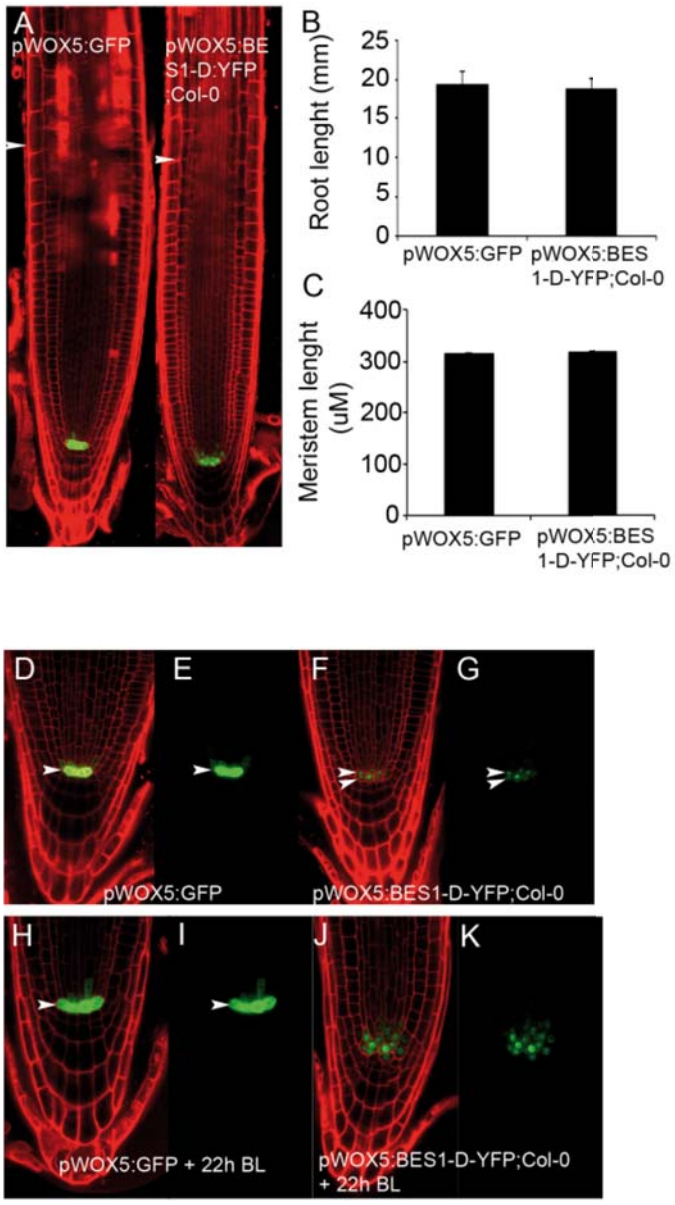

Figure 13. Local activation of BR signaling promotes QC divisions.

(A, D-K) Confocal images of six-day-old seedlings, counterstained with PI. (A) Col-0 and pWOX5:BES1-D-YFP;Col-0. (B) Root length quantification, (C) Meristem length quantification. (D-E) pWOX5:GFP expression, (H-I) pWOX5:GFP expression after 22 hours BL treatment. (F-G) pWOX5:BES1-D-YFP;Col-0, (J-K) pWOX5:BES1-D-YFP;Col-0 after 22 hours BL treatment.

CHAPTER 3

RESULTS

MECHANISTIC BASIS FOR THE BRASSINOSTEORID-MEDIATED QUIESCENT CENTRE CELL DIVISIONS

SUMMARY

The quiescent center (QC) maintains the activity of the surrounding stem cells within the root stem cell niche, yet specific molecular players sustaining the low rate of QC cell division remain poorly understood. Here, we identified a R2R3-MYB transcription factor, *BRAVO (BRASSINOSTEROIDS AT VASCULAR AND ORGANIZING CENTRE),* acting as cell-specific repressor of QC divisions in the primary root of Arabidopsis. Ectopic BRAVO expression restricts overall root growth and ceases root regeneration upon damage of the stem cells, demonstrating the role of BRAVO in counteracting Brassinosteroid (BR)-mediated cell division in the QC cells. Interestingly, BR-regulated transcription factor BES1 (BRI1-EMS SUPRESSOR 1) directly repress and physically interacts with BRAVO *in vivo*, creating a bistable switch that modulates QC divisions at the root stem cell niche. Together, our results define a mechanism for BR-mediated regulation of stem cell quiescence in plants.

INTRODUCTION

Cellular quiescence is a temporary and reversible cell cycle arrest characterized by programmed events that avoid proliferation. However, in eukaryotes, the molecular determinants for the quiescent state remain unknown (Cheung and Rando, 2013). Self-renewal of quiescent cells acts as a replenishment source, e.g. in the hematopoietic case it ensures long term maintenance of multipotent stem cells throughout the organismal lifespan (Wilson and Trumpp, 2006). In plants, the root stem cell niche is composed of different sets of stem cells that give rise to specific root cell lineages, which are surrounding a group of cells with low proliferation rate termed quiescent centre (QC) (Scheres, 2007) (Figure 1 A). The QC cells maintains the stemness of neighbouring cells, which functions as a major signaling hub maintaining the proliferation/differentiation rates (Cheung and Rando, 2013; Scheres, 2007), where retinoblastoma (RBR) plays an autonomous control in the regulation of QC division (Cruz-Ramírez et al., 2013; Wachsman et al., 2011). The proper balance between quiescence and proliferation ensures organismal longevity and prevents both genetic damage and stem cell exhaustion (Cheung and Rando, 2013; Stuart and Brown, 2006).

Plant steroid hormones, Brassinosteroids (BRs), are essential regulators of plant architecture, growth and development (Zhu et al., 2013). BR perception through the plasma membrane-localized BRASSINOSTEROID INSENSITIVE 1 (BRI) (Clouse et al., 1996; Kinoshita et al., 2005; Li and Chory, 1997; Wang et al., 2001), a Leucine-Rich-Repeat Receptor-Like-Kinase (LRR-RKL) protein, results in mutual transphosphorylation events with several SOMATIC EMBRYOGENESIS RECEPTOR KINASE (SERK) coreceptors (Karlova and de Vries, 2006; Li et al., 2002; Russinova et al., 2004). Downstream of BRI1 and SERKs, the members of BRASSINOSTEROID SIGNALING KINASE (BSK) cytoplasmatic kinase family are phosphorylated by BRI1 (Tang et al., 2008). Subsequent, BSK activation triggers inactivation of BRASSINOSTEROID INSENSITIVE 2 (BIN2) (Tang et al., 2008) kinase which promotes nuclear

translocation of BRI1 EMS SUPPRESSOR 1 (BES1) and BRASSINAZOLE-RESISTANT1 (BZR1) (Wang et al., 2002; Yin et al., 2002) to the nucleus where they regulate gene expression (Sun et al., 2010; Yu et al., 2011). Despite the high degree of knowledge existing on the BR-signaling components, how the regulatory events downstream of BES1 and BRZ1 are translated into specific developmental outputs remain poorly understood.

The majority of the BR signaling components are ubiquitously expressed in the plant, yet cell-specific components have not hitherto been identified. The local action of BRs in stomata patterning and establishment of organ boundary (Bell et al., 2012; Gendron et al., 2012; Gudesblat et al., 2012; Kim and Wang, 2010) argues for cell specific BR pathways in different organs. In the primary root, BRs are essential regulators of growth and development (Fabregas et al., 2013; González-García et al., 2011; Müssig et al., 2003; Zhu et al., 2013). BRs promote the division of QC cells at the root stem cell niche, suggesting that counteracting BR-signaling is a mechanism to preserve the low rates of cell division in the QC (González-García et al., 2011; Heyman et al., 2013), yet cell-specific repressors of the BR pathway remain to be identified.

In this study, we used a cell-based transcriptomic approach to identify novel cell-specific regulators of the BR-mediated signaling in the root stem cell niche. We report the identification of a R2R3-MYB transcription factor, *BRAVO (BRASSINOSTEROIDS AT VASCULAR AND ORGANIZING CENTRE),* acting as cell-specific repressor of BR-mediated divisions in the stem cell niche of the Arabidopsis root. We have found that BRAVO overexpression under an inducible promoter represses root growth leading to root growth exhaustion upon genotoxic stress. BES1 directly represses and physically interacts with BRAVO *in vivo*, creating a bistable circuit that modulates the rate of QC divisions at the root stem cell niche. Our study unveils that the BRAVO/BES1 signaling module defines a novel mechanism for BR-mediated regulation of stem cell quiescence in plants.

PREVIOUS RESULTS OBTAINED AT OUR LABORATORY

A cell-specific transcriptomic approach was conducted to obtain a spatio-temporal map of the transcriptional response of BRs in the stele. Primary roots of the stele maker *WOODEN LEG* (pWOL:GFP) (Mähönen et al., 2000) were treated with 10 nM Brassinolide (BL, the most active BR compound) at 0.5, 1, 2 and 4 hours. The stele cells were isolated by fluorescent-activated cell sorting (FACS) of green fluorescent protein (GFP) and subjected to microarrays analysis (Birnbaum et al., 2005). Further analysis revealed a total of 309 significantly differentially regulated genes (fold change>1.5; p-value<0.01). Time-course analysis showed a peak of 120 deregulated genes after 2 hour BL treatment, whereas Gene-ontology (GO) enrichment analysis (López-Bigas et al., 2008) disclosed cell cycle, histone modification, gravitropism and phloem/xylem histogenesis among the most enriched categories, in agreement with previously described BR developmental responses (Zhu et al., 2013). To further refine our search the *CORONA/ATHB15* (Zhiponova et al., 2013) (*pAthb15:YFP*) marker that labels a few provascular meristematic cells was used to perform FACS and microarray analysis after 2 hour of BL treatment (724 genes, fold change>1.5; p-value<0.01).

BRAVO defines a BR-regulated transcription factor specific of root stem cells

We have previously describe the role of BRs in the control of stem cell homeostasis in the root apical stem cell niche (see chapter 2). Specifically, BRs regulate both the quiescent center (QC) divisions and the columella stem cell (CSC) differentiation (González-García et al., 2011), yet specific molecular components controlling this process remain unknown. To identify novel cell-specific BR-signaling components regulating the stem cell niche, we used a previously generated dataset of the BRs response in the stele cells labeled by WOL or ATHB15 (Mähönen et al., 2000; Zhiponova et al., 2013). Time-course analysis of the WOL domain showed a peak of deregulation 2 hour after BL treatment. In order to identify specific genes expressed in the stem cell niche we used previously publish cell-type specific transcriptomics of the root stem cell niche (Brady et al., 2007; Nawy et al., 2005). Moreover, to unsure that the BL-regulated genes were regulated by downstream BR-signaling components we used previously publish targets of both BES1 and BZR transcription factors (Sun et al., 2010; Yu et al., 2011). Venn-diagram comparison of genes enriched at; (i) the vascular initial/quiescent centre (QC), (ii) direct targets of BR-regulated BES1 and (iii) BZR1 transcription factors and (iv) WOL and ATHB15 genes deregulated identified a single gene that matched all criteria (Figure 1 B). The gene corresponds to an R2R3-MYB transcription factor, MYB56 (At5g17800), hereafter-renamed *BRAVO* (*BRASSINOSTEROIDS AT VASCULAR AND ORGANIZING CENTER*).

In agreement with the microarray data, BRAVO expression *(pBRAVO:GFP)* appeared to be specific to vascular initial and QC cells of the root apical meristem (Figure 1 C). BRAVO transcription was downregulated by BRs in a dose- and time-dependent manner (Figure 1 D, E; Figure 2 A-H, M). BRAVO repression appeared to be specific to BRs, since exogenous treatments with different plant hormones previously related to root stem-cell maintenance failed to significantly modify its expression (Figure 2 K-M). Similarly, BRAVO protein (*pBRAVO:BRAVO-GFP*) was localized at the nuclei of vascular initials

and QC cells and disappeared rapidly upon short-term BL treatment (Figure 1 F, G). The BR-activated *bes1-D* mutants that accumulates the active (dephosphorylated) form of BES1 (BES1-D) (Yin et al., 2002) exhibited a dramatic reduction in BRAVO levels (Figure 1 C, H; Figure 2 N).. Collectively, these results show that the BRAVO locus defines a cell-specific component of the BR signaling pathway at the root stem cell niche of Arabidopsis.

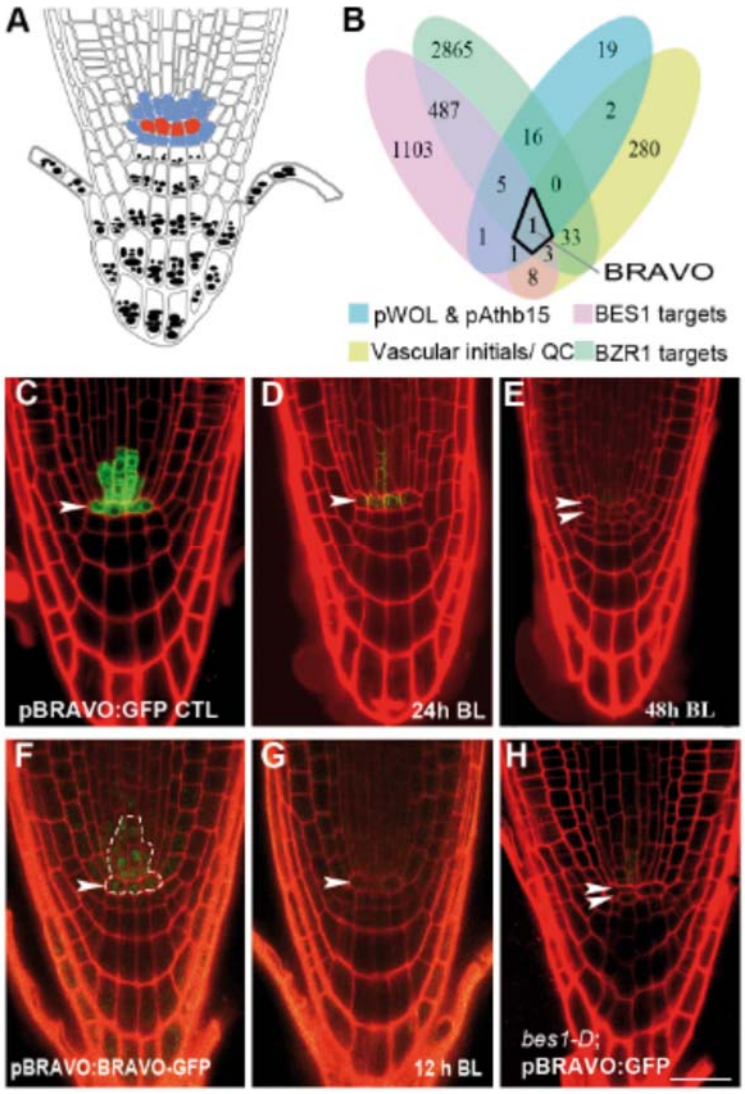

Figure 1. BRAVO encodes a R2R3-MYB transcription factor directly regulated by BR-regulated BES1 in the vascular initials and the QC cells. **(A)** Schematic representation of the root stem cell niche in *Arabidopsis thaliana* (Arabidopsis) stem cells (blue) surrounds QC cells (red)**.** (**B**) Venn diagram of the deregulated transcription factors in *pWOL:GFP* and *pAthb15:YFP*, enriched in the vascular initials /QC together with BES1 and BZR1 targets; only one gene

fit all criteria, i.e. *BRAVO*. (**C-H**) Six-day-old seedlings counterstained with propidium iodide (PI). (**C**) Expression of *pBRAVO:GFP* is restricted to the vascular initials and the QC cells (**D-E**) BL treatment of *pBRAVO:GFP*, 24 and 48 hours respectively promotes BRAVO repression. (**F-G**) BRAVO a nuclear transcription factor repressed after BL application. (**H)** Reduction of *pBRAVO:GFP* expression in the *bes1-D* mutant background. White arrows point QC cells. Scale bar in H represents 25*μ*m.

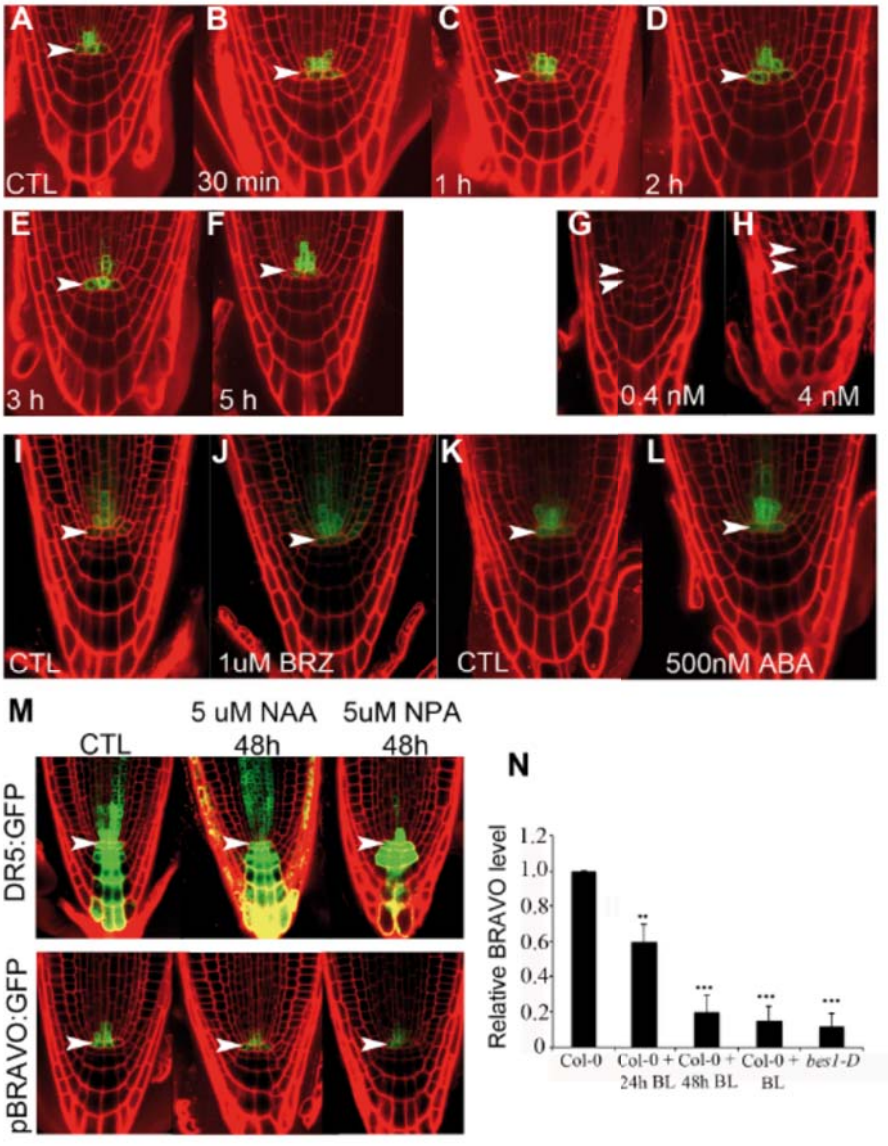

Figure 2. Brassinosteroids control BRAVO expression.

(**A-M**) Six-day-old seedlings counterstained with propidium iodide (PI). (**A-L**) *pBRAVO-GFP* control (**A**), 0.5 (**B**), 1 (**C**), 2 (**D**), 3 (**E**), 5 (**F**) hours after BL

treatment. (**G, H**) Continuous treatment with 0.4 and 4 nM BL. (**I-J**) continuous treatment with 1μM brassinazole (BRZ), (**K-L**) continuous treatment with 500nM ABA. (**M**) 48 hour treatment with NAA and NPA. (**N**) Relative *BRAVO* levels of Col-0, 24, 48 hours, continuous BL treatment and *bes1-D* root tips.(** p-value<0.05; *** p-value<0.005). White arrows point QC cells. Error bars ± SEM.

BRAVO is a negative regulator of QC divisions

Previous analyses established that BR-activated BRI1 and downstream BES1 signaling promotes the division of QC cells in the root stem cell niche (Chapter 2) (González-García et al., 2011), yet the mechanism for such regulation is not known. To determine whether the BR-signaling component BRAVO controls QC divisions, we analysed loss-of-function *bravo* mutants in the primary root apex. Two insertional mutants harbouring a T-DNA fragment in the second exon displayed a dramatic reduction in BRAVO transcriptional levels (Alonso, 2003)(Figure 3 A, B), thus being loss-of-function mutants. Microscopic analysis of primary root tips from six-day-old seedlings revealed a dramatically increased frequency of QC divisions in both *bravo* mutants as compared to Col-0 wild-type (WT) roots (70% vs. 15%; N>100 for each genotype). Moreover, QC divisions frequency was restored to wild-type levels with the expression of BRAVO under control of its endogenous promoter in *pBRAVO:BRAVO-YFP*; *bravo* plants. In contrast to the *bes1-D,* the *bravo* mutants did not exhibit defects in the distal stem cell differentiation (González-García et al., 2011)(Chapter 2 and Figure 1 C, E), indicating that BRAVO specifically functions as a local repressor of QC self-renewal in the primary root.

Defects in the QC patterning or identity have been shown to directly promote root growth defects (Sabatini et al., 2003), while in other cases over proliferation or premature differentiation of stem cells did not affect root growth (Sarkar et al., 2007; Wildwater et al., 2005). Root growth in *bravo* mutants was similar to wild-type plants (Figure 4 A, B). Moreover, analysis of six-day-old

meristems of *bravo* mutants did not reveal any difference compared to wild-type plants (Figure 4 C, D). Expression of BRAVO in the provascular cells could render defects in vascular organization of *bravo* mutants. Radial sections of six-day-old *bravo* roots did not reveal any defects in vascular organization compared to wild-type plants (Figure 4 E).

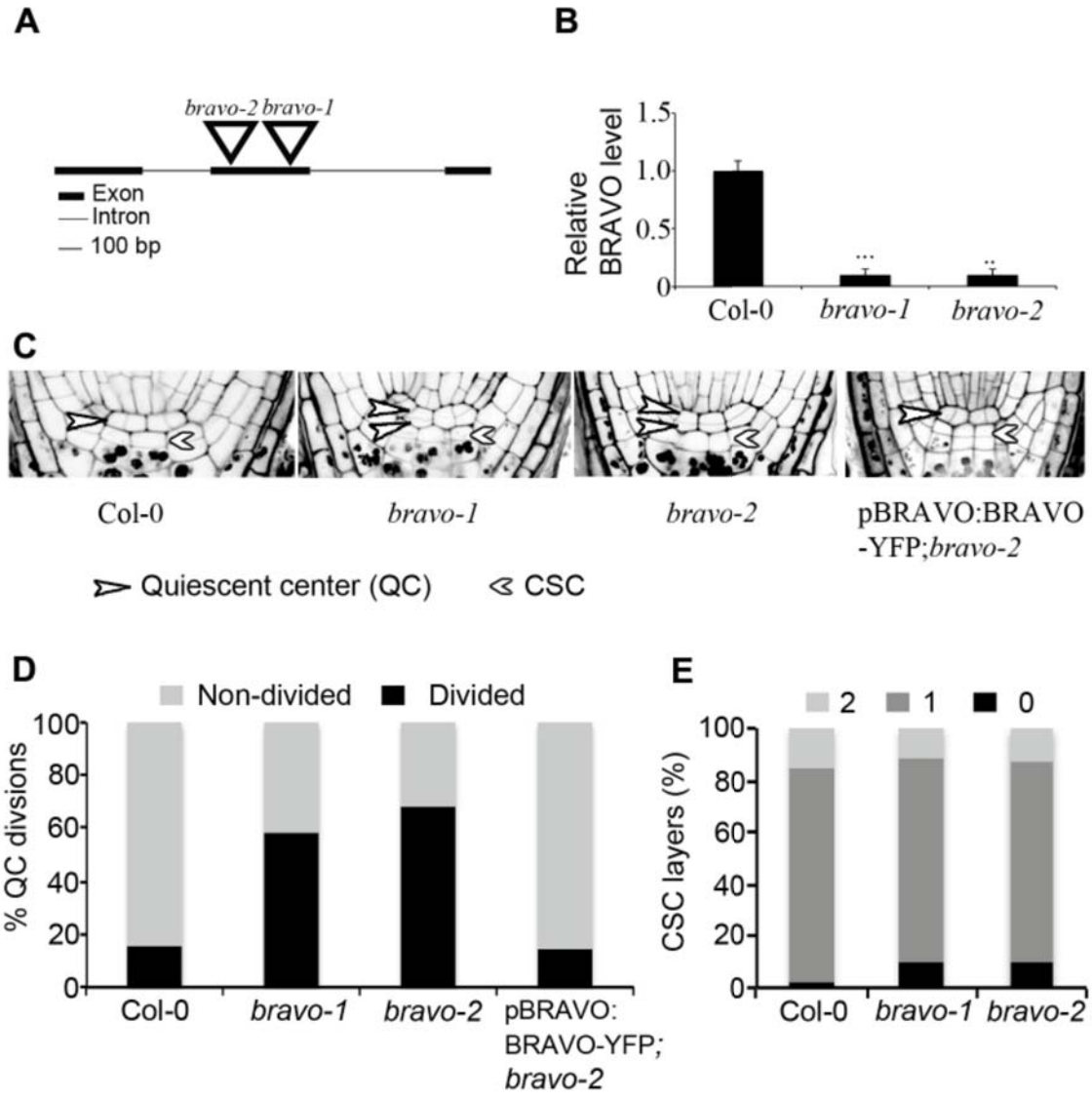

Figura 3. BRAVO represses QC cell divisions.

(**A**) Schematic representation of T-DNA insertions of *bravo* mutants in the BRAVO coding sequence. (B) Relative levels of BRAVO in Col-0 (wild-type) compared to *bravo* mutants. *** indicate p-value<0.001. (C) Confocal images of six-day-old primary root tips mPS-PI stained of the indicated genotypes. CSC

stands for columella stem cells. (D) Quantification of the frequency of QC divisions in six-day-old primary root tips of the indicated genotypes (n>100). (E) CSC layers distribution, represented by the number of cell layers given between the QC and the first differentiated columella cells that contain starch granules (n>100).

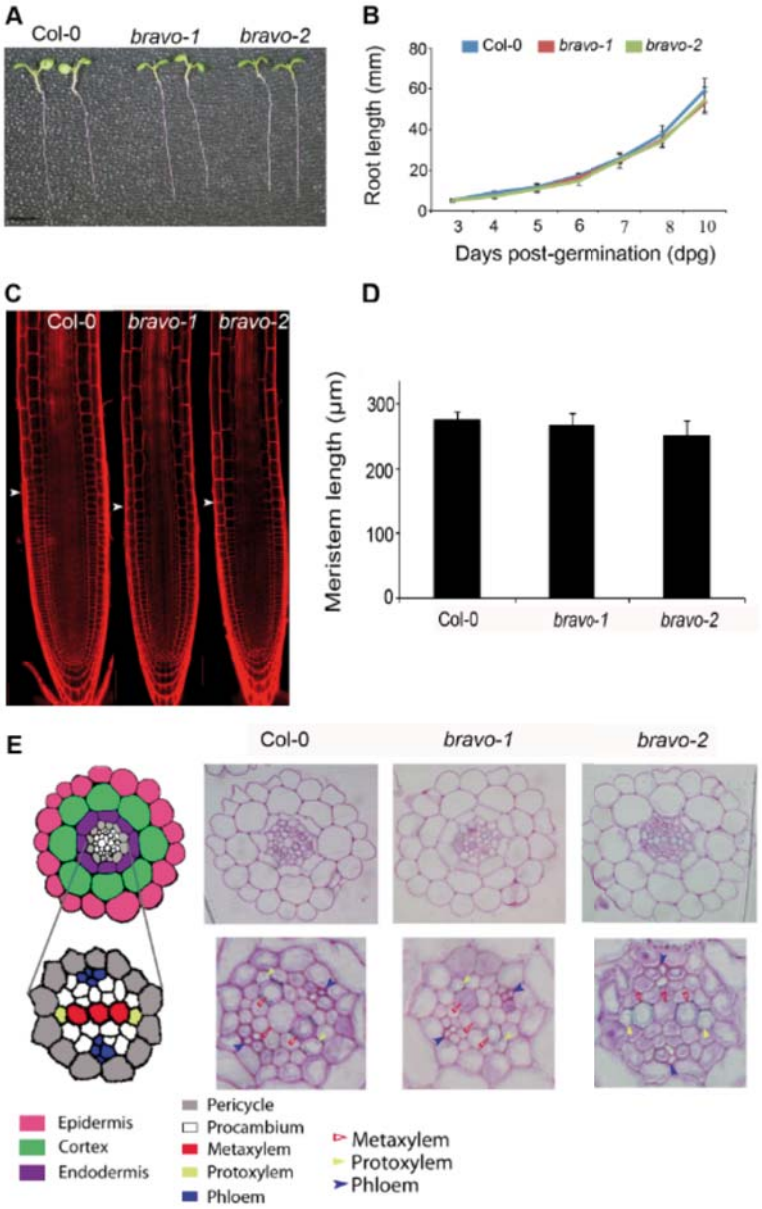

Figure 4*.* Phenotypic analysis of *bravo* mutants in the primary root.

(**A**) Six-day-old seedlings of Col-0, *bravo-1* and *bravo-2.* **(F)** Root length of Col-0, *bravo-1* and *bravo-2.* (**G**) Six-day-old roots counterstained with PI, white

arrow indicates the end of meristematic cells. (**H**) Quantification of meristem length of Col-0, *bravo-1* and *bravo-2.* (**I**) Transverse root sections of six-day-old col-0, *bravo-1*, *bravo-2* seedlings stained with toluidine blue. Error bars ± SEM.

To address the identity of the newly formed cells in *bravo* mutants, we used identity markers. Divided cells of *bravo* express the QC and endodermis identity marker SCARECROW (Sabatini et al., 2003). A progressive fade-out of SCR expression over time in the newly rootward QC cells indicates an asymmetric QC division (Figure 5 A-H)., The fade-out in GFP expression of the rootward QC cells loosing the QC identity progressively generates new columella stem cells , in agreement with previous work (Wachsman et al., 2011). Furthermore, QC markers *WUSCHEL-RELATED HOMEOBOX 5* (*WOX5*) (Sarkar et al., 2007) and *AGL42* (Nawy et al., 2005) were also present in the divided QC cells of *bravo* mutants, yet their expression appeared to be below wild-type levels (Figure 5 I-L, O, P). Moreover expression domains of the provascular markers pAthb15:YFP and pARF7:GFP did not change in the *bravo* mutant background (Figure 5). Altogether BRAVO represents a new negative regulator of QC divisions.

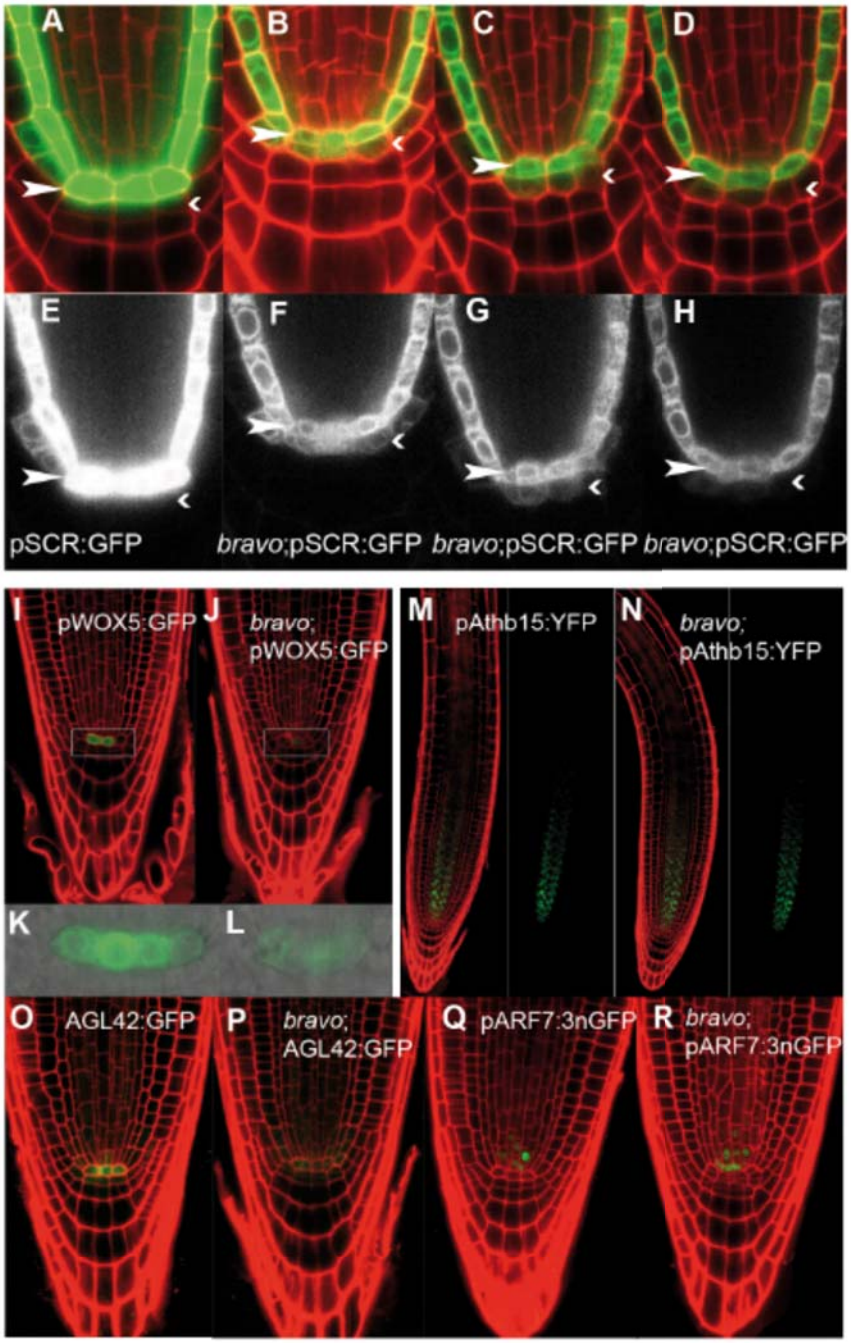

Figure 5. Asymmetric QC division on *bravo* mutants.

(**A-D**) Confocal images of primary roots from six-day-old seedlings counterstained with propidium iodide (PI), (**E-H**) GFP fluorescent signal in white. (**A, E**) pSCR:GFP, (**B-D**, **F-H**,) *bravo*; pSCR:GFP. Note the reduced GFP intensity of the lower QC cells. White arrows point QC cells (right), and newly formed QC cells (left). (**I, K**) *pWOX5-GFP*, (**J, L**) *bravo;pWOX5-GFP*. (**M**)

pAthb15-YFP$_{nls}$, (**N**) *bravo;*pAthb15-YFP$_{nls}$. (**O**) *ALG42-GFP*, (**P**) *bravo;*ALG42-GFP, (**Q***)* pARF7-3GFP$_{nls}$, (**R**) *bravo;*pARF7-3GFP$_{nls}$.

BRAVO loss-of-function mutant phenotype resembles that of plants with excess of BR-signaling, such as the gain-of-function *bes1-D* (70%) and plants exogenously treated with BL (0.04 nM; 70%; Figure 6 A-D, M). In agreement, the additional QC divisions observed in plants with excess of BRs appeared concomitantly with *BRAVO* downregulation (Figure 1 C-E). Next, we investigated whether QC divisions are downstream BES1 or BZR1 in the BR-pathway. Analysis of RNAi BES1 roots indicated that the BR-mediated QC divisions are downstream of BES1 (Figure 6 G-H, M). Notably, the mild QC defects in *bzr1*-D mutants together with the dramatically reduced BZR1 expression in the QC cells suggest that the BR-mediated QC divisions occur preferentially downstream BES1 (Figure 6 I, M, Q-S). Additionally, BES1 showed high expression in the QC cells (Figure 6 N-P). Analysis of *bes1-D*/*bravo* and *bri1-116/bravo* double mutants pointed to the requirement of active BES1 in order to promote QC divisions (Figure 6 J, K, M). In the same direction, local expression of BES1-D in the QC cells in *pWOX5:BES1-D-GFP,* displayed a stronger phenotype than *bravo* mutants. Thus, QC divisions are both activated by *BES1* and repressed by *BRAVO* to preserve quiescence in the root meristem.

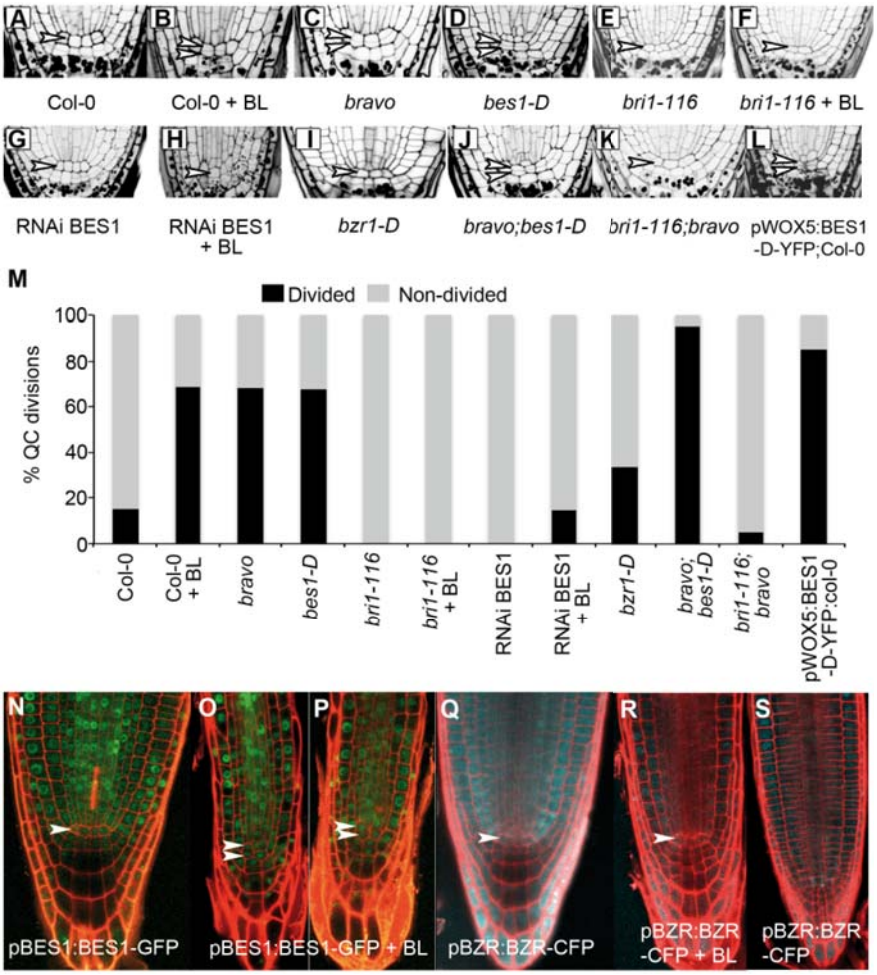

Figure 6. BR-mediated QC divisions are downstream of BES1

(A-L) Confocal images of primary roots mPS-PI stained. Col-0 (wild-type) untreated (A) and treated with BL (B). (C) *bravo*, (D) *bes1-D*, (E) *bri1-116*, (F) *bri1-116* + BL, (G) RNAi BES1, (H) RNAi BES1 treated with BL, (I) *bzr1-D*, (J) *bravo;bes1-D*, (K) *bri1-116;bravo*, (L) pWOX5:BES1-D-YFP;col-0. (M) Quantification of QC divisions. (N-S) Confocal images of primary root tips counterstained with propidium iodide (PI). Untreated pBES1:BES1-YFP (A) and treated with BL (O, P). Untreated pBZR1:BZR1-CFP (Q, S) and treated with BL (R). Note the enriched expression of pBZR1:BZR1-CFP in the root epidermis, while no expression is detected in the QC cells.

Biological significance of the BRAVO pathway in root development

In the stem cell niche the activation of stress-associated BR-signaling triggers increased QC division, premature stem cell differentiation that results in aberrant root growth (González-García et al., 2011; Heyman et al., 2013). Our data indicates that BRAVO acts as a highly regionalized repressor counteracting BR-mediated divisions in the QC. To further investigate the role as a repressor of cell division, we expressed BRAVO outside its native expression domain by generating inducible BRAVO lines. Ectopic induction of BRAVO led to a 50% reduction in root length of six day-old seedlings and a reduction of the meristematic cell number (Figure 7 A-D). Furthermore, this induction led to a significant downregulation of widely expressed cell cycle regulators such as *CYCB1;2, CYCD3;3, CYD2;2, RBR* and *WEE1* (Figure 7 E) concomitantly with BRAVO induction, in agreement with microarray data of BR responsive genes. Thus, BRAVO can repress cell divisions by downregulating cell cycle genes.

The continuous renewal of stem cells ensures the proper root growth and development (Scheres, 2007). In light of our results, we hypothesized that BRAVO functions in conferring the QC the capacity to overcome external stresses, i.e. DNA stress (Heyman et al., 2013). Thus, using a radiolabeled drug (Cruz-Ramírez et al., 2013; Fulcher and Sablowski, 2009; Heyman et al., 2013), we investigated the role of *BRAVO* in controlling stem cell regeneration by chemical induction of DNA damage. Upon bleomycin treatment, wild-type plants expressing *pBRAVO:GFP* undergo a downregulation of BRAVO concomitantly with QC division. This indicates that BRAVO regulates the preceding QC division necessary to guarantee replenishment of the stem cell compartment and to promote root growth (Figure 7 F, G). In contrast, bleomycin treatment of plants that ectopically express *BRAVO* blocked root growth, and end-up with organ exhaustion (Figure 7 G, H). Moreover, BRs promoted DNA damage-mediated death of the QC cells (Figure 8). Hence, BR-mediated regulation of BRAVO functions to restrict quiescence and ensures the maintenance of

regeneration potential of stem cells upon damage. Together, these analyses uncover a novel role for the BR-mediated BRAVO pathway in root development.

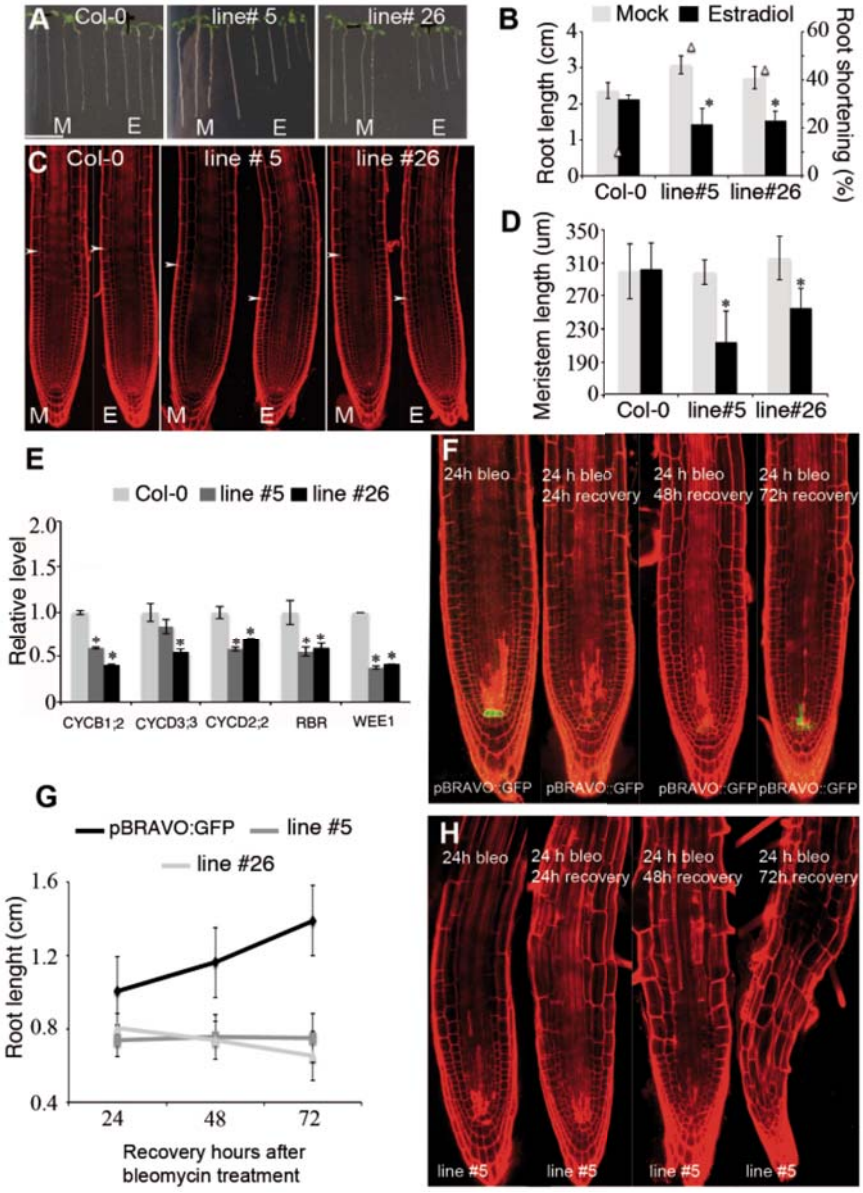

Figure 7. Ectopic *BRAVO* expression results in reduced and root growth, and organ exhaustion upon DNA damage.

(**A**) Six-day-old seedlings of Col-0 and BRAVO estradiol inducible lines #5 and #26, (mock (M), 20μM estradiol (E)). (**B**) Root-length measurement of six-day-old seedlings. Left y axis represents cm (bars), and right y axis represents the percentage of root shortening (triangles). (**C**) Six-day-old roots of indicated genotypes counterstained with PI, white arrow represents the end of the meristematic zone. (**D**) Quantification of meristem length in mock and estradiol-

induced BRAVO lines. (**E**) Relative levels of indicated cell cycle genes after induction of BRAVO overexpression. (**F**) Expression of pBRAVO:GFP counterstained with PI, 4 days-post germination seedlings were treated with bleomycin for 24 hours (24h bleo) and transfer to free-drug media after 24 hours (24h bleo, 24h recovery), 48 hours (24h bleo, 48h recovery) and 72 hours (24h bleo, 72h recovery). (**G**) Root length after 24 hours bleomycin treatment. (**H**) Ectopic expression of BRAVO, same treatments as G. . (* p-value < 0.05). Scale bars in A represents 1cm, and 50 μm in C.

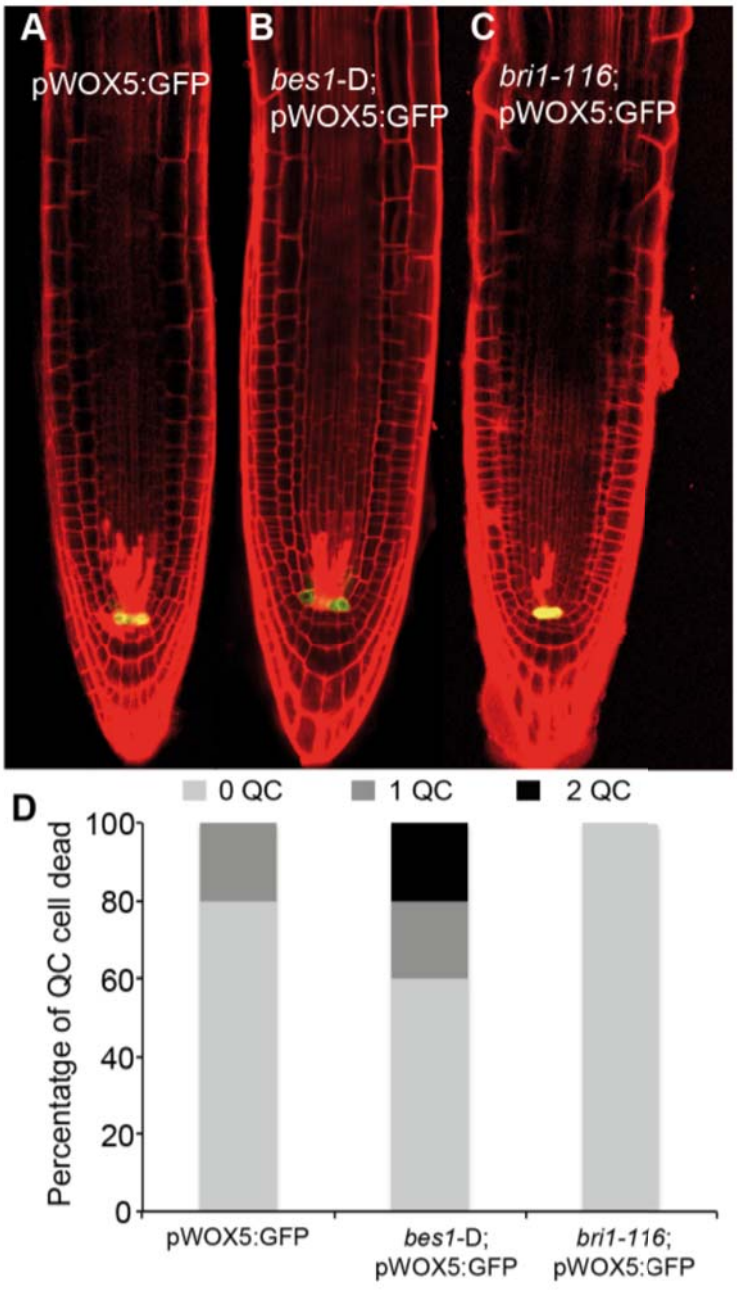

Figure 8. DNA damage responses in BR-signaling mutants.

(**A-D**) 6 day-old seedlings treated with bleomycin for 24 hours, counterstained with propidium iodide (PI). (**A**) pWOX5:GFP, (**B**) *bes1*-D;pWOX5GFP, (**C**) *bri1-116*;pWOX5:GFP. (**D**) Quantification of QC cell dead. 0 QC, 1 QC and 2 QC, stays for the number of QC dead cells (incorporated PI).

WOX5 promotes BRAVO expression.

Local regulators of QC and stem cell activities have been extensively screen, yet few specific locus have been identified and characterized. WOX5 is specifically expressed in the QC cells, were non-cell autonomously maintains the undifferentiation of the CSC (Sarkar et al., 2007). Unlike its homolog in the shoot apical meristem, WUSCHEL (WUS), *wox5-1* loss-of function mutants grow normally and do not show the pleotropic effects of *wus-1* mutants. Previously, we have shown that BRAVO promotes WOX5 expression in the QC cells (Figure 5 I-L), not interfering with WOX5 maintenance of the CSC (Figure 3 C-D). We test whether WOX5 was also regulating BRAVO expression in the QC cells. We used WOX5 dexamethasone (DEX)-inducible overexpression lines, previously shown to promote dedifferentiation of distal columella stem cells (Sarkar et al., 2007). Continuous treatment with DEX led to an ubiquitously expression of BRAVO along the root, and in the newly formed CSC in 35S:WOX5-GR; *pBRAVO:GFP* plants (Figure 9 A-C). Moreover, short DEX treatments suggest that this regulation may be direct (Figure 9 E-H). Continuous treatments with both DEX and BL promote root exhaustion, while short treatments unveil the antagonistic role of WOX5 and BRs in the regulation of BRAVO (Figure 9 I-K). Additionally, BRAVO expression in *wox5-1* loss-of-function mutants disappears from the QC cells and remains in the provascular Figure (L, M). Altogether, WOX5 promotes BRAVO expression in the QC cells.

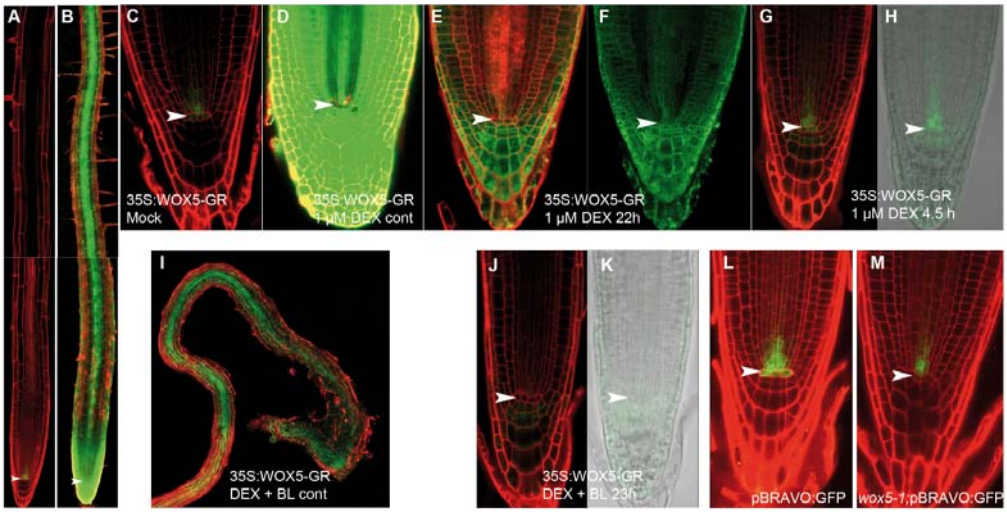

Figure 9. WOX5 promotes BRAVO expression

(**A-M**) Confocal images from primary roots of six-day-old seedlings. 35S:WOX5-GR;pBRAVO:GFP untreated (A) and treated with 1 µM of dexamethasone (DEX) continuously. 35S:WOX5-GR;pBRAVO:GFP untreated (**C**) and treated with 1 µM of dexamethasone (DEX) continuously (**D)** for 22 hours (**E-F**) and 4.5 hours (**G-H**). (I) 35S:WOX5-GR;pBRAVO:GFP continuously treated with 1 µM of DEX and BL. (J) 35S:WOX5-GR;pBRAVO:GFP treated with DEX and BL for 23 hours. (L) pBRAVO:GFP, (M) *wox5-1*;pBRAVO:GFP.

BRAVO/ BES1 signaling module establish fine mechanism for the control of cellular quiescence

Since both BRAVO and BES1 are found in QC cells and drive antagonistic effects on QC divisions we evaluated whether they regulate each other to ensure a univocal response. First, we tested whether BES1-D downregulation of BRAVO is transcriptional. Chromatin Inmunoprecipitation (ChIP) data showed that BES1-D protein binds to the E-boxes of the BRAVO promoter (Figure 10 A, B). This transcriptional repression was confirmed by transactivation assays in *Nicotiana benthamiana* and it was released in the presence of BRAVO (Figure 1 H; 4 C). In addition, both ChIP and transactivation analysis revealed that BRAVO binds to and promotes its own expression (Figure 4 C, D) demonstrating that BRAVO can be transcriptionally regulated by both BRAVO and BES1-D. The heterodimerization of BES1-D with other MYB transcription factors has been previously reported (Li et al., 2009). We next tested BRAVO/BES1-D heterodimerization by Fluorescence Resonance Energy Transfer (FRET)- Fluorescence Lifetime Imaging Microscopy (FLIM) (FRET-FLIM) and by co-immunoprecipitation experiments *in planta.* Our results showed that BRAVO interacts with BES1-D (dephosphorylated form) in the nucleus (Figure 10 E, F).

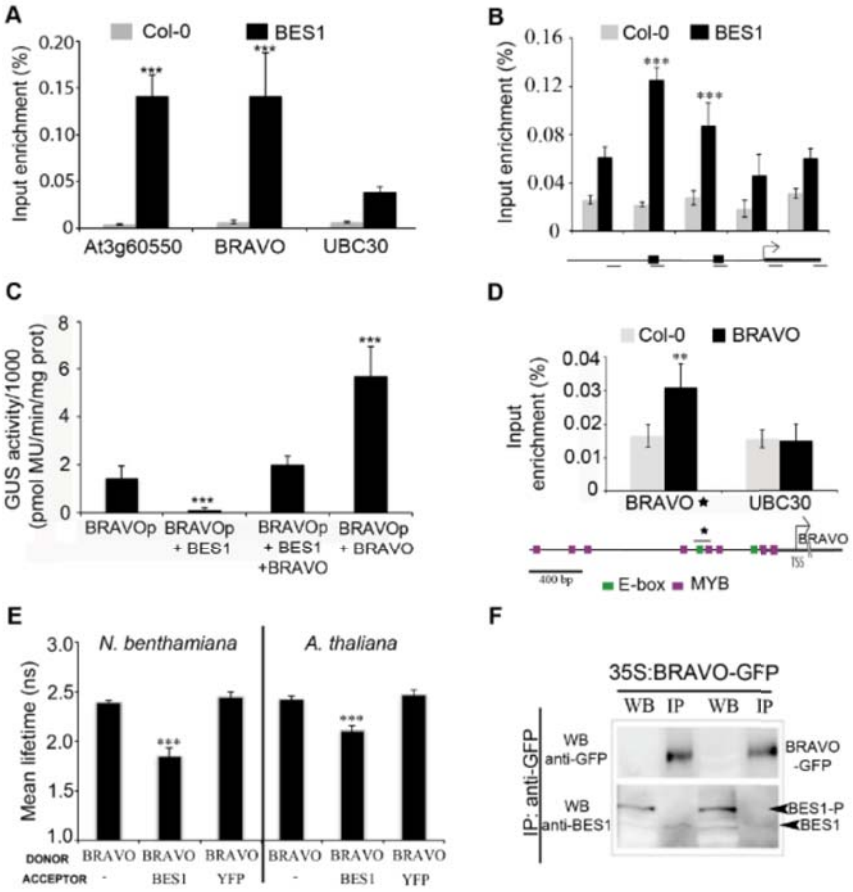

Figure 10. BES1 targets, interacts and represses BRAVO expression.

(**A**) Input enrichment of BES1 targets At3g60550 and BRAVO promoter. (**B**) Input enrichment of BRAVO promoter containing E-boxes after ChIP-PCR, PCR fragments indicated in horizontal axis, E-boxes highlighted in black. (**C**) Transient transactivation of *pBRAVO* by BES1 and BRAVO in *N. benthamiana* leaves. (**D**) Input enrichment of *BRAVO* promoter containing E-boxes and MYB-boxes, bottom; schematic representation of BRAVO promoter containing both E-boxes and MYB-boxes, star indicates PCR fragments used in ChIP-PCR. **(E)** Graphical representation of reduced CFP lifetime in nuclei co-expressing BRAVO-CFP (cyan fluorescence protein; donor) and BES1-YFP (yellow fluorescence protein; acceptor), as compared to nuclei expressing BRAVO-CFP alone; nuclear YFP was used as specificity control. (**F**) Co-immunoprecipitation using 35S:BRAVO-GFP, top panel; enriched BRAVO-GFP protein complex after IP, lower panel; BES1 dephosphorylated form (black arrow) is detected using anti-BES1 antibodies in the BRAVO-GFP protein immunoprecipitated fraction.

(**, ***, represent p-value<0.05 and < 0.001, respectively), error bars indicate ±SEM. WB; western blot; IP: inmunoprecipitation.

To assess the biological activity of BRAVO/BES1 interaction *bes1*-D; *pBRAVO:BRAVO-GFP* double mutants were generated. Increased levels of BRAVO were able to suppress the QC division phenotype of *bes1-D*, similarly to the exogenous BL treatment of *pBRAVO:BRAVO-GFP* (Figure 11 A-F). In the same direction, transactivation assays with BES1/BRAVO dimer suppress repression/activation activities of BES1 and BRAVO (Figure 10 C), respectively. Altogether, these results show that BRAVO/BES1 proteins heterodimerize in the QC cells.

Finally, to understand how the opposite effects of BES1 and BRAVO over the QC divisions are combined into a final QC cell decision, we built a mathematical model that takes into account the mutual regulations between BES1 and BRAVO (Figure 11 G, see Methods). Computational results showed that these interactions drive robustly a univocal response since they ensure opposite amounts of BRAVO and BES1-D (Figure 11 H). Therefore, a high amount of free BRAVO arises concomitantly with low amounts of free BES1-D (i.e. (HIGH, LOW) state) and viceversa (i.e. (LOW, HIGH) state). The (HIGH, LOW) state arises for low dephosphorylation rates and prevents QC divisions, whereas the (LOW, HIGH) state arises at higher level of BES1-D (e.g. upon BL treatment) and induces QC divisions. Most importantly, these states are in agreement with the extent of BRAVO and BES1-D in the QC of WT plants and in plants treated with BL.

A sharp transition from the (HIGH, LOW) to the (LOW, HIGH) state when the dephosphorylation rate increases is indicated by the model (Figure 11 H). This sharp transition involves bistability (Cruz-Ramírez et al., 2012; Krishnan et al., 2003; Novak et al., 2007) of both states (Figure 11 H). Most importantly, the transition is relevant since it prevents the existence of states with similar amounts of BRAVO and BES1-D which, given the opposing functions of BES1 and BRAVO, would drive an unclear signal as to whether the

QC needs or not to divide. Taken together, these results provide a mechanism for BR-controlled QC divisions.

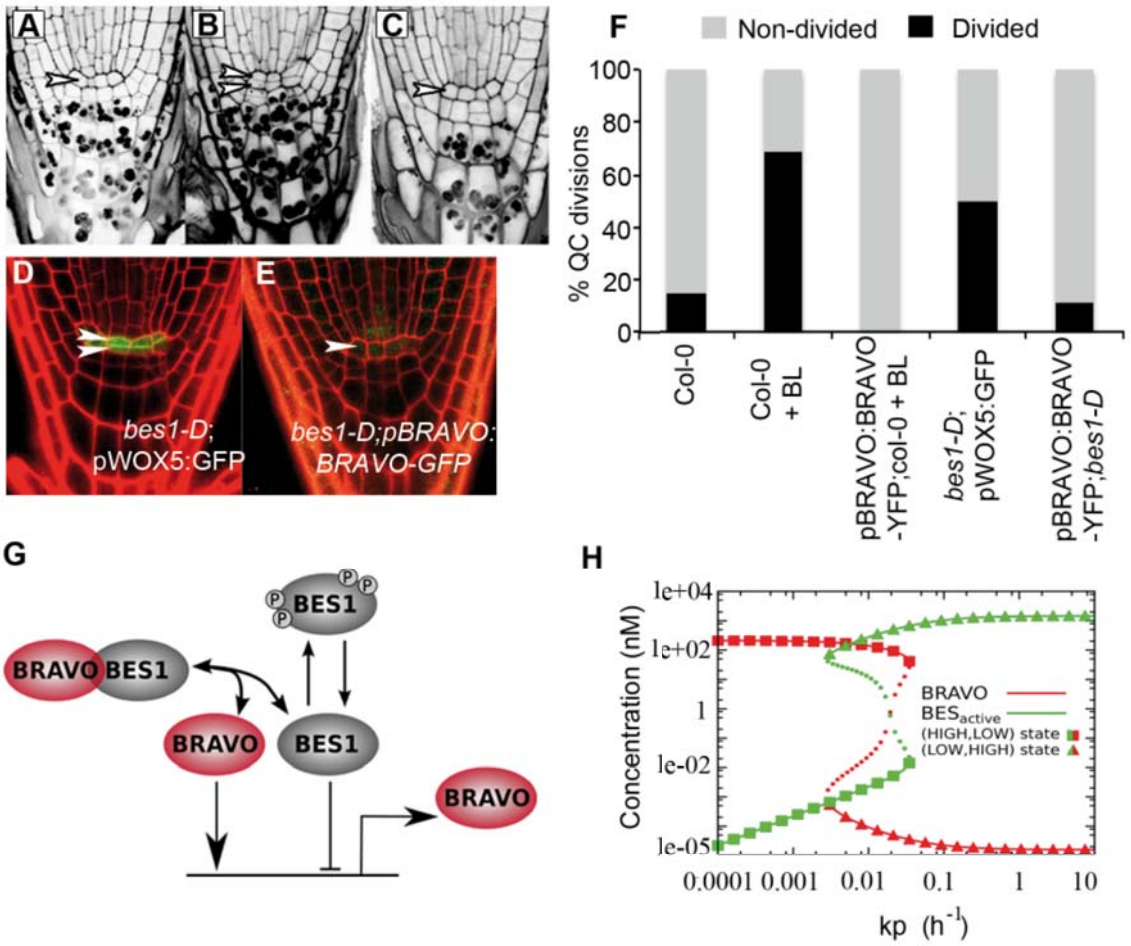

Figure 11. BES1/BRAVO defines a bistable switch that controls QC divisions in the root apex.

(**A-E**) Confocal pictures of primary root from 6 day-old-seedlings, (**A-C**) mPS-PI stained, (**D, E**) counterstained with PI. White arrows point QC cells. (**F**) Quantification of QC divisions. (**G**) Schematics of the reactions considered in the model. Production of BES1 and degradation of all molecules is omitted for simplicity. (**H**) Amounts of free BRAVO (red) and free dephosphorylated BES1 (green) as a function of the dephosphorylating rate k_{P-}. When free BRAVO is at high amounts, free dephosphorylated BES1 is almost absent. We term this state as (HIGH, LOW) (squares). Analogously, when BRAVO is absent, active dephosphorylated BES1 is at high amounts. We term this state (LOW, HIGH)

(triangles). The dephosphorylating rate controls a sharp transition between these two sates.

CHAPTER 4

RESULTS

IDENTIFICATION OF BRAVO

DOWNSTREAM REGULATED GENES

SUMMARY

The elucidation of downstream factors from signaling pathways is a major challenge to understand morphogenetic processes. In this chapter, we present the identification of *BRAVO* deregulated genes by using a cell-based transcriptomic approach. We identify a set of genes deregulated in the divided QC cells of *bravo* mutants. This set of genes comprises signaling pathways that control both stimuli and circadian regulation. Additionally, identification of BRAVO direct targets using the endogenous expression of *BRAVO* is presented. Unfortunately, statistical analysis of genome-wide comparison of *BRAVO* inmunoprecipitation compared to wild-type plants did not raise any specific binding region. Altogether, we identified a set of genes regulated by *BRAVO* that will unveil the downstream components of the BRAVO pathway.

INTRODUCTION

Genome scale analyses enable to address the function of molecular players from signaling pathways and the construction of gene regulatory networks (Hyduke and Palsson, 2010). The recent genome sequencing of a vast number of model organisms opens new avenues for genome-wide profile. Until recently, the main profiling source of information came from oligonucleotide-based microarrays, which required knowledge of the genomic sequence (Schena et al., 1995). The released of the *Arabidopsis thaliana* genome (Arabidopsis Genome Initiative, 2000), came accompanied by the emergence of microarray technologies covering a limited number of loci (Wisman and Ohlrogge, 2000; Zhu and Wang, 2000). Genome-wide studies in different organs, developmental stages and in response to stimuli, shed light in the identification of gene regulatory networks (Goda et al., 2008; Schmid et al., 2005).

Different approaches have been develop to gain an insight into developmental or cell-type specific processes, diluted in genome-wide studies of whole plant or plant parts (Brady et al., 2007). Laser Capture Microdissection (LCM) proof advantages for selection of cells based on morphology and/or position, avoiding any specific cell marker (Palovaara et al., 2013). It have been successfully applied to roots and shoots (Kerk et al., 2003), but it is labor-intensive, need specialized equipment and yield poor RNA quality relegating its use in species where not transgenic markers can be generated. Another method involves the expression of a tagged protein intrinsic to ribosomes under a tissue-specific promoter, that upon inmunoprecipitation reflects the mRNA that is being translated (Zanetti et al., 2005). INTACT uses a two-component transgenic labeling system, with a specific promoter driving a nuclear lamina-localized GFP with *in vivo* biotinylated site (nuclear tagging factor) and a biotin ligase in the same plant. When both are coexpressed biotin –tagged nuclei can be efficiently isolated from a crude nuclear preparation. It have been successfully carried in different root cell types (Deal and Henikoff, 2011). Finally, sorting marked cells using a fluorescent activated cell sorted (FACS)

has been the most productive strategy for profiling different tissues. It requires transgenic plants which express a fluorophore in a tissue-specific manner, such as promoter fusions or enhancer trap lines (Birnbaum et al., 2005). Prior to FACS sorting, cell walls are digested resulting in protoplasts, this represents the main disadvantage as the cell isolation disrupts intercellular signaling and could have an impact on the transcriptional state. However, it have been demonstrated that the overall global gene profile remains unchanged after protoplasting, excluding a small subset of genes (Birnbaum, 2003). To date, FACS has been the most efficient approach to isolate specific cell-types in Arabidopsis and it allowed the profiling of all cell-types of the root under both stressed and non-stressed conditions (Birnbaum, 2003; Brady et al., 2007; Dinneny et al., 2008; Gifford et al., 2008).

The gain of detail in genome wide profiling have uncovered that whole-profiling misses many gens that are expressed in a regionalized manner (Iyer-Pascuzzi et al., 2011; Schmid et al., 2005). Surprisingly, gene expression fluctuations during root development reconsidered the view of development as not an unidirectional processes (Brady et al., 2007). Cyclic pulses of expression mark the position of future lateral roots (Moreno-Risueno et al., 2010), a mechanism that also drives somite formation and neural development (Kageyama et al., 2009). In another example, profiles of apical meristem, quiescent center and root cap of maize primary roots, revealed the upregulation of metabolic genes in the root cap (Jiang et al., 2006). The differences between whole-organ and cell-type specific expression are not limited to the root. In leafs, isolation of guard cells from mesophyll cells uncovered the expression of guard cell-specific ABA-responsive genes (Leonhardt, 2004). Isolation of different cell domains within the shoot apical meristems (SAM), populations of stem cells surrounded by differentiated cells in the shoot apex, revealed enrichment of DNA repair and chromatin modification pathways (Yadav et al., 2009). Thus, the increasing amount of cell-type specific profiling data uncovered new and specific developmental regulators that shape the final plant body.

To gain an insight in the networks regulating development, two types of data are needed; (i) mRNA expression data, and (ii) transcription factor binding

data. Efforts in increasing mRNA expression data resolution have far overcome transcription factor binding data. Genome-wide identification of binding sites for different transcription factors identified a wide-range of sites, ranging from less than 100 to several hundred and up to thousand (Lee et al., 2007; Morohashi and Grotewold, 2009; Thibaud-Nissen et al., 2006). Interestingly, in most of the cases reported less than 10% of the genes bound responded transcriptionally to altered levels of the transcription factor (Lee et al., 2007; Morohashi and Grotewold, 2009; Zheng et al., 2009). Combination of both expression and transcription factor binding data have been fruitfully in integrating growth, patterning, and hormonal pathways (Kaufmann et al., 2010).

The development of novel high-throughput DNA sequencing technologies provides another degree in the genome-wide profiling. Early RNA-seq studies identified previously unknown transcript and novel non-coding RNA, while it provides a deeper view of the eukaryotic genomes (Wang et al., 2009). RNA-seq in different cell-types and developmental regions of the Arabidopsis roots detected over 60 novel miRNAs (Breakfield et al., 2012). Recent development of single cell RNA-seq technologies allowed an unprecedented degree of resolution, at single cell level (Ramsköld et al., 2012; Tang et al., 2009). Development of quantitative statistical methods to distinguish biological relevance from technical noisy in single-cell profiling experiments will allow the study of transcriptional heterogeneity within homogenous cell-types (Brennecke et al., 2013; Chang et al., 2008). Massive profiling of single cells revealed the *in vivo* transcriptional state of thousands of cells, allowing researchers to address cell-type heterogeneity (Jaitin et al., 2014).

Here we report, the combination of cell-based sequencing approaches for; (i) mRNA expression data of the QC cells in *bravo* mutant background and (ii) transcription factor binding of BRAVO in the endogenous expression domain. In our knowledge, such degree of cell-specificity of a highly regionalized transcription factor has not been carried out in plants before.

A cell-based transcriptomic approach to identify BRAVO regulated genes

Transcriptomic profiling has been a major source of information to unveil downstream-deregulated genes. To identify genes regulated by BRAVO we used a cell-based transcriptomic approach. We choose a cell based transcriptomic because the BRAVO phenotype appear in the QC cells, around 12 cells per root, and reasoned that traditional transcript profiling of seedlings or roots will experience a dilution effect that will mask BRAVO deregulated genes. Fluorescence activated cell sorting (FACS) is the most common methodology to profile specific cellular domains. The FACS methodology implies the generation of protoplasts prior to flow cytometer, which in total take at least 2.5 hours. To avoid these long time sample processing we used manual selection of GFP positive protoplasts, with a microinjection needle, which reduce the sample processing time. Thus, to elucidate BRAVO downstream deregulated genes six-day-old roots from pWOX5::GFP and *bravo*;pWOX5::GFP were protoplasted, and GFP positive protoplasts were visualized under a fluorescence microscope to detect and subsequently selected positive protoplasts using a mouth-pipetting device (Figure 1, see Methods pg.119),). Two independent biological replicates were selected for sequencing, after quality check of the cDNA samples prior library generation. Successful cDNA amplification should yield a peak spanning from 400bp to 9000bp, peaked at ~2000bp, and yielding approximately 2-7ng of cDNA (Figure 2 A-C).

After sequencing, a quality control analysis was performed on the raw sequencing data in order to remove the adaptor sequences and obtain high quality reads, with a minimum length of 25bp. This yield to ~50 million reads per sample after the quality control. Upon analysis of the high quality reads (see methods for more information), the HTsFilter, based on global Jaccardi index, was used to identify the minimum normalized read counts to remove transcripts with very low and excessively variable expression across samples (Figure 2 D). As a result, 3.9 read count were used subsequently with TMM to normalize the read counts.

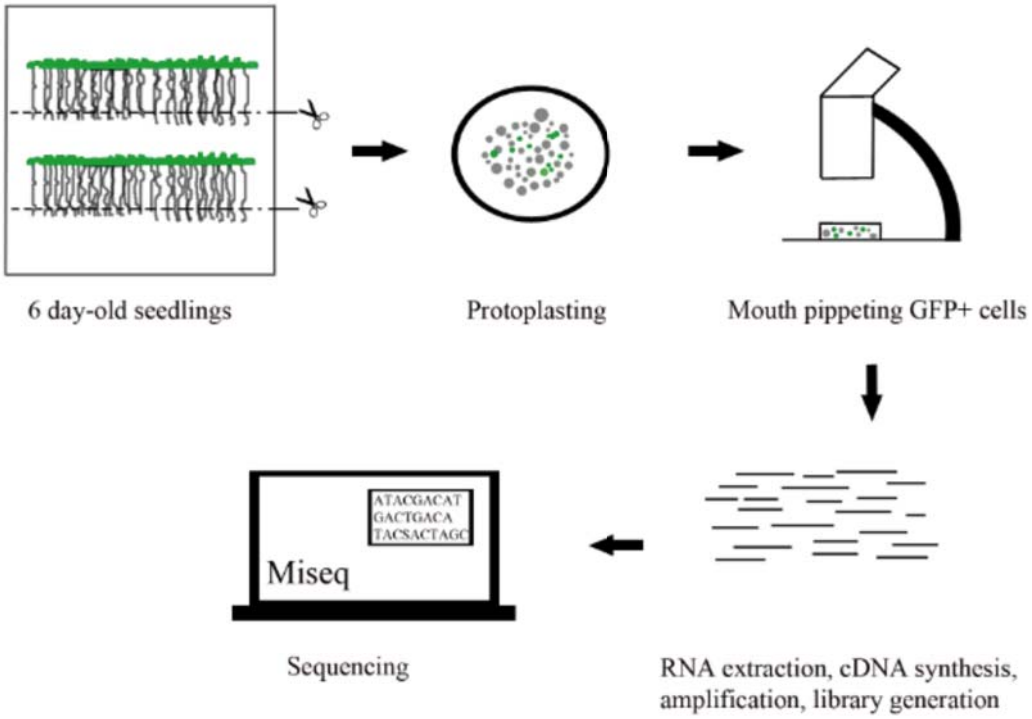

Figure 1. **Schematic procedure of a cell based RNA-seq to uncover deregulated genes in *bravo* mutants**. pWOX5:GFP and *bravo*;pWOX5GFP seedlings were grown for 6 days vertically in square plates. Protoplasting of root tips according to (Birnbaum et al., 2005), was performed at room temperature checking the release of fluorescent cells under the microscope to avoid excessive digestion time. Subsequently, selection of the GFP positive cells under the microscope was performed as fast as possible, within 20 minutes from the moment that QC cells were released, samples were flash frozen using liquid nitrogen. RNA isolation, cDNA synthesis and library preparation were performed in a clean bench (see methods for details). Sequencing was performed using a Ilumina Mi-seq equipment (http://www.illumina.com/systems/miseq.ilmn).

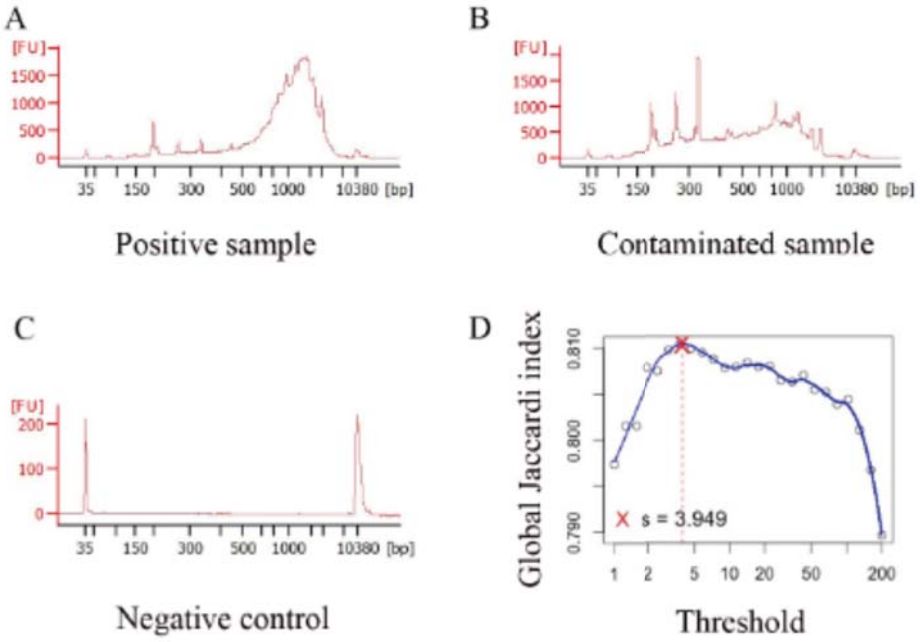

Figure 2. Quality control of amplified cDNA and sequences. (A-C) Validation of the amplified cDNA using Agilent 2100 Bioanalyzer. (A) A positive selected sample with a peak extending from 400bp to 9000bp, peaking at 2000bp. (B) Contaminated sample with a broader peak. (C) No amplification in the negative control. (D) Graphic representation of the HTSFilter analysis, showing that for s=3.9 the samples have the highest similarity, thus this value was used as a threshold.

Identification of BRAVO regulated genes

After quality filtering, the final dataset was analyzed with TCC to identify differentially expressed transcripts between *bravo* and wild-type plants, in single comparisons. As a result, 38 genes appear deregulated (q-value<0.05. p-value<0.05). A general down regulation was observed in *bravo* mutants compared to wild-type plants, were 35 out of 38 genes were down-regulated and 3 genes up-regulated (Table 1). None of the deregulated genes appear in datasets generated in response to BRs exogenous treatment (Goda, 2002; Goda et al., 2008; 2004; Mouchel et al., 2006; Müssig et al., 2002; Nemhauser et al., 2006; 2004; Vert et al., 2005). Nevertheless, we used the cell-type specific transcriptome in response to BRs (see also Chapter 3) and compare it to the deregulated genes uncover in the RNA-seq analysis. Surprisingly, 11 genes appear among the deregulated genes (FC>1.2 p-value<0.001), 8 up regulated and 3 down regulated respectively. Further analysis of the deregulated genes revealed 8 genes targeted by BES1 or BZR and BL regulated in our cell-type specific transcriptomics in response to BR. From those a single gene matches all the criteria; deregulated by BRAVO, target of BES1 and BZR and deregulated in response to BRs. These gene corresponds to At2g22330, which encodes a cytochrome P450 protein which converts tryptophan to indole-3-acetaldoxime (IAOx) (Zhao et al., 2002), highly expressed in the QC cells (Table 1). Further analysis of cis-elements in the deregulated genes uncover an enrichment with p-value<0.05 of both ATHB5ATCORE and TATA-box motif.

Gene Locus	Gene model description	m-value	p-value	q-value	remarks
AT1G01470	Late embryogenesis abundant 14 (LEA 14)	-5.047	6,90E-06	1,79E-02	1, 2
AT1G02920	Glutathione S-tranferase 7 (GSTF 7)	-6,224	3,42E-05	4,81E-02	
AT1G05680	Uridine diphosphateglycosyltransferase (UGT74E2)	-7,308	4,98E-06	1,72E-02	
AT1G10682	Other RNA	-3,454	5,23E-07	3,71E-03	
AT1G12080	Vacuolar calcium-binding protein related	-3,513	2,43E-06	9,07E-03	3,
AT1G13670	Unknown protein	-11,052	2,56E-06	9,07E-03	3,
AT1G25230	Calcineurin-like metallo-phosphoesterase superfamily protein	-3,538	4,01E-06	1,22E-02	
AT1G50060	Cysteine-rich secretory protein (CAP)	-6,160	2,12E-08	4,40E-04	
AT1G52050	Mannose-binding lectin superfamily protein	-5,800	5,99E-06	1,78E-02	3,
AT1G52070	Mannose-binding lectin superfamily protein	-4,170	8,42E-06	1,94E-02	3,
AT1G62480	Vacuolar calcium-binding protein related	-3,062	2,34E-11	2,75E-07	3,
AT1G75750	GA-responsive GAST1 protein homolog regulated by BR and GA antagonistcally	-2,435	1,31E-05	2,79E-02	1, 2
AT1G80240	Protein of unknown function, DUF642	-5,585	9,88E-06	2,05E-02	4
AT2G01008	Unknown protein	2,287	2,35E-06	9,19E-03	
AT2G16586	Unknown protein	2,233	1,03E-07	8,03E-04	
AT2G20670	Protein of unknown function (DUF506)	-2,736	1,90E-05	3,38E-02	
AT2G22330	Cytochrome P450, family 79, subfamily B, polypeptide 3 (CYCP79B3)	-4,840	8,73E-11	5,98E-07	1, 2, 3, 4
AT2G30040	Mitogen-activated protein kinase kinase kinase 14 (MAPKKK14)	-3,204	2,56E-05	3,88E-02	
AT2G33830	Dormancy/auxin associated family protein	-6,998	3,29E-14	6,77E-10	
AT2G37040	Phenylalanine ammonia-lyase (PAL1)	-9,002	1,25E-05	2,37E-02	
AT2G43610	Chitinase family protein	-4,191	3,42E-06	1,53E-02	3,
AT2G46830	Circadian clock associate 1 (CCA1)	-6,300	1,52E-05	2,94E-02	
AT3G12320	Unknown protein	-7,740	2,46E-05	3,88E-02	1
AT3G15450	Aluminium induced protein with YGL and LRDR motifs	-2,610	1,18E-06	4,86E-03	3, 4
AT3G47340	Glutamine-dependent asparagine synthase 1 (ASN1)	-6,334	1,31E-06	6,41E-03	
AT3G53420	Plasma membrane intrinsic protein 2ª (PIP2A)	-3,097	4,09E-08	8,71E-04	1, 2
AT3G59370	Vacuolar calcium-binding protein related	-4,045	1,02E-05	2,32E-02	3,
AT4G14130	Xyloglucanendotransglucosylase/hydrolase 15 (XTH15)	-4,596	2,62E-07	2,79E-03	
AT4G26320	Arabinogalactan protein 13 (AGP13)	-2,870	8,82E-06	2,35E-02	

AT4G27450	Aluminium induced protein with YGL and LRDR motifs	-10,723	4,09E-06	1,26E-02	3
AT4G39260	Cold circadian rhythm, and RNA binding 1 (CCR1)	2,585	3,62E-05	4,81E-02	
At5g02230	Haloacid dehalogenase-like hydrolase (HAD)	-10,850	3,70E-06	1.53E-02	
AT5G05600	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase	-7,862	2,52E-05	4,37E-02	
AT5G14180	Myzus persicae-induced lipase 1 (MPL1)	-11,271	1,28E-06	6,80E-03	3,
AT5G16970	Alkenal reductase (AER)	-10,191	1,14E-05	3,99E-02	
AT5G20230	Blue-copper binding protein (BCB)	-4,861	1,10E-05	2,60E-04	
AT5G54370	Late embryogenesis abundant (LEA) protein-related	-4,766	3,09E-06	1,53E-02	3,
AT5G65683	WAV3 homolog 2 (WAVH2)	-6,922	2,77E-05	4,42E-02	1, 2

Table 1. Differentially expressed transcripts between *bravo* and wild-type. M-value= fold change in logarithmic scale, p-value= p-value calculated during the exact test, q-value= corresponds to the FDR. Remarks; 1= Bes1 target, 2= high stringency BZR target, 3=BR-regulated FC<1.2 p-value<0.001 (our previous arrays, chapter 3), 4= R2R3-type MYB motif.

Moreover, to investigate whether genes encoding for specific proteins functions were present in our dataset, we performed gene ontology studies using AgriGo (http://bioinfo.cau.edu.cn/agriGo) (Du et al., 2010). This analysis revealed that genes with a function in response to stimuli are over-represented in our dataset, with further enrichment in the subcategories response to; abiotic stimulus, external stimulus, stress and chemical induction (Figure 3 A). No GO enrichment was detected for molecular function nor for cellular components. To identify possible contaminations in the sample from other cell-types, a clustering was performed between the normalized counts from the RNA-seq and the transcriptomic data from different cell-types available (Brady et al., 2007; Nawy et al., 2005). As a result, BRAVO deregulated genes cluster with S4 domain corresponding to xylem.

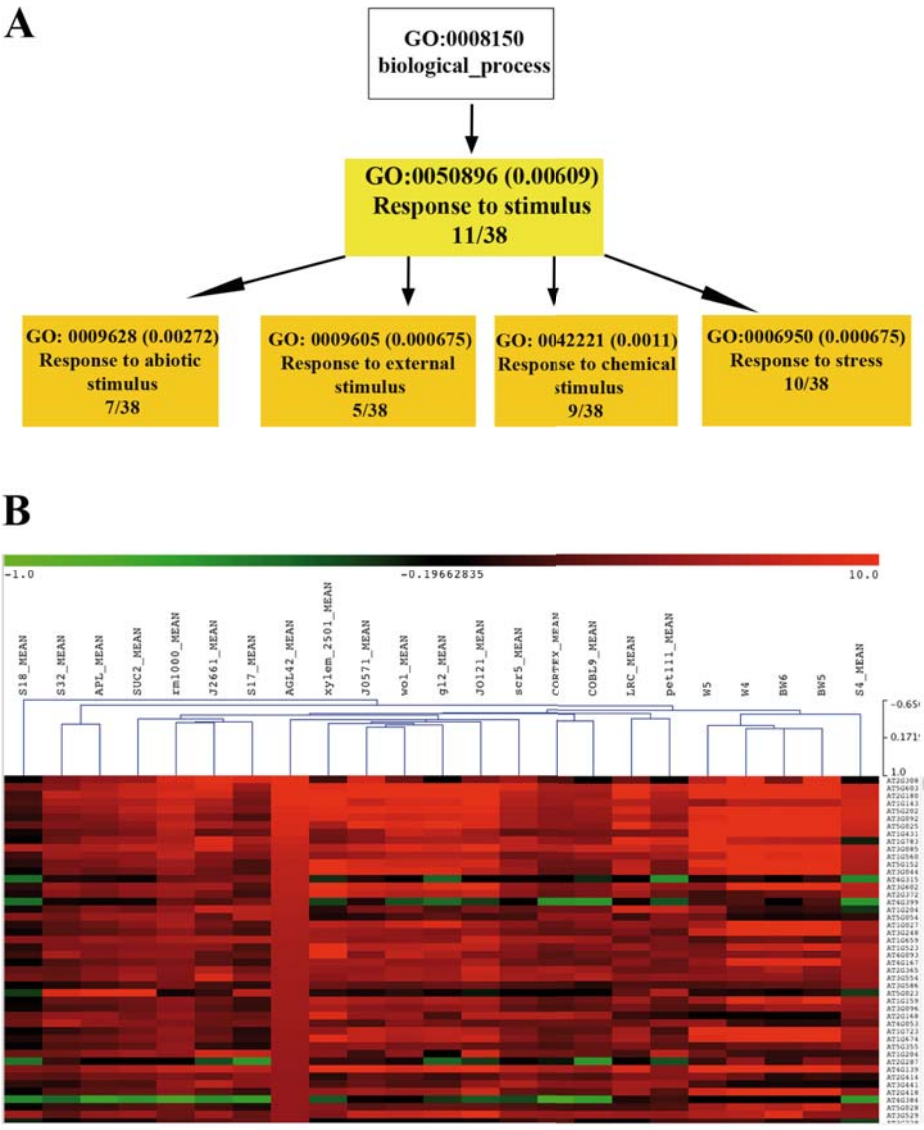

Figure 3. GO and clustering analysis of BRAVO deregulated genes. (A) GO analysis of the deregulated genes using AgriGO. (B) Clustering analysis of BRAVO deregulated genes and cell-type transcriptomic data from(Brady et al., 2007; Nawy et al., 2005).

Identification of BRAVO direct targets thought a cell-type specific chromatin inmunoprecipitation approach

The identification of direct targets of transcription factors, have been a straightforward strategy to unveil the biological processes regulated by specific DNA-binding proteins (Busch et al., 2010; Huang et al., 2012; Lee et al., 2007). While an increasing amount of data elucidates the binding regions thought the genome with sequencing data or tilling microarrays, none of the current datasets represents a cell-type, rather a whole seedling. In addition most of the ChIp assays are conducted using overexpression lines, which may affect binding affinity by increasing the number of molecules present, and false positive binding sites. Thus, we aimed to performed cell-type specific chromatin profiling using pBRAVO:BRAVO-GFP seedlings. First we treated 4-5 day old seedling with NPA for an additional 4 days, to increase the expression domain, without affecting the cellular fate (Sabatini et al., 1999). Blocking auxin transport results in an expansion of the BRAVO domain, thought the respecification of the distal cell fates (Figure 1 A, B) (Sabatini et al., 1999). ChIP experiments were performed using root tip samples, and ChIP-PCR of the BRAVO promoter region was used as a positive control, previously identified as direct target (Chapter 3) (Figure 3 A). Three independent replicas, from eight pooled ChIP experiments, were subsequently sequenced for both wild-type plants and pBRAVO:BRAVO-GFP.

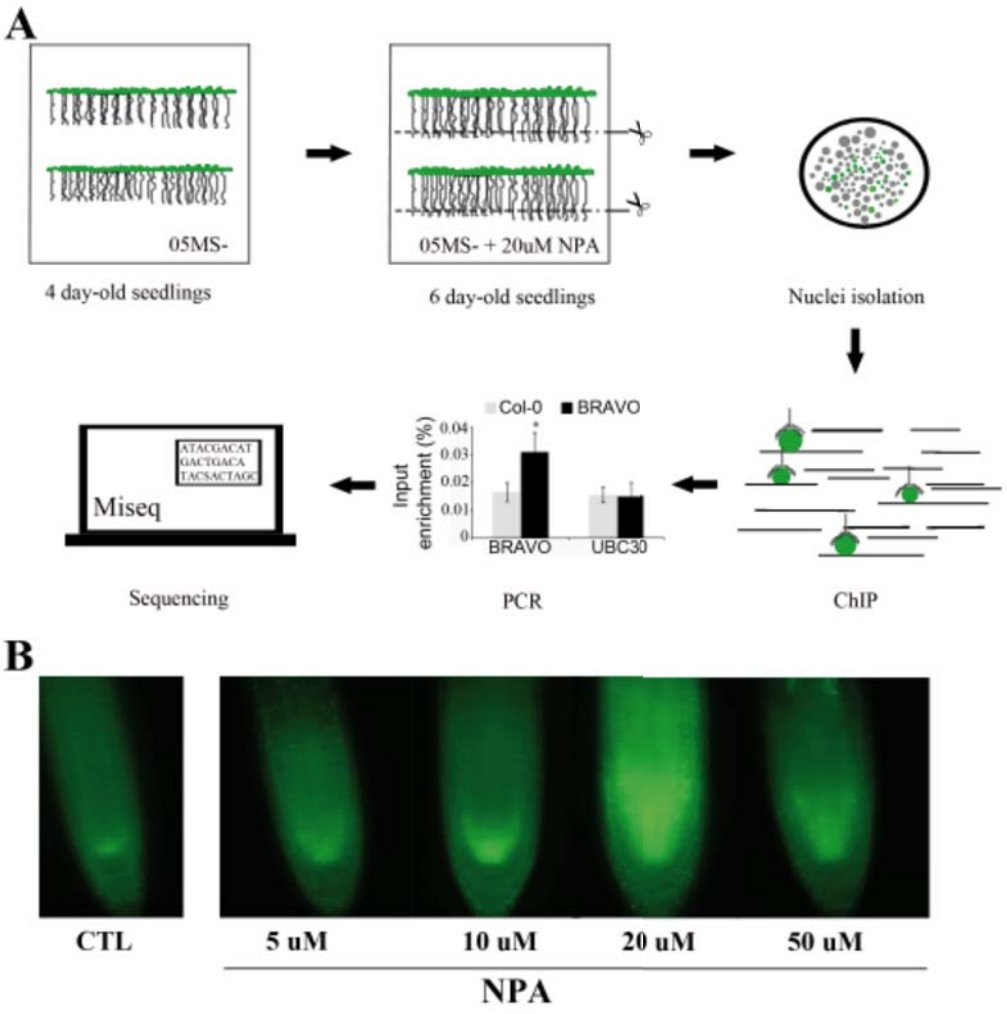

Figure 4. Schematic procedure of cell-type specific chromatin inmunoprecipitation (ChIP) approach to identify BRAVO direct targets. (A) pBRAVO:BRAVO-GFP seedling were grown in vertical using square plates, 4 days after germination seedlings were transferred to plates containing 20uM NPA. 6 day-old root tips were collected and used for the subsequent steps; nuclei isolation, ChIP, ChIP-PCR and sequencing (see also methods). (B) Expression of pBRAVO:BRAVO-GFP in control media (CTL) and increasing amounts of NPA.

Analysis of genome wide BRAVO binding sites

After sequencing raw reads were trimmed and the adapters removed in order to obtain high quality reads (minimum length of 25 bp and a minimum Phred Quality of 35). The obtained reads were mapped against the Arabidopsis reference genome with Bowtie2 and only uniquely mapping reads were retained for further analyses. Despite using several FDR thresholds (ranging from 10 to 0.001) it was not possible to detect any statistically significant peak in the Col-0 samples neither with Homer nor with SPP.

A closer look at the mapping results by mean of genome viewers showed that the distribution of the reads in the two groups of samples is quite uniform across the genome and it is very similar between Col-0 and BRAVO. The promoter region of BRAVO was analyzed and indeed no difference between pBRAVO:BRAVO-GFP (BR) and the negative control (Col-0_reads) could be observed (Figure 5). These results might suggest either a lack of specificity of the antibody used for the ChIP or that the quantity of the immunoprecipitated DNA was not enough for the analysis.

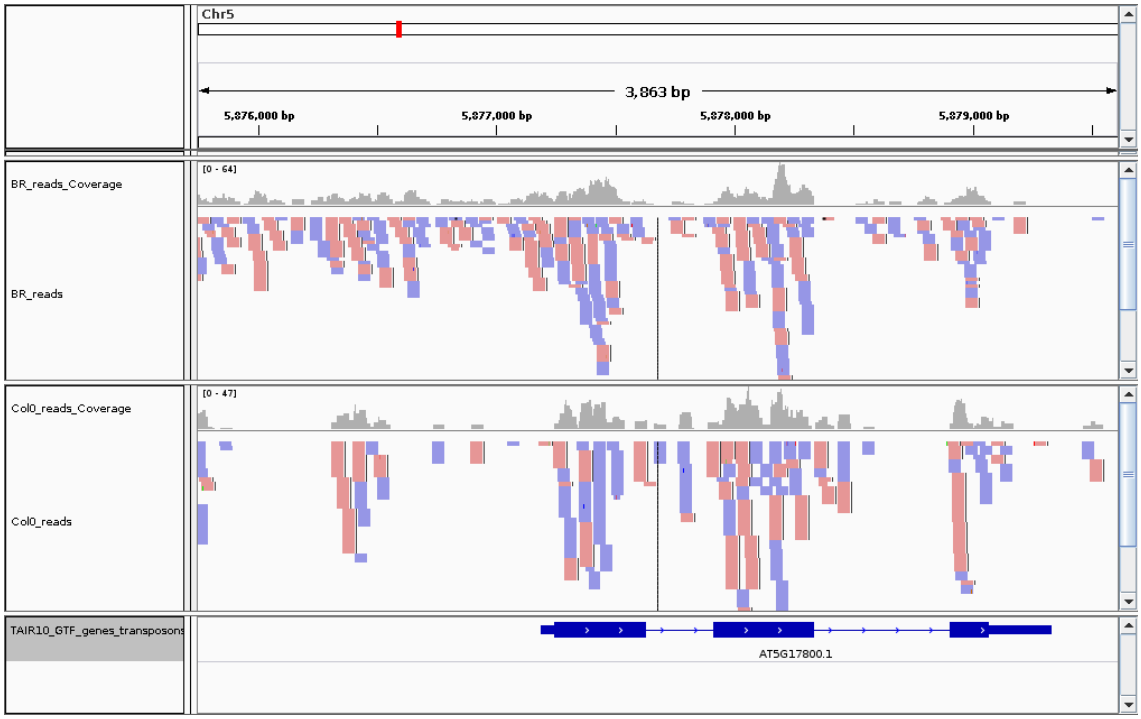

Figure 5. ChIP analysis. Screenshot of the MACS viewer for BRAVO (At5g17800) promoter region. Note no significant peaks were called in the BR_reads (BRAVO, upper) ChIP, compared to Col-0 (wild-type) ChIP.

GENERAL DISCUSSION

Brassinosteroid (BRs) are important regulators of plant development, yet the mechanisms for BR control of these processes have only started to be elucidated. The present PhD thesis work describes a role for BRs in the control of the stem cell homeostasis in the Arabidopsis primary root. In particular, it reports the identification of BRAVO, a novel BR-signaling component controlling the QC divisions within the stem cell niche. BRAVO is a cell-type specific BR-signaling component that encodes a R2R3 MYB transcription factor that acts as a local repressor of the BR-mediated QC divisions. While the positive actions of steroids in stem cells have been described in animals, in plants those have remained unknown. Our data shows that BRAVO provides both robustness and the plasticity to the stem cell niche to respond to environmental challenges opening new scenarios for cell-type specific signaling events.

First, a new role for BRs in the regulation of the root stem cell niche has been demonstrated. Genetic and physiological treatment with BRs showed that BRs acts upstream or independently of both PLETHORA and SCARECROW pathways in the maintenance of the meristematic activities. Local expression of BRI1 receptor in *bri1-116* loss-of-function mutants showed that BRI1-mediated signaling controls root growth non-cell autonomously from the dividing cells. Moreover, BRs promote QC division and columella stem cell (CSC) differentiation downstream of BES1 transcription factor. Genetic analysis placed BRs-mediated CSC differentiation upstream or independent of SCR, PLT and WOX5 pathways. Importantly, BRs control CSC differentiation independently or downstream of auxins. Local expression of BRI1 in the QC cells non-cell autonomously promotes CSC differentiation, while expression of BRI1 in the provascular tissues, endodermis or epidermis did not affect CSC differentiation. Importantly, local expression of BES1 in the QC cells promotes cell-autonomously QC divisions. Thus, attenuation of the BRI1 receptor mediated

signaling may occur in the QC cells preventing BR-mediated cell cycle progression and subsequent divisions in the QC cells.

Second, the identification and characterization of a new BR-signaling component acting as a highly regionalized repressor of QC divisions has been carried out. A metagenomic approach identified BRAVO as a stem cell niche BR-regulated transcription factor target of both BES1 and BZR transcription factors. Characterization of *bravo* mutants revealed that BRAVO acts as a repressor of QC divisions, and ectopic expression showed that BRAVO controls divisions within the meristematic cells. Mathematical modeling taking into account BES1/BRAVO interaction, BRAVO auto activation and repression of BRAVO by BES, proposed that a bistable switch between two states control BES1/BRAVO mediated QC divisions. Importantly, BRAVO acts as a DNA-damage sensor in the QC cells allowing stem cell replenishment after DNA damage of the stem cells. Together, a new specific branch of the BR-signaling pathway that maintains the low cell cycle status of the QC has been identified, while provides a mechanism to rapidly replenish stem cell upon damage.

Third, a cell-based transcriptomic approach was used to identify downstream targets of the BRAVO pathway. RNA-seq analyses of QC cells in *bravo* mutants elucidate cell-response to stimuli as overrepresented gene ontology in the divided QC cells. Interestingly, a number of response pathways were deregulated in the divided QC in response to; abiotic stimulus, external stimulus, stress and chemical stimulus. The deregulated genes by BRAVO provides a new set of genes controlling the availability of various macro-micronutrients, pointing out the importance of complex microenvironments controlling the stem cell niche. The maintenance of the metabolic status in the stem cell niches has been shown to be crucial in stem cell maintenance (Jasper and Jones, 2010). Together, complex environmental conditions of the stem cell niche provide the bases to sense external stimuli and act as a long-term reservoir of stem cells.

Altogether, regionalized signaling pathways may account for specific developmental outputs, providing a broader picture to the present knowledge of hormone signaling in plants.

Spatial analysis of Brassinosteroids action during root development

The Arabidopsis root provides an excellent model to study organogenesis, with a spatio-temporal scenario that allows to easily distinguish between different root cell-types and developmental zones (Dolan et al., 1993; Ioio et al., 2008). In multicellular organism, a proper balance between cell proliferation and cell differentiation shapes final organ growth. In plants, the root meristems integrate information from hormones and reactive species to shape their final size (Achard et al., 2009; Garay-Arroyo et al., 2012; Ioio et al., 2008; Tsukagoshi et al., 2010; Ubeda-Tomás et al., 2008). BRs have been historically associated with cell elongation (Kauschmann et al., 1996), but elongation is not sufficient to explain BR mutants' phenotype, being additional developmental defects elusive. Our work and others uncover a role for BRs in meristem maintenance and cell cycle progression (González-García et al., 2011; Hacham et al., 2011). In contrast to the balance between CK and GA necessary to define the final meristem length, BRs act in the speed of the cell cycle of the dividing cells. Cell cycle defects in BR-mutants were recovered by CYCD3;1 overexpression or silencing of retinoblastoma in loss-of-function *bri1-116* mutants. Cell cycle markers defects together with the recovery of *bri1-116* loss-of-function mutants strongly suggest that BR-signaling mediates cell cycle progression. Interestingly, the effect on cell division and cell elongation takes part in other plant tissues (Zhiponova et al., 2013), suggesting that BR control plant growth by modulating both cell expansion and cell division across organs.

The availability of tissue specific promoters shed light to the spatial action of different hormonal pathways in regulating final root growth. A compilation of tissue specific expression studies of different downstream hormone controlled factors, established a scenario were different plant hormones control root

growth from different cell types. As an example, the elongating epidermal cells are primary tissue targets of auxin during root gravitropic response (Swarup et al., 2005), triggering differential root growth. Additionally, the GA response in the endodermis controls both elongation and meristematic activities (Ubeda-Tomas et al., 2009; Ubeda-Tomás et al., 2008). BRs perception or biosynthesis in the epidermal layer of the shoot apical meristem rescues the dwarf plants of BRs receptor and biosynthetic loss-of-function mutants (Savaldi-Goldstein et al., 2007). Similarly, expression of BRI1 receptor in the root epidermis rescues root growth defects of *bri1* loss-of-function mutants (Hacham et al., 2011). Our local expression of BRI1 in the meristematic dividing cells rescuing the plant root growth points to an additional regulation of BR-mediated root growth in the dividing cells, were they exert major effects controlling meristem lenght and cell number. Thus, is tempting to speculate that the dividing epidermal cells are the driving force of BR-mediated growth. Moreover, local expression of BRI1 in the QC cells promotes meristem shortening upon BR application. Recently, local expression of SCR in the QC cells have been shown to coordinate stem cell division and cell differentiation though the control of auxin biosynthesis in the QC cells (Moubayidin et al., 2013). Whether BRs control SCR activities autonomously in the QC or act independently of both SCR and auxins remains an open question.

The correct coordination of root growth involves tissue-to-tissue communication, to drive a final univocal response. Tissue communication though plasmodesmata is essential for proper root and lateral root development (Benitez-Alfonso et al., 2013; Vatén et al., 2011). A number of transcription factors have been shown to move across different cell-types including; SHORT-ROOT (SHR) and UPBEAT (UPB) (Nakajima et al., 2001; Tsukagoshi et al., 2010), but we are far to understand how hormone mediated signals communicate between tissue layers. Mobile signals set the radial organization in the vascular cylinder, producing differential accumulation of target mRNAs that determine different cell fates(Carlsbecker et al., 2010). In the BRs case no evidence was presented for movement of neither BES1 nor BZR (Hacham et al.,

2011), and differential accumulation may modulate signaling outputs (Gendron et al., 2012). Identification of mobile signals coordinating hormonal response will certainly advance our understanding of root growth plasticity.

Mechanical forces triggered by the rigid plant cell walls encompasses another level of coordination of growth, thus cell growth of one tissue layer exerts force to the neighbor cell promoting growth (Coen et al., 2004). BR regulate a wide range of cell wall enzymes, hence BR regulate mechanical forces that ultimately drive growth (Sun et al., 2010; Yu et al., 2011). BRI1-mediated signaling in the epidermis could activate cell wall enzymes that ultimately promote growth of meristematic tissue thought mechanical forces. In the other hand, been the activation of cell cycle in the meristem a stochastic event, BRI1 signaling in the dividing cell should have a reduced impact in mechanical forces. Overall, further studies integrating mechanical forces with local signaling event should uncover how final growth is shaped. Which environmental stimuli trigger different plant hormones and how they are coordinated to provide plasticity to root growth remains an exciting future question.

The hormone distribution along the different tissues further reinforces the local action of hormones in different cell types. In this direction, GA levels in the endodermis correlate with the previously proposed roles (Shani et al., 2013). In addition, auxin quantification in different cell types (Petersson et al., 2009) and development of auxin sensors (Brunoud et al., 2012; Sabatini et al., 1999), correlates with the proposed roles of auxin along development. BRs concentrations are very low compared to other hormones (Fujioka and Sakurai, 1997), and technical difficulties deprived their quantification *in planta.* The development of a fluorescently bioactive BR analog (Irani et al., 2012), enable the visualization of BRI1 endocytosis *in planta*. Interestingly, quantification of BRI1 receptors have been carried out in Arabidopsis roots where higher density correlates with dividing tissues, whereas lower concentrations were found in QC cells and differentiation tissues (van Esse et al., 2011). Altogether, BRs control cell division autonomously from the epidermis and meristematic dividing cells.

Our results further reinforce the spatial distribution of hormone signaling pathways that ultimately coordinate growth.

Brassinosteroids control stem cell niche homeostasis

Continuous root growth requires production of new cells thought asymmetric divisions of the stem cells (Scheres, 2007). The stem cell niche is composed by different sets of stem cells surrounding a set of low proliferating cells termed quiescent cells (QC), which maintain and replenish damaged stem cells (van den Berg et al., 1997; 1995). The fate of the stem cell can be used as readout of the QC activity. By looking at the expression of QC–specific markers and the differentiation of CSC, we found that BRs modulate the activity of the stem cell niche at the root apex. The ectopic expression of QC identity markers WOX5, SHR, SCR in plants with increased BR-signaling, and the insensitivity of *bri1-116* loss-of-function mutants to BR-mediated affects on the stem cell niche suggest that BRs control stem cell niche activities mainly though the BRI1 receptor. Moreover, the specific BRL3/BRL1/BAK1 signalosome fine tunes the BR-mediated homeostasis of the stem cell niche (Fabregas et al., 2013). Interestingly, divisions in the QC were not detected in *bzr1*-D gain-of-function mutants, suggesting that BRs act in the stem cell niche thought the BES1 signaling branch. In addition, BR treatment or *bes1*-D gain-of-function mutants promote CSC differentiation further supporting a specific role of BES1-mediated signaling in the stem cell niche.

Stem cell maintenance in the root apex have been shown to require; (i) proper QC patterning by two independent pathways SCR-SHR and PLT, (ii) cell cycle control by RBR and (iii) WOX5 which maintains the CSC non-cell autonomously thought the CLE40-WOX5-ACR4/CLV1 QC-distal stem cell regulatory pathway (Aida et al., 2004; Sabatini et al., 2003; Sarkar et al., 2007; Stahl et al., 2009). The SCR-SHR pathway interacts with RBR, and specifies asymmetric cell division in the ground tissue stem cell (Cruz-Ramírez et al., 2012). Analysis of double mutant combinations showed that BRs act upstream

or independently of the PLT and SCR-SHR pathways in the maintenance of the CSC. The synergic phenotype of *scr-4* and *plt1plt2* mutants after BL application, similarly to *wox5-1;scr-4* double mutants (Sarkar et al., 2007), reinforces an independent role of BRs, SCR-SHR and PLT pathways in the stem cell maintenance. The unchanged levels of PLT upon BL treatment further reinforce independent roles of PLT and BRs in the stem cell niche. RBR downregulation (*Rbi*) promotes QC self-renewal and CSC proliferation (Wildwater et al., 2005), *bri1-116;Rbi* and *bes1-D;Rbi* double mutants have a decreased proliferation of CSC similarly to exogenous BL application to *Rbi*. As discussed above BR promote cell cycle progression and similarly RBR modulates cell cycle, thus we can not exclude that the observed phenotypes are due to cell cycle defects and not specific to the stem cell niche. Interestingly, the lack of functional BRI1 receptor abolishes RBR-mediated QC divisions pointing to an uncoupled role of BRs in the QC and CSC self renewal.

Our genetic and physiological treatments showed that WOX5 control CSC differentiation downstream of BRs. Auxin have been shown to repress WOX5 expression, leading to CSC differentiation. In this context, lower WOX5 levels in *bri1-116* should delay CSC differentiation, while gain-of-function mutants should increase the auxin maximum, restrict WOX5 expression and accelerate differentiation. As none of these features were observed, BRs have to play a role on their own in the stem cell niche homeostasis beyond their interactions with auxin. In agreement, neither auxin exogenous application nor blocking auxin transport promotes CSC differentiation in *bri1-116* mutants supporting an independent role of BRs in CSC maintenance. Interestingly BRs promote QC divisions independently of CSC differentiation upon WOX5 overexpression, reinforcing a dual action of BRs in the QC self-renewal and CSC differentiation.

Spatial analysis of the BR-mediated CSC differentiation pointed to the local requirement of BRI1 signaling in the QC cells to promote CSC differentiation. While BRI1 expression in the epidermis recovers root growth and regulates the activity of AGL42 in the stem cell niche (Hacham et al., 2011), the

control of the stem cell niche homeostasis requires BRI1 in the QC cells. Our spatial analyses shows that BRI-signaling mediates stem cell homeostasis locally.

Altogether, BR-mediated cell cycle defects could be traced back to the stem cell niche where they promote QC division. Additionally, BRs promote CSC differentiation independently of the canonical patterning pathways and auxins. Importantly, both QC divisions and CSC differentiation are independent processes regulated by BRs.

BRAVO a new BR signaling component that represses QC divisions

BRs play key roles in cell division associated to developmental programs such as root meristem (Fabregas et al., 2013; González-García et al., 2011), the formation of organ boundaries (Bell et al., 2012; Gendron et al., 2012) and stomata patterning (Gudesblat et al., 2012; Kim et al., 2012), yet novel BR components operating at a cellular scale have not been disclosed. Our data unveils how BR signaling operate with cellular resolution, and defines BRAVO as a molecular repressor counteracting steroid mediated divisions in the stem cell niche. This mechanism ensures the low rates of cell proliferation in the QC, whereas BRAVO/BES1 bistable behaviour confers the QC cells with the plasticity to adapt to environmental changing conditions. Collectively, our results support that BRAVO is a master regulator of cellular quiescence in plants.

The identification of BRAVO as the single gene appearing in a Venn-diagram to ascertain stem cell specific BR-signaling components using fluorescence-activated cell sorting (FACS) coupled to transcriptomics hinted the potential significance of this locus. Despite that BRAVO gene belongs to a large multigene family, MYB transcription factors (Dubos et al., 2010), the *bravo* knock-out mutants exhibit cell-specific defects at the quiescent center cells of the root stem cell niche.

The regulation of quiescence in the stem cell niche, where the quiescent cells are surrounded by rapidly dividing stem cells, has been a long outstanding question in developmental biology (Hsu and Fuchs, 2012; Morrison and Spradling, 2008). In the plant root, the quiescent cells provides short range signals to maintain stemness of the surrounding cells (van den Berg et al., 1997). Our findings represent a major step forward in the present understanding of how steroids control stem cell division in eukaryotes. The positive actions of steroids on stem niches is well established across phyla (Ables and Drummond-Barbosa, 2010; Simões and Vivanco, 2011) including mammals, and excessive activation may result in pathologies, such as breast or prostate cancer (Lin et al., 2010; Risbridger et al., 2010). However little is known about negative regulators that maintain homeostasis and the long-term function of stem cell niches. In this context, the identification of a novel negative regulator, BRAVO, that inhibits the steroid hormonal pathway of the root stem niche will serve as a novel paradigm that will be of relevance for other stem cell niches, beyond the root.

We propose that BRAVO negatively regulates QC divisions by acting as a safe-lock to retain QC cells in a mitotic inactive status. The lack of BRs signaling in the non-dividing QC cells supported by: (i) the specific BRAVO expression, (ii) the lack of BRs-promoted ERF115 expression (Heyman et al., 2013), suggests that the activation of the BR-signaling pathway is detrimental for proper QC function. This notion is further supported by radiolabeled drug treatment of BR-signaling mutants. Importantly, BRAVO dynamics upon DNA damage suggest its involvement in promoting quiescence, ensuring proper root growth regeneration. Oppositely, BRAVO down-regulation would release the BR-dependent ERF115 expression (Heyman et al., 2013). QC cells are a reservoir of both, auxin transport and biosynthesis (Overvoorde et al., 2010). Despite any specific quantification of BRs *in planta* have been carried out it is attractive to speculate that low levels of BRs in the QC will result in low proliferation activities. Understanding the hierarchy of those among other regulators will further refine our understanding of quiescence.

Cell response to stimuli is fundamental for proper plant adaptation to environmental cues, and these reversible responses account for the renowned plasticity (Siegal-Gaskins et al., 2011). QC cells divide upon stimulation by hormones (González-García et al., 2011; Ortega-Martínez et al., 2007; Scheres, 2007; Zhang et al., 2010), stem cell damage (Heyman et al., 2013) or cell cycle interference (Cruz-Ramírez et al., 2013; Wachsman et al., 2011) enabling replenishment of the stem cells upon damage. To preserve the QC function as a reservoir of stem cells, the transition between divided and non-divided QC cells should be reversible. The proposed mathematical modelling taking into account BES1/BRAVO mutual interaction and BRAVO autoactivation results in a robust response to stimuli that is threshold-like. Upon BR hormone stimuli, the response switches sharply from quiescence to the induction of QC divisions. This sharp transition is reversible and can involve bistable behaviour. This mechanism differs from the SCARECROW/RETINOBLASTOMA bistable circuit that promotes asymmetric cell divisions in the stem cell niche (Cruz-Ramírez et al., 2012), where auxin biases the circuit. The mechanism provided by BES1/BRAVO signaling module gives an example for a selective control of cellular quiescence in eukaryotes, which may be instructive to investigate related mechanisms for preventing cancer in humans.

Identification of BRAVO regulated genes

Increasing knowledge of gene regulatory networks controlling developmental outputs have been crucial for understanding how signaling pathways coordinate. Identification of deregulated genes upon different hormone conditions has been pivotal in understanding the molecular nature of developmental defects (Nemhauser et al., 2006). Recent development of next-generation sequencing technologies allowed massive profiling without the microarray limitation of spotted loci. The combination of deregulated gens with binding regions of transcription factors offers a good approach to elucidate candidate genes to coordinate specific developmental process.

By combining both transcript profiling of divided QC cells in *bravo*, and binding regions of BRAVO in the endogenous expression domain we aimed to disclosed downstream players in the BRAVO pathway. Unfortunately, our ChIP-seq experiments did not raise any specific binding peak after a wide range of analysis. The technical challenge of ChIP experiments coupled with the low number of cells containing BRAVO transcription factor may account for the final negative results. To our knowledge, the analysis of specific binding regions for cell-type specific transcription factors have not been reported. In contrast, publish sets of targets are generated with overexpressing lines or transcription factors ubiquitously expresses. To overcome technical limitations of cell-type specific ChIP experiments efficient isolation of specific cell populations needs to be address. The optimization of FACS, INTACT and other methods to recover higher yields of desiderate low abundant cells represent a major bottleneck. The requirement of large population of cells, compared to transcript profiling experiments, together with the optimization of inmunoprecipitation procedure represents a major challenge in profiling chromatin binding sites of cell-type specific transcription factors. In contrast, cell-type specific profiling of histone binding sites, which are enriched proteins compared to transcription factors, may provide in the future the first steps to overcome technical limitations.

Future perspectives

The role of BRs in different cell-types addresses the existence of cell-type specific regulators. The identification of the cell-specific BRAVO signaling pathway opens a new avenue on the spatial control of hormonal signaling. The proposed role of BRAVO on the regulation of stem cell replenishment, opens a new scenario were BRs control plant survival through the regulation of stem cells. Further understanding of the coordination between stem cells and QC cells, upon stem cell damage will shed light on the mechanistic basis of plants longevity and adaptation to environmental changing conditions.

CONCLUSIONS

1. **Plant roots use Brassinosteroid signaling in the meristem for proper root growth and development. In particular:**
    - **BRs control meristematic activities independent or upstream of PLETHORA and SCARECROW.**
    - **A non-cell autonomous action of BRs from the dividing cells at the meristem is required for normal root growth.**
2. **Brassinosteroid signaling controls the stem cell homeostasis at the root apex.**
    - **BRs control the identity and activity of the quiescent center (QC).**
    - **BRs promote columella stem cell differentiation upstream or independently of auxin.**
    - **Local activation of BRI-signaling in the QC cells promotes columella stem cell differentiation.**
3. **BRAVO defines a novel cell-type specific BR-signaling component**
    - **BRAVO acts as a repressor of QC divisions.**
    - **BRAVO mediates root regeneration upon external damage of root stem cells.**
    - **BRAVO interacts with the BR-regulated transcription factor BES1.**
    - **BRAVO/BES1 establish a ultrasensitive mechanism for the control of quiescent cells at the stem cell niche.**

MATERIALS AND METHODS

Methods in plant biology

Plant material and growth conditions

Arabidopsis thaliana (L.) Heyhn. was in Columbia (Col-0) background. To avoid ecotype variability, the *bes1*-D mutant, originally in Enkheim-2 (En-2) background was introgressed into Col-0 ecotype. In addition to the transgenic lines and high order mutants generated in this work, lines reported in this work were generated in the publications listed in Table 1.

Table 1. Mutants used in this PhD thesis.

Name	Gene	Description	Comments	Reference
bri1-116	At4g39400	BRI strong mutant allele	loss-of-function	(Li and Chory, 1997)
bes1-D	At1g19350	BR-signaling	Gain-of-function	(Yin et al., 2002)
bzr1-D	At1g75080	BR-signaling	Gain-of-function	(Wang et al., 2002)
CYCD3;1ox	At4g34160	Cell cycle	overexpression	(Riou-Khamlichi et al., 1999)
Rbi	At3g12280	Cell cycle	Knock-down	(Wildwater et al., 2005)
plt1plt2	At3g20840 At1g51190	Stem cell patterning	loss-of-function	(Aida et al., 2004)
scr-4	At3g54220	Root patterning	loss-of-function	(Fukaki et al., 1998)
wox5-1	At3g11260	Stem homeostasis	loss-of-function	(Sarkar et al., 2007)
35S:WOX5-GR	At3g11260	Stem homeostasis	inducible overexpression	(Sarkar et al., 2007)
bravo-1	At5g17800	Stem homeostasis	loss-of-function	This work
bravo-2	At5g17800	Stem homeostasis	loss-of-function	This work
RNAi BES1	At1g19350	BR-signaling	Knock-down	(Yin et al., 2002)
pWOX5:GFP	At3g11260	Transcriptional fusion	QC maker	(Sarkar et al., 2007)
pSCR:GFP	At3g54220	Transcriptional	Endodermis/	(Sabatini et al., 1999)

		fusion	QC marker	
pSHR:SHR-GFP	At4g37650	Translational fusion	Stele/endodemis	(Nakajima et al., 2001)
pPLT1:PLT1-YFP	At3g20840	Translational fusion	Stem cell marker	(Galinha et al., 2007)
pPLT2:PLT2-YFP	At1g51190	Translational fusion	Stem cell marker	(Galinha et al., 2007)
pPLT3:PLT3-YFP	At5g10510	Translational fusion	Stem cell marker	(Galinha et al., 2007)
DR5:GUS		Transcriptional fusion	Auxin marker	(Sabatini et al., 1999)
pBRAVO:GFP	At5g17800	Transcriptional fusion		(Lee et al., 2006)
pBRAVO:BRAVO-GFP	At5g17800	Translational fusion		(Lee et al., 2006)
pAthb15:YFP	At1g52150	Transcriptional fusion	Provascular marker	(Zhiponova et al., 2013)
pARF7:GFP	At5g20730	Transcriptional fusion	Provascular marker	(Rademacher et al., 2011)
AGL42:GFP	At5g62165	Transcriptional fusion	QC/vascular initial marker	(Nawy et al., 2005)
pBES1:BES1-GFP	At1g19350	Translational fusion	Endogenous expression	(Yin et al., 2002)
pBZR1:BZR1-CFP	At1g75080	Translational fusion	Endogenous expression	(Wang et al., 2002)
pRP5a:BRI1-YFP	At4g39400	Translational fusion	Meristematic expression	This Work
pWOX5:BRI1-YFP	At4g39400	Translational fusion	QC expression	This Work
pWOX5:BES1-D-YFP	At1g19350	Translational fusion	QC expression	This Work
pGL2:BRI1-YFP	At4g39400	Translational fusion	Epidermis expression	(Hacham et al., 2011)
pSHR:BRI1-YFP	At4g39400	Translational fusion	Stele expression	(Hacham et al., 2011)
pSCR:BRI1-YFP	At4g39400	Translational fusion	Endodermis/QC expression	(Hacham et al., 2011)
BRAVO inducible	At5g17800	Translational fusion	Inducible overexpression	This work

Seeds were surface sterilized with 35% sodium hypochlorite, followed by 4 water washes. To synchronize germination, seeds were vernalized for 72 hours at 4 ºC in darkness. Seeds were grown for 6 days in vertically oriented plates containing ½ Murashige and Skoog (MS) salt mixture (pH=5.7) and 0.8 % of

agar. Pharmacological treatment were performed in plates adding hormones/drugs in the media. Hormones used include; brassinolide (BL) ($C_{28}H_{48}O_6$; Wako, Osaka, Japan), brassinozole (BRZ_{220}) gift from T. Nakano (RIKEN, Japan), N-(1-naphthyl) phthalamic acid (NPA) (Duchefa), 1-naphthaleneacetic acid (NAA) (Duchefa), Absisic acid (ABA) (Duchefa), β-Estradiol (Sigma-Aldrich).

Statistical analysis of root measurements

The root lenght, cell lenght and the number of meristem cells of seedlings grown in vertically orientated plates. Root were scanned and root lenght measured and analyzed with ImageJ software (http://imagej.nih.gov/ij/). All experiments were repeated at least 3 times, measuring 15 different seedling per replica. The number of epidermal cells in individual cell files was used to gauge the meristem length. The meristematic zone was defined as the region of isodiametric cells from the QC up to the cell that was twice the length of the immediately preceding cell. Student's t-test was used to show statistical difference of root lengths. Error bars in the graphic representation show standard deviations

Methods in molecular biology

Molecular cloning and generation of trangenic lines

Trangenic lines were generated by DNA transformation in wild-type (Col-0) plants or bri1-116 heterozygous plants. pPR5:BRI1-YFP, pWOX5:BRI1-YFP, pWOX5:BES1-D-YFP constructs were cloned using recombination Gateway Multisite Cloning system. DNA sequences were amplified from genomic DNA or cDNA from wild-type plants. Primers used for all cloning are detailed in table 2. The purified gene PCR products were placed into the gateway pDONOR221 donor vector by BP reaction mixing 50 fmol of PCR product with 150 ng of the pDONOR221 and 1 µl of BP clonase enzyme diluted up to 5 µl in TE pH 8.0. The same procedure was used for promoter sequences placed in the gateway

P4P1R vector. For tagged YFP a P2RP3 donor vector was used. Recombination LR reaction was performed by mixing the three sequenced pDONOR vectors (10 fmol each one) in a three-component 25 fmol pDEST vector (pB7m34GW) adding 2 µl LR clonase enzyme, diluted up to 8 µl in TE buffer pH 8.0. For estradiol inducible lines pDONOR221 containing the BRAVO coding sequence was recombined with pER8-GW. All recombination reactions were incubated overnight at 25 ºC. All LR recombination reactions described above were transformed into Agrobacterium GV2260 by electroporation. Agrobacterium was selected by antibiotic resistance and transformed to plants and described in (Zhang et al., 2006).

Quantitative real-time PCR

Total RNA from root tips was extracted with Plant RNeasy Mini Kit (Qiagen, Hilden, Germany), DNA contaminations were removed with the DNA-free Kit (Ambion, Austin, TX, USA) and cDNA synthesized with SuperScript III Reverse Transcriptase (Invitrogen), all according to the manufacturer's instructions. Oligonucleotides were design with the Primer Express software (Applied Biosystems by Life Technologies, Carlsbad, CA, USA) and listed in table 2. All primers efficiencies were evaluated by a dilution series, primers with efficiency between 1.9-2.1 were used subsequently. PCR products were detected with the SYBR Green I Master (Roche Diagnostics, Mannheim, Germany). Lightcycler480 software 1.5.0 release (Roche Diagnostics) was used to calculate relative change in expression levels, with three technical replicas. Melting curves analysis at the end of the process and no template controls were carried out to ensure product-specific amplification without primer-dimer artifacts. To evaluate genomic DNA contamination, a control reaction was run without reverse transcriptase.

Table 2. Primers used in this PhD thesis

Primer	Sequence (5'-3')	Use
Cloning		
BRI1f	GGGGACAAGTTTGTACAAAAAAGCAGGCTCTATGAAGA CTTTTTCAAGCTTCTTTCTC	Cloning coding sequence in pDONOR221
BRI1r	GGGGACCACTTTGTACAAGAAAGCTGGGTATAATTTTC CTTCAGGAAC TTCTTTTATACTC	Cloning coding sequence in pDONOR221
BES1-Df	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGAAAA GATTCTTCTATAATTCC	Cloning coding sequence in pDONOR221
BES1-Dr	GGGGGACCACTTTGTACAAGAAAGCTGGGTAACTATG AGCTTTACCATTTCC	Cloning coding sequence in pDONOR221
pRP5af	GGGGACAAGTTTGTACAAAAAAGCAGGCTCTATGAATC CAAATCTCCTTGAGAAA	Cloning 1.7 kb promoter sequence in pDONORP4P1R
pRP5ar	GGGGACCACTTTGTACAAGAAAGCTGGGTAGGAAGCT CCAACTCCAAGAA	Cloning 1.7 kb promoter sequence in pDONORP4P1R
pWOX5f	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATTAGCC AACGTTACAACTTACAA	Cloning 5 kb promoter sequence in pDONORP4P1R
pWOX5r	GGGGACCACTTTGTACAAGAAAGCTGGGTTGTTCAGAT GTAAAGTCCTCAACTG	Cloning 5 kb promoter sequence in pDONORP4P1R
pBRAVOf	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCTCCATCA AAATTATGTGGTTGC	Cloning 2 kb promoter sequence in pDONOR207
pBRAVOr	GGGGACCACTTTGTACAAGAAAGCTGGGTTGTTTCTGG GTTTAGGGATTAAGG	Cloning 2 kb promoter sequence in pDONOR207
BRAVOf	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGAATC CAAATCTCCTTGAGAAA	Cloning coding sequence in pDONOR207
BRAVOr	GGGGACCACTTTGTACAAGAAAGCTGGGTCGGAAGCT CCAACTCCAAGAA	Cloning coding sequence in pDONOR207
Genotyping		
plt1f	ATCCAACCACCTAGTAGCTTACAACGACTC	Genotype *plt1-4*
plt1r	TCGAGCCACCACCGTACTGGAAACT	Genotype *plt1-4*
plt2f	GTTACCTACAGTCGTCACTTGTGC	Genotype *plt2-2*
plt2r	ACTCTTGTCTCGTCATGTTTTTCA	Genotype *plt2-2*
JL202	CATTTTATAATAACGCTGCGGACATCTAC	Border primer for *plt1-4* and *plt2-2* genotyping
wox5-1f	TAGATGGAACAGAAGCCTAGATAGGTTAGGA	Genotype *wox5-1*
wox5-1r	TCTGTGATGCAAATAGAACTATTCGTTAATG	Genotype *wox5-1*
SalkLBa1	TGGTTCACGTAGTGGGCCATCG	Border primer for *wox5-1* genotyping
bri1-116f	CAT CG G AAC CAT TGT TAT CAA ACG TC	Genotype *bri1-116*, PCR followed by MseI digestion
bri1-116r	CAA TCT TAA CTG GAT TTC TCT GTC	Genotype *bri1-116*, PCR followed by MseI digestion
bravo-1f	TCTCTGCACACACTGACCATC	Genotype *bravo-1*
bravo-1r	TTTTGTTACTCCAAATTCCGC	Genotype *bravo-1*
bravo-2f	TCCCTTAATCCCTAAACCCAG	Genotype *bravo-2*
bravo-2r	CCTGATGCAAGGGTACTATCG	Genotype *bravo-2*
SalkLBb1.3	ATTTTGCCGATTTCGGAAC	Border primer genotype *bravo-1* and *bravo-2*

ChIP		
5'UTR pBRAVOf	ATTGAACTTTTGTTTGTCACCATTC	BRAVO promoter, ChIP.
5'UTR pBRAVOf	TAGGTACGAAGGGAACATAGTTTTT	BRAVO promoter, ChIP.
E-box /MYB pBRAVOf	CACTTAACGTACGTGCAATATCTGT	BRAVO promoter, ChIP.
E-box/MYB pBRAVOr	ACAGTCTCGGAAACTCCAAAAA	BRAVO promoter, ChIP.
E-box 2 pBRAVOf	GATTATTAGGCCACAATTTGGTAAT	BRAVO promoter, ChIP.
E-box 2 pBRAVOr	TTATTCACACACCAAAAGAAATTGA	BRAVO promoter, ChIP.
TSS pBRAVOf	TCTTTCCTCTTCCACCATATAAACA	BRAVO promoter, ChIP.
TSS pBRAVOr	GGGATTAAGGGAAGAGAAACTAAAA	BRAVO promoter, ChIP.
Exon pBRAVOf	TGGCATATATGGTAAAAAGATGGAC	BRAVO promoter, ChIP.
Exon pBRAVOr	GTAATAGCCACTCCTCATCTTCATC	BRAVO promoter, ChIP.
At3g60550f	GGTCTCCTTCTGGTATTTCAAACTT	Positive control BES1 ChIP
At3g60550r	AGGAACCACCTGATGACACGTA	Positive control BES1 ChIP
UBC30f	CAAATCCAAAACCCTAGAAACCGAA	Negative control ChIP, and reference for quantitative PCR
UBC30r	AACGACGAAGATCAAGAACTGGGAA	Negative control ChIP, and reference for quantitative PCR
Real time		
BRAVO left f	CTGTTAGCAGCTCATCGAGCC	Real time PCR, left side T-DNA insertion.
BRAVO left r	ATGACGTGCCAATGGTTCTTG	Real time PCR, left side T-DNA insertion.
BRAVO right f	TGTTAGCAGCTCATCGAGCCT	Real time PCR, right side T-DNA insertion.
BRAVO right r	GATGACGTGCCAATGGTTCTT	Real time PCR, right side T-DNA insertion.
CYCB1;2F	CCTGAACAAGTCAGAGGTGCT	Real time PCR
CYCB1;2R	CCTCCTTGAACTCCGGGAAC	Real time PCR
CYCD3;3F	ACTCAAAGTTGATTCGGAGAAGG	Real time PCR
CYCD3;3R	ATCGGACTAGCGGGTTGTTG	Real time PCR
CYCD2;2F	ACAGGAGAGCATTGGGTGTG	Real time PCR
CYCD2;2R	TCCTGAGATCTTGAAATTGACGGA	Real time PCR
RBRf	TTGAACAACAGCAGCAGCAACC	Real time PCR
RBRr	TCTGTTGGCTCGGTTTTAAGGG	Real time PCR
WEE1f	AGATGCCAATTGCGGAGAGA	Real time PCR
WEE1r	TCCTGTGAAACTCTCCTGGC	Real time PCR

Chromatin Immunoprecipitation

For ChIP experiments 35S:BES1-D-GFP and wild-type control plants were grown in half MS under long-day conditions for 6 days. Seedlings were fixed with 1% of formaldehyde. Nuclei extraction was performed according to (Deal

and Henikoff, 2011). ChIP experiments using anti-GFP antibodies were performed according to (Gendrel et al., 2005). Detection of PCR products was performed using Absolute qPCR SYBR Green mix (Thermo scientific) in a Biorad termocycler. Three different biological replicas were performed for each region of interest. BRAVO ChIP were performed using 3 grams of roots tips expressing *pBRAVO:BRAVO-GFP* treated 5 days with 20uM NPA, subsequent steps were conducted as BES1-D ChIP, three biological replicates were performed.

RNA-seq

Plant material & isolation

The pWOX5:GFP plants and *bravo*;pWOX5GFP seeds were surface sterilized in 35% sodium hypochlorite, vernalized for 72 hours at 4C in darkness and grown on vertically oriented plates, containing ½ Murashige and Skoog (MS) salt medium, without sucrose and 0.8% agar. Plants were subsequently grown vertically for 6 days in constant light conditions at 22C and 45% humidity.

QC cells were isolated as previously described(Birnbaum et al., 2005). Briefly, root tips of the fluorescent marker lines were cut off using a scalpel and transferred to solution B for protoplasting (0.6 M Mannitol, 10 mM MgCl2, 10 mM KCl, 10 mM CaCl2, 1mg/ml BSA, 0.39 mg/ml MES, pH 5.5, 1.5% Cellulase R10 (Yakult), 0.1% Pectolyase Y-23 (Yakult). Root tips were protoplasted on a platform shaker for 50 min, release of protoplasts was facilitated by gently streaking root tips on a 75 uM cell strainer every 15 min. GFP Cells of interest were identified by GFP signal (Olympus, stereo zoom binocular microscope) and washed with solution A (0.6M Mannitol, 10 mM KCl, 10 mM MgCl2, 10 mM CaCl2, 1 mg7ml BSA, 0.39 mg/ml MES, pH 5.5). After 3 washes, cells were transferred within 1 ul of solution A in PCR tubes and immediately frozen with liquid nitrogen.

RNA and cDNA libraries preparation

RNAs from 3 pooled samples of approximately 100 cells each were extacted using Qiagen micro RNA extraction following manufactures recommendations. cDNA preparation and amplification was performed using SMARTer Ultra low RNA kit for Illumina sequencing following manufactures recommendations, with X PCR amplification cycles. Libraries were fragmented with the Covaris S2 system using Covaris mircoTUBEs (#cat3520045) and a volume 130ul for shearing. After fragmentation, the volume was reduced to 44 µl using a SpeedVac concentrator, and samples were subjected to standard Illumina library preparation using the NEBNext ChIP-Seq Sample Prep Master Mix Set 1 kit according to the manufacturer's instructions. Illumina PE adapters multiplexing barcodes were ligated (the amount of adapters was adjusted according to the amount of input material), and Illumina PE primers (PE PCR Primer 1.0 and PE PCR Primer 2.0) were used for the PCR enrichment step (15 cycles) of the NEBNext protocol. The final purification step was performed using AMPure XP beads (Beckman Coulter) rather than columns, and clusters were generated by following the standard Illumina protocol. Samples were sequenced on an Illumina MiSeq machine.

Alignment of reads & stadistical analysis

The high quality reads were aligned against the *Arabidopsis thaliana* reference genome sequence (TAIR 10) with TopHat (version 2.0.9). The resulting alignment files were used as input for HtSeq-count (version 0.5.4p2) together with the TAIR10 annotation file to calculate transcript expression values (read counts). All stadistical analyses were performed with R using the libraries; SERE, ArrayQualityMetrix, HTSFilter and TCC. The overall quality of the experiment was evaluated on the basis of the similarity between replicates by using several approaches. The algorithm SERE calculates similarity scores among samples assuming a binomial distribution of the read counts. HTSFilter was used to identify the minimum normalized read count to remove transcripts with very low and excessively variable expression across the samples. The final dataset was composed by all the transcripts passing the HTSFilter step. The final dataset was analysed with TCC to identify differentially expressed (DE)

transcripts between *bravo*;pWOX5:GFP and pWOX5:GFP. TMM normalization was used to normalize counts across the experiments, then exact test was used with an FDR treshold of 0.05. A total of 16 transcripts were identified as differentially expressed. 1 gene and 15 genes resulted up- and down-regulated, respectively.

Imaging

Confocal microscopy

A FV 1000 confocal microscope (Olympus, Tokyo, Japan) was used. Six-day-old roots were stained with 10 µg/ml propidium iodide (PI) for 2-5 minutes, and visualized after excitation by a Kr/Ar 488-nm laser line. PI and GFP were detected with a band-pass 570-670 nm filter and 500-545 nm filter, respectively. For yellow fluorescent proteins, the excitation wavelengths were 488nm and 405 nm, and fluorescence was collected in the ranges of 493-536 nm. Starch granules in columella cells were visualized by a modified Pseudo-Schiff (mPS)-PI staining method (Truernit et al., 2008). Images were processed with ImageJ (http://imagej.nih.gov/ij/), and assembled with Photoshop CS (Adobe Systems, San Jose, CA, USA).

Fluorescence lifetime microscopy and data analysis

Fluorescence lifetime of the donor was experimentally measured in the presence and absence of the acceptor. FRET efficiency (E) was calculated by comparing the lifetime of the donor in the presence (τ_{DA}) or absence (τ_D) of the acceptor: $E=1-(\tau_{DA})/(\tau_D)$. Statistical comparisons between control (donor) and assay (donor + acceptor) lifetime values were performed by Student *t* test. FRET-FLIM measurements were performed using a FLIM system coupled to a streak camera. The light source (l = 439 nm) was a pulsed diode laser working at 2 MHz (Hamamatsu Photonics, Japan). All images were acquired with a 60x oil immersion lens (Plan Apo 1.4 numerical aperture, IR) mounted on an inverted microscope (Eclipse TE2000E, Nikon, Japan) coupled to the FLIM

system. The fluorescence emission was directed back out into the detection unit through a band pass filter. The FLIM unit was composed of a streak camera (Streakscope C4334, Hamamatsu Photonics, Japan) coupled to a fast and high sensitivity CCD camera (model C8800-53C, Hamamatsu). For each nucleus, average fluorescence decay profiles were plotted and lifetimes were estimated by fitting data with tri-exponential function using a non-linear least-squares estimation procedure.

Fluorimetric GUS Assays

For GUS reporter assays, the indicated constructs were transiently expressed in *N. benthamiana* leaves using *Agrobacterium*. Leaf discs were collected 36 h after agroinoculation, frozen in liquid nitrogen and stored at -80°C until processing. After protein extraction, 1 mg of total protein was used in replicates to measure enzymatic activities of individual samples. GUS activity was measured using the substrate 4-methylumbelliferyl-b-D-glucuronidase as described (Froidure et al., 2010).

Methods in biochemistry

Co-Immunoprecipitation

Approximately 2 g (1-2 leafs) were ground in liquid nitrogen using a mortar. The frozen, powdered material was transferred to a 50 mL falcon tube and 2 volumes (20 mL approx.) of extraction buffer was added (50 mM Tris/HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, and Proteases inhibitors PMSF, Leupeptine, Aprotinine, Pepstatin A and E-64). After thawing, the powdered material was homogenized by shaking. The resulting extract was left on ice for 20 min, followed by sonication [15sec/paused/15sec] x 3 times at 10% of Amplitude Intensity, on ice. Samples were incubated 10 min on ice and subjected to centrifugation at 10.000 rpm 2 times for 10 min at 4°C. The resulting supernatant (17,5mL) was incubated, under rotation at 4°C, with 75 μl

of magnetic beads attached to anti-GFP antibodies (Miltenyi Biotec) for 1 hour. Magnetic beads with attached proteins were immobilized on a magnetic separator (MACS, Miltenyi Biotec) and washed 2 times with 200μl extraction buffer. Bound proteins were eluted from the immobilized beads with 50 μl hot (95°C) SDS-PAGE loading buffer (1,6% SDS, 0.1M DTT, 5% Glycerol, 0.08M TrisHCl pH 6.8, Bromophenol blue).

Eluted proteins were separated in 15% acrylamide SDS denaturing gel. Proteins were transferred to a nitrocellulose membrane (Hybonnd-ECL, GE Healthcare$^{@}$) by blotting 1 hour at 100mV on ice under agitation. Membranes containing the immunoprecipitated proteins were blocked during 1 hour in 3% milk in PBS-T (0.1% Tween). One membrane was incubated with anti-GFP primary antibody for 1 hour and the other membrane was incubated with anti-BES1 primary antibody for 2.5 hours. Both membranes were incubated 1 hour in secondary anti-rabbit antibody

Methods in cell biology

GUS activity staining

Six-day-old roots were fixed on ice-cold 90% acetone for 20 min on ice, rinsed with water and incubated with 100nM sodium phosphate buffer (pH 7.2), 10 mM sodium EDTA, 0.1 % Triton X-100, 1 mg/ml 5-bromo-4-chloro-3indolyl-b-D-glucuronide (X-Gluc; Duchefa, Haarlem, The Netherlands), 10 mM potassium Ferrocyanide (K3[Fe(CN)6]) and potassium Ferricyanide K4[Fe(CN)6]). Penetration of the solution was ensures by 10 minutes vaccum. Finally the samples were incubated at 37ºC until the signal appeared. Samples were mounted in Chloral-hydrate (8:3:1 chloral hydrate: distilled water:glycerol) and visualized with upright widefield fluorescence microscope, Axiophot, Zeiss.

Historesin embedding

Six-day-old seedlings were fixed at 4 ºC overnight in 1.25% Glutaraldehyde in 0.1M sodium cacodylate buffer (pH 7.4; Electron MIcroscopy Science). Fixer solution was removed by water rinsed (x2). Samples were dehydrated through increasing graded series of etanol: 30%, 50%, 70%, 90% and 100% etanol dilutions every 30 minutes. Next, 50% Historesin-I (Technkovit) in etanol solution was added and incubated for 30 min, followed by Historesin-I 100% solution wash for 1 hour. New Historesin-I 100% solution was added and samples were kept at 4 ºC overnight. Blocks were done by placing samples into plastic molds, which were filled with 100% Historesin-II solution (Technkovit). Each mold was covered with parafilm and kept overnight at 4 ºC to accelerate their solidification.

Historesin-I (for 100ml): 100ml Historesin + 1ml Hardener-I

Historesin-II (for 15ml): 15ml Historesin-I + 1ml Hardener-II

Transverse root sections (3µM) were done using a Leica Microtome (Microtome RM2265, Leica). Sections were stainedwith 0.1% Toluidine blue 0.1M NaPO4 buffer pH 7.0, rinsed and mounted in water for microscopical visualitzation in Axiophot widefield fluorescence microscope, Axiophot, Zeiss.

Mathematical model

The mathematical model was entirely done by David Frigola and Dr. Marta Ibañes (Faculty of Physics, Univ. of Barcelona). It is summarize here for overall clarity of the BRAVO studies. To model the BRAVO-BES1D interaction module, we considered that *BRAVO* transcription is repressed by BES1D and activated by BRAVO, and that BES1D and BRAVO form a heterodimer which is inactive (i.e. does not bind to the *BRAVO* promoter). We assumed: (1) transcription is independently controlled by BES1D and BRAVO, (2) BRAVO autoactivates itself in a non-cooperative way and BES1-D represses *BRAVO* non-cooperatively too, (3) reversible dephosphorylation and phosphorylation of

BES1, and (4) a constant production rate for BES1. From this, we obtained the following 17 chemical equations:

$$D_1 + M \underset{k_{M-}}{\overset{k_{M+}}{\rightleftharpoons}} D_{1M} \; ; \; D_2 + B_d \underset{k_{B-}}{\overset{k_{B+}}{\rightleftharpoons}} D_{2B}$$

$$D_1 + D_2 \xrightarrow{\alpha} D_1 + D_2 + M$$

$$D_{1M} + D_2 \xrightarrow{\varepsilon_1 \alpha} D_{1M} + D_2 + M$$

$$D_1 + D_{2B} \xrightarrow{\varepsilon_2 \alpha} D_1 + D_{2B} + M$$

$$D_{1M} + D_{2B} \xrightarrow{\varepsilon_3 \alpha} D_{1M} + D_{2B} + M$$

$$B \underset{k_{P+}}{\overset{k_{P-}}{\rightleftharpoons}} B_d$$

$$M \xrightarrow{d_M} \varnothing$$

$$\varnothing \xrightarrow{\beta} B \xrightarrow{d_B} \varnothing$$

$$B_d \xrightarrow{d_{B_d}} \varnothing$$

$$M + B_d \underset{k_{D-}}{\overset{k_{D+}}{\rightleftharpoons}} C \xrightarrow{d_C} \varnothing$$

where all variables are concentrations of the following molecular species: *M* stands for free BRAVO, *B* for free phosphorylated BES1, B_d for free dephosphorylated (active) BES1, *C* for the BRAVO-dephosphorylated BES1 heterodimer, D_1 and D_2 stand for the free binding sites for BRAVO and dephosphorylated BES1 respectively in the *BRAVO* promoter, D_{1M} and D_{2B} correspond to these binding sites bound to BRAVO and dephosphorylated BES1 respectively. Rates for each reaction are indicated. Explicit mRNA dynamics with linear mRNA degradation and protein production proportional to mRNA concentration have been omitted for simplicity. Accordingly, the production rate of BRAVO protein α stands for the transcription rate times the translation rate over the *BRAVO* mRNA degradation rate. The deterministic stationary solutions computed do not depend on this simplification. α gives the production rate of BRAVO protein when the binding sites for BRAVO and dephosphorylated BES1 are free. Hereafter we call this production rate the basal production rate. ε_1 is the ratio between the production rate of BRAVO when *BRAVO* is bound to its promoter over the basal production rate. Therefore, $\varepsilon_1 > 1$ stands for *BRAVO* auto-activation. ε_2 is the ratio between the BRAVO

production rate when dephosphorylated BES1 is bound to the *BRAVO* promoter over the basal production rate. Notice that ε_2<1 stands for BES1 repression of *BRAVO* transcription. ε_3 is the ratio between the BRAVO production rate when both BRAVO and dephosphorylated BES1 are bound to the *BRAVO* promoter over the basal production rate. ε_3<1 and ε_3>1 stand for inhibition and activation, respectively, driven when both dephosphorylated BES1 and BRAVO are bound to the *BRAVO* promoter, whereas ε_3=1 indicates that production is not modified from the basal one.

Using mass action kinetics we can translate these reactions into the following 6 ordinary differential equations

$$\dot{D}_1 = k_{M-} D_{1M} - k_{M+} M D_1 \; ; \quad D_1 + D_{1M} = D_{tot1}$$
$$\dot{D}_2 = k_{B-} D_{2B} - k_{B+} B_d D_2 \quad ; \quad D_2 + D_{2B} = D_{tot2}$$
$$\dot{M} = \alpha(D_1 D_2 + \varepsilon_1 D_{1M} D_2 + \varepsilon_2 D_1 D_{2B} + \varepsilon_3 D_{1M} D_{2B}) + k_{M-} D_{1M} + k_{D-} C - k_{M+} M D_1 - k_{D+} M B_d - d_M M$$
$$\dot{B}_d = k_{p-} B + k_{B-} D_{2B} + k_{D-} C - k_{P+} B_d - k_{B+} B_d D_2 - k_{D+} M B_d - d_{B_d} B_d$$
$$\dot{B} = \beta + k_{p+} B_d - k_{p-} B - d_B B$$
$$\dot{C} = k_{D+} M B_d - k_{D-} C - d_C C$$

BIBLIOGRAPHY

Ables, E.T., and Drummond-Barbosa, D. (2010). The Steroid Hormone Ecdysone Functions with Intrinsic Chromatin Remodeling Factors to Control Female Germline Stem Cells in Drosophila. Stem Cell *7*, 581–592.

Achard, P., Gusti, A., Cheminant, S., Alioua, M., Dhondt, S., Coppens, F., Beemster, G.T.S., and Genschik, P. (2009). Gibberellin signaling controls cell proliferation rate in Arabidopsis. Curr Biol *19*, 1188–1193.

Aida, M., Beis, D., Heidstra, R., Willemsen, V., Blilou, I., Galinha, C., Nussaume, L., Noh, Y.-S., Amasino, R., and Scheres, B. (2004). The PLETHORA genes mediate patterning of the Arabidopsis root stem cell niche. Cell *119*, 109–120.

Alonso, J.M. (2003). Genome-Wide Insertional Mutagenesis of Arabidopsis thaliana. Science *301*, 653–657.

Arabidopsis Genome Initiative (2000). Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. Nature *408*, 796–815.

Azpiroz, R., Wu, Y., LoCascio, J.C., and Feldmann, K.A. (1998). An Arabidopsis brassinosteroid-dependent mutant is blocked in cell elongation. Plant Cell *10*, 219–230.

Bai, M.-Y., Shang, J.-X., Oh, E., Fan, M., Bai, Y., Zentella, R., Sun, T.-P., and Wang, Z.-Y. (2012). Brassinosteroid, gibberellin and phytochrome impinge on a common transcription module in Arabidopsis. Nat Cell Biol *14*, 810–817.

Bajguz, A., and Hayat, S. (2009). Effects of brassinosteroids on the plant responses to environmental stresses. Plant Physiol Biochem *47*, 1–8.

Bao, F., Shen, J., Brady, S.R., Muday, G.K., Asami, T., and Yang, Z. (2004). Brassinosteroids interact with auxin to promote lateral root development in Arabidopsis. Plant Physiol *134*, 1624–1631.

Beemster, G.T., and Baskin, T.I. (1998). Analysis of cell division and elongation underlying the developmental acceleration of root growth in Arabidopsis thaliana. Plant Physiol *116*, 1515–1526.

Bell, E.M., Lin, W.-C., Husbands, A.Y., Yu, L., Jaganatha, V., Jablonska, B., Mangeon, A., Neff, M.M., Girke, T., and Springer, P.S. (2012). Arabidopsis lateral organ boundaries negatively regulates brassinosteroid accumulation to limit growth in organ boundaries. Proc Natl Acad Sci USA *109*, 21146–21151.

Benitez-Alfonso, Y., Faulkner, C., Pendle, A., Miyashima, S., Helariutta, Y., and Maule, A. (2013). Symplastic Intercellular Connectivity Regulates Lateral Root Patterning. Dev Cell *26*, 136–147.

Bergmann, D.C., and Sack, F.D. (2007). Stomatal development. Annu Rev Plant Biol *58*, 163–181.

Birnbaum, K. (2003). A Gene Expression Map of the Arabidopsis Root. Science *302*, 1956–1960.

Birnbaum, K., Jung, J.W., Wang, J.Y., Lambert, G.M., Hirst, J.A., Galbraith, D.W., and Benfey, P.N. (2005). Cell type-specific expression profiling in plants via cell sorting of protoplasts from fluorescent reporter lines. Nat Methods *2*, 615–619.

Brady, S.M., Orlando, D.A., Lee, J.-Y., Wang, J.Y., Koch, J., Dinneny, J.R., Mace, D., Ohler, U., and Benfey, P.N. (2007). A high-resolution root spatiotemporal map reveals dominant expression patterns. Science *318*, 801–806.

Breakfield, N.W., Corcoran, D.L., Petricka, J.J., Shen, J., Sae-Seaw, J., Rubio-Somoza, I., Weigel, D., Ohler, U., and Benfey, P.N. (2012). High-resolution experimental and computational profiling of tissue-specific known and novel miRNAs in Arabidopsis. Genome Res *22*, 163–176.

Brennecke, P., Anders, S., Kim, J.K., odziejczyk, A.A.K.L., Zhang, X., Proserpio, V., Baying, B., Benes, V., Teichmann, S.A., Marioni, J.C., et al. (2013). Brennecke13_single cell RNA-seq. Nat Methods *10*, 1093–1095.

Brunoud, G., Wells, D.M., Oliva, M., Larrieu, A., Mirabet, V., Burrow, A.H., Beeckman, T., Kepinski, S., Traas, J., Bennett, M.J., et al. (2012). A novel sensor to map auxin response and distribution at high spatio-temporal resolution. Nature 1–6.

Busch, W., Miotk, A., Ariel, F.D., Zhao, Z., Forner, J., Daum, G., Suzaki, T., Schuster, C., Schultheiss, S.J., Leibfried, A., et al. (2010). Transcriptional control of a plant stem cell niche. Dev Cell *18*, 849–861.

Caño-Delgado, A., Lee, J.-Y., and Demura, T. (2010). Regulatory mechanisms for specification and patterning of plant vascular tissues. Annu Rev Cell Dev Biol *26*, 605–637.

Caño-Delgado, A., Yin, Y., Yu, C., Vafeados, D., Mora-García, S., Cheng, J.-C., Nam, K.H., Li, J., and Chory, J. (2004). BRL1 and BRL3 are novel brassinosteroid receptors that function in vascular differentiation in Arabidopsis. Development *131*, 5341–5351.

Carlsbecker, A., Lee, J.-Y., Roberts, C.J., Dettmer, J., Lehesranta, S., Zhou, J., Lindgren, O., Moreno-Risueno, M.A., Vatén, A., Thitamadee, S., et al. (2010).

Cell signaling by microRNA165/6 directs gene dose-dependent root cell fate. Nature *465*, 316–321.

Casamitjana-Martinez, E., Hofhuis, H.F., Xu, J., Liu, C.-M., Heidstra, R., and Scheres, B. (2003). Root-specific CLE19 overexpression and the sol1/2 suppressors implicate a CLV-like pathway in the control of Arabidopsis root meristem maintenance. Current Biology *13*, 1435–1441.

Ceserani, T., Trofka, A., Gandotra, N., and Nelson, T. (2009). VH1/BRL2 receptor-like kinase interacts with vascular-specific adaptor proteins VIT and VIK to influence leaf venation. Plant J *57*, 1000–1014.

Chang, H.H., Hemberg, M., Barahona, M., Ingber, D.E., and Huang, S. (2008). Transcriptome-wide noise controls lineage choice in mammalian progenitor cells. Nature *453*, 544–547.

Cheng, Y., Zhu, W., Chen, Y., Ito, S., Asami, T., and Wang, X. (2014). Brassinosteroids control root epidermal cell fate via direct regulation of a MYB-bHLH-WD40 complex by GSK3-like kinases. eLife.

Cheung, T.H., and Rando, T.A. (2013). Molecular regulation of stem cell quiescence. Nat Rev Mol Cell Biol *14*, 329–340.

Choe, S., Dilkes, B.P., Fujioka, S., Takatsuto, S., Sakurai, A., and Feldmann, K.A. (1998). The DWF4 gene of Arabidopsis encodes a cytochrome P450 that mediates multiple 22alpha-hydroxylation steps in brassinosteroid biosynthesis. Plant Cell *10*, 231–243.

Choe, S., Fujioka, S., Noguchi, T., Takatsuto, S., Yoshida, S., and Feldmann, K.A. (2001). Overexpression of DWARF4 in the brassinosteroid biosynthetic pathway results in increased vegetative growth and seed yield in Arabidopsis. Plant J *26*, 573–582.

Chory, J., Nagpal, P., and Peto, C.A. (1991). Phenotypic and Genetic Analysis of det2, a New Mutant That Affects Light-Regulated Seedling Development in Arabidopsis. Plant Cell *3*, 445–459.

Clouse, S.D., Langford, M., and McMorris, T.C. (1996). A brassinosteroid-insensitive mutant in Arabidopsis thaliana exhibits multiple defects in growth and development. Plant Physiol *111*, 671–678.

Clouse, S.D. (2011). Brassinosteroid Signal Transduction: From Receptor Kinase Activation to Transcriptional Networks Regulating Plant Development. Plant Cell.

Coen, E., Rolland-Lagan, A.-G., Matthews, M., Bangham, J.A., and Prusinkiewicz, P. (2004). The genetics of geometry. Proc Natl Acad Sci USA *101*, 4728–4735.

Colón-Carmona, A., You, R., Haimovitch-Gal, T., and Doerner, P. (1999).

Technical advance: spatio-temporal analysis of mitotic activity with a labile cyclin-GUS fusion protein. Plant J *20*, 503–508.

Cruz-Ramírez, A., Diaz-Trivino, S., Blilou, I., Grieneisen, V.A., Sozzani, R., Zamioudis, C., Miskolczi, P., Nieuwland, J., Benjamins, R., Dhonukshe, P., et al. (2012). A bistable circuit involving SCARECROW-RETINOBLASTOMA integrates cues to inform asymmetric stem cell division. Cell *150*, 1002–1015.

Cruz-Ramírez, A., Diaz-Trivino, S., Wachsman, G., Du, Y., Arteaga-Vázquez, M., Zhang, H., Benjamins, R., Blilou, I., Neef, A.B., Chandler, V., et al. (2013). A SCARECROW-RETINOBLASTOMA Protein Network Controls Protective Quiescence in the Arabidopsis Root Stem Cell Organizer. PLoS Biol *11*, e1001724.

Deal, R.B., and Henikoff, S. (2011). The INTACT method for cell type-specific gene expression and chromatin profiling in Arabidopsis thaliana. Nat Protoc *6*, 56–68.

Di Laurenzio, L., Wysocka-Diller, J., Malamy, J.E., Pysh, L., Helariutta, Y., Freshour, G., Hahn, M.G., Feldmann, K.A., and Benfey, P.N. (1996). The SCARECROW gene regulates an asymmetric cell division that is essential for generating the radial organization of the Arabidopsis root. Cell *86*, 423–433.

Ding, Z., and Friml, J. (2010). Auxin regulates distal stem cell differentiation in Arabidopsis roots. Proc Natl Acad Sci USA *107*, 12046–12051.

Dinneny, J.R., Long, T.A., Wang, J.Y., Jung, J.W., Mace, D., Pointer, S., Barron, C., Brady, S.M., Schiefelbein, J., and Benfey, P.N. (2008). Cell identity mediates the response of Arabidopsis roots to abiotic stress. Science *320*, 942–945.

Dolan, L., Janmaat, K., Willemsen, V., Linstead, P., Poethig, S., Roberts, K., and Scheres, B. (1993). Cellular organisation of the Arabidopsis thaliana root. Development *119*, 71–84.

Domagalska, M.A., Schomburg, F.M., Amasino, R.M., Vierstra, R.D., Nagy, F., and Davis, S.J. (2007). Attenuation of brassinosteroid signaling enhances FLC expression and delays flowering. Development *134*, 2841–2850.

Du, Z., Zhou, X., Ling, Y., Zhang, Z., and Su, Z. (2010). agriGO: a GO analysis toolkit for the agricultural community. Nucleic Acids Res *38*, W64–W70.

Dubos, C., Stracke, R., Grotewold, E., Weisshaar, B., Martin, C., and Lepiniec, L. (2010). MYB transcription factors in Arabidopsis. Trends Plant Sci *15*, 573–581.

Fabregas, N., Li, N., Boeren, S., Nash, T.E., Goshe, M.B., Clouse, S.D., de Vries, S., and Cano-Delgado, A.I. (2013). The BRASSINOSTEROID INSENSITIVE1-LIKE3 Signalosome Complex Regulates Arabidopsis Root Development. Plant Cell.

Fàbregas, N., and Caño-Delgado, A.I. (2014). Turning on the microscope turret: a new view for the study of brassinosteroid signaling in plant development. Physiol Plant n/a–n/a.

Fletcher, J.C. (2002). Shoot and floral meristem maintenance in arabidopsis. Annu Rev Plant Biol *53*, 45–66.

Fridman, Y., Elkouby, L., Holland, N., Vragovi, K., Elbaum, R., and Savaldi-Goldstein, S. (2014). Root growth is modulated by differential hormonal sensitivity in neighboring cells. Genes Dev *28*, 912–920.

Friedrichsen, D.M., Joazeiro, C.A., Li, J., Hunter, T., and Chory, J. (2000). Brassinosteroid-insensitive-1 is a ubiquitously expressed leucine-rich repeat receptor serine/threonine kinase. Plant Physiol *123*, 1247–1256.

Friml, J., Vieten, A., Sauer, M., Weijers, D., Schwarz, H., Hamann, T., Offringa, R., and Jürgens, G. (2003). Efflux-dependent auxin gradients establish the apical-basal axis of Arabidopsis. Nature *426*, 147–153.

Froidure, S., Canonne, J., Daniel, X., Jauneau, A., Brière, C., Roby, D., and Rivas, S. (2010). AtsPLA2-alpha nuclear relocalization by the Arabidopsis transcription factor AtMYB30 leads to repression of the plant defense response. Proc Natl Acad Sci USA *107*, 15281–15286.

Fujioka, S., and Sakurai, A. (1997). Brassinosteroids. Nat. Prod. Rep. *14*, 1.

Fukaki, H., Wysocka-Diller, J., Kato, T., Fujisawa, H., Benfey, P.N., and Tasaka, M. (1998). Genetic evidence that the endodermis is essential for shoot gravitropism in Arabidopsis thaliana. Plant J *14*, 425–430.

Fulcher, N., and Sablowski, R. (2009). Hypersensitivity to DNA damage in plant stem cell niches. Proc Natl Acad Sci USA *106*, 20984–20988.

Galinha, C., Hofhuis, H., Luijten, M., Willemsen, V., Blilou, I., Heidstra, R., and Scheres, B. (2007). PLETHORA proteins as dose-dependent master regulators of Arabidopsis root development. Nature *449*, 1053–1057.

Gampala, S.S., Kim, T.-W., He, J.-X., Tang, W., Deng, Z., Bai, M.-Y., Guan, S., Lalonde, S., Sun, Y., Gendron, J.M., et al. (2007). An essential role for 14-3-3 proteins in brassinosteroid signal transduction in Arabidopsis. Dev Cell *13*, 177–189.

Garay-Arroyo, A., La Paz Sanchez, de, M., García-Ponce, B., Azpeitia, E., and Alvarez-Buylla, E.R. (2012). Hormone symphony during root growth and development. Dev. Dyn. *241*, 1867–1885.

Geldner, N., Hyman, D.L., Wang, X., Schumacher, K., and Chory, J. (2007). Endosomal signaling of plant steroid receptor kinase BRI1. Genes Dev *21*, 1598–1602.

Gendrel, A.-V., Lippman, Z., Martienssen, R., and Colot, V. (2005). Profiling histone modification patterns in plants using genomic tiling microarrays. Nat Methods *2*, 213–218.

Gendron, J.M., Liu, J.-S., Fan, M., Bai, M.-Y., Wenkel, S., Springer, P.S., Barton, M.K., and Wang, Z.-Y. (2012). Brassinosteroids regulate organ boundary formation in the shoot apical meristem of Arabidopsis. Proc Natl Acad Sci USA *109*, 21152–21157.

Gifford, M.L., Dean, A., Gutiérrez, R.A., Coruzzi, G.M., and Birnbaum, K.D. (2008). Cell-specific nitrogen responses mediate developmental plasticity. Proc Natl Acad Sci USA *105*, 803–808.

Goda, H. (2002). Microarray Analysis of Brassinosteroid-Regulated Genes in Arabidopsis. Plant Physiol *130*, 1319–1334.

Goda, H., Sasaki, E., Akiyama, K., Maruyama-Nakashita, A., Nakabayashi, K., Li, W., Ogawa, M., Yamauchi, Y., Preston, J., Aoki, K., et al. (2008). The AtGenExpress hormone and chemical treatment data set: experimental design, data evaluation, model data analysis and data access. Plant J *55*, 526–542.

Goda, H., Sawa, S., Asami, T., Fujioka, S., Shimada, Y., and Yoshida, S. (2004). Comprehensive comparison of auxin-regulated and brassinosteroid-regulated genes in Arabidopsis. Plant Physiol *134*, 1555–1573.

Gonzalez, N., de Bodt, S., Sulpice, R., Jikumaru, Y., Chae, E., Dhondt, S., van Daele, T., de Milde, L., Weigel, D., Kamiya, Y., et al. (2010). Increased leaf size: different means to an end. Plant Physiol *153*, 1261–1279.

González-García, M.P., Vilarrasa-Blasi, J., Zhiponova, M., Divol, F., Mora-García, S., Russinova, E., and Caño-Delgado, A.I. (2011). Brassinosteroids control meristem size by promoting cell cycle progression in Arabidopsis roots. Development *138*, 849–859.

Graham, L.E., Cook, M.E., and Busse, J.S. (2000). The origin of plants: body plan changes contributing to a major evolutionary radiation. Proc Natl Acad Sci USA *97*, 4535–4540.

Gudesblat, G.E., and Russinova, E. (2011). Plants grow on brassinosteroids. Curr Opin Plant Biol.

Gudesblat, G.E., Schneider-Pizoń, J., Betti, C., Mayerhofer, J., Vanhoutte, I., van Dongen, W., Boeren, S., Zhiponova, M., de Vries, S., Jonak, C., et al. (2012). SPEECHLESS integrates brassinosteroid and stomata signaling pathways. Nat Cell Biol *14*, 1–9.

Hacham, Y., Holland, N., Butterfield, C., Ubeda-Tomas, S., Bennett, M.J., Chory, J., and Savaldi-Goldstein, S. (2011). Brassinosteroid perception in the epidermis controls root meristem size. Development *138*, 839–848.

He, J.-X., Gendron, J.M., Yang, Y., Li, J., and Wang, Z.-Y. (2002). The GSK3-like kinase BIN2 phosphorylates and destabilizes BZR1, a positive regulator of the brassinosteroid signaling pathway in Arabidopsis. Proc Natl Acad Sci USA *99*, 10185–10190.

Heyman, J., Cools, T., Vandenbussche, F., Heyndrickx, K.S., Van Leene, J., Vercauteren, I., Vanderauwera, S., Vandepoele, K., De Jaeger, G., Van Der Straeten, D., et al. (2013). ERF115 Controls Root Quiescent Center Cell Division and Stem Cell Replenishment. Science.

Hothorn, M., Belkhadir, Y., Dreux, M., Dabi, T., Noel, J.P., Wilson, I.A., and Chory, J. (2011). Structural basis of steroid hormone perception by the receptor kinase BRI1. Nature 1–6.

Hsu, Y.-C., and Fuchs, E. (2012). A family business: stem cell progeny join the niche to regulate homeostasis. Nat Rev Mol Cell Biol *13*, 103–114.

Huang, W., Perez-Garcia, P., Pokhilko, A., Millar, A.J., Antoshechkin, I., Riechmann, J.L., and Mas, P. (2012). Mapping the Core of the Arabidopsis Circadian Clock Defines the Network Structure of the Oscillator. Science *336*, 75–79.

Hyduke, D.R., and Palsson, B.Ø. (2010). nrg2750. Nat Rev Genet *11*, 297–307.

Ibañes, M., Fàbregas, N., Chory, J., and Caño-Delgado, A.I. (2009). Brassinosteroid signaling and auxin transport are required to establish the periodic pattern of Arabidopsis shoot vascular bundles. Proc Natl Acad Sci USA *106*, 13630–13635.

Ioio, dello, R., Linhares, F.S., Scacchi, E., Casamitjana-Martinez, E., Heidstra, R., Costantino, P., and Sabatini, S. (2007). Cytokinins determine Arabidopsis root-meristem size by controlling cell differentiation. Current Biology *17*, 678–682.

Ioio, dello, R., Nakamura, K., Moubayidin, L., Perilli, S., Taniguchi, M., Morita, M.T., Aoyama, T., Costantino, P., and Sabatini, S. (2008). A genetic framework for the control of cell division and differentiation in the root meristem. Science *322*, 1380–1384.

Irani, N.G., Di Rubbo, S., Mylle, E., Van den Begin, J., nacute, J.S.-P., aacute, J.H., a, M.S.I.S., Buyst, D., Vilarrasa-Blasi, J., ri, A.-M.A.R.S.A., et al. (2012). Fluorescent castasterone reveals BRI1 signaling from the plasma membrane. Nat Chem Biol *8*, 583–589.

Iyer-Pascuzzi, A.S., Jackson, T., Cui, H., Petricka, J.J., Busch, W., Tsukagoshi, H., and Benfey, P.N. (2011). Cell Identity Regulators Link Development and Stress Responses in the Arabidopsis Root. Dev Cell *21*, 770–782.

Jaillais, Y., Belkhadir, Y., Balsemão-Pires, E., Dangl, J.L., and Chory, J. (2011a).

Extracellular leucine-rich repeats as a platform for receptor/coreceptor complex formation. Proc Natl Acad Sci USA.

Jaillais, Y., Hothorn, M., Belkhadir, Y., Dabi, T., Nimchuk, Z.L., Meyerowitz, E.M., and Chory, J. (2011b). Tyrosine phosphorylation controls brassinosteroid receptor activation by triggering membrane release of its kinase inhibitor. Genes Dev *25*, 232–237.

Jaitin, D.A., Kenigsberg, E., Keren-Shaul, H., Elefant, N., Paul, F., Zaretsky, I., Mildner, A., Cohen, N., Jung, S., Tanay, A., et al. (2014). Massively Parallel Single-Cell RNA-Seq for Marker-Free Decomposition of Tissues into Cell Types. Science *343*, 776–779.

Jasper, H., and Jones, D.L. (2010). Metabolic Regulation of Stem Cell Behavior and Implications for Aging. Cell Metabolism *12*, 561–565.

Jiang, K., Zhang, S., Lee, S., Tsai, G., Kim, K., Huang, H., Chilcott, C., Zhu, T., and Feldman, L.J. (2006). Transcription Profile Analyses Identify Genes and Pathways Central to Root Cap Functions in Maize. Plant Mol Biol *60*, 343–363.

Kageyama, R., Niwa, Y., and Shimojo, H. (2009). Rhythmic gene expression in somite formation and neural development. Mol Cells *27*, 497–502.

Karlova, R., and de Vries, S.C. (2006). Advances in understanding brassinosteroid signaling. Sci STKE *2006*, pe36.

Kaufmann, K., Wellmer, F., Muiño, J.M., Ferrier, T., Wuest, S.E., Kumar, V., Serrano-Mislata, A., Madueño, F., Krajewski, P., Meyerowitz, E.M., et al. (2010). Orchestration of floral initiation by APETALA1. Science *328*, 85–89.

Kauschmann, A., Jessop, A., Koncz, C., Szekeres, M., Willmitzer, L., and Altmann, T. (1996). Genetic evidence for an essential role of brassinosteroids in plant development. Plant J *9*, 701–713.

Kerk, N.M., Ceserani, T., Tausta, S.L., Sussex, I.M., and Nelson, T.M. (2003). Laser capture microdissection of cells from plant tissues. Plant Physiol *132*, 27–35.

Kim, S.K., Chang, S.C., Lee, E.J., Chung, W.S., Kim, Y.S., Hwang, S., and Lee, J.S. (2000). Involvement of brassinosteroids in the gravitropic response of primary root of maize. Plant Physiol *123*, 997–1004.

Kim, T.-W., and Wang, Z.-Y. (2010). Brassinosteroid signal transduction from receptor kinases to transcription factors. Annu Rev Plant Biol *61*, 681–704.

Kim, T.-W., Michniewicz, M., Bergmann, D.C., and Wang, Z.-Y. (2012). Brassinosteroid regulates stomatal development by GSK3-mediated inhibition of a MAPK pathway. Nature 1–5.

Kinoshita, T., Cano-Delgado, A., Seto, H., Hiranuma, S., Fujioka, S., Yoshida,

S., and Chory, J. (2005). Binding of brassinosteroids to the extracellular domain of plant receptor kinase BRI1. Nature *433*, 167–171.

Krishnan, R.V., Masuda, A., Centonze, V.E., and Herman, B. (2003). Quantitative imaging of protein-protein interactions by multiphoton fluorescence lifetime imaging microscopy using a streak camera. J Biomed Opt *8*, 362–367.

Kuppusamy, K.T., Chen, A.Y., and Nemhauser, J.L. (2009). Steroids are required for epidermal cell fate establishment in Arabidopsis roots. Proc Natl Acad Sci USA *106*, 8073–8076.

Lee, J.-Y., Colinas, J., Wang, J.Y., Mace, D., Ohler, U., and Benfey, P.N. (2006). Transcriptional and posttranscriptional regulation of transcription factor expression in Arabidopsis roots. Proc Natl Acad Sci USA *103*, 6055–6060.

Lee, J., He, K., Stolc, V., Lee, H., Figueroa, P., Gao, Y., Tongprasit, W., Zhao, H., Lee, I., and Deng, X.W. (2007). Analysis of transcription factor HY5 genomic binding sites revealed its hierarchical role in light regulation of development. Plant Cell *19*, 731–749.

Leonhardt, N. (2004). Microarray Expression Analyses of Arabidopsis Guard Cells and Isolation of a Recessive Abscisic Acid Hypersensitive Protein Phosphatase 2C Mutant. Plant Cell *16*, 596–615.

Li, J., and Chory, J. (1997). A putative leucine-rich repeat receptor kinase involved in brassinosteroid signal transduction. Cell *90*, 929–938.

Li, J., Nagpal, P., Vitart, V., McMorris, T.C., and Chory, J. (1996). A role for brassinosteroids in light-dependent development of Arabidopsis. Science *272*, 398–401.

Li, J., Wen, J., Lease, K.A., Doke, J.T., Tax, F.E., and Walker, J.C. (2002). BAK1, an Arabidopsis LRR receptor-like protein kinase, interacts with BRI1 and modulates brassinosteroid signaling. Cell *110*, 213–222.

Li, L., Yu, X., Thompson, A., Guo, M., Yoshida, S., Asami, T., Chory, J., and Yin, Y. (2009). Arabidopsis MYB30 is a direct target of BES1 and cooperates with BES1 to regulate brassinosteroid-induced gene expression. Plant J *58*, 275–286.

Lin, S.-X., Chen, J., Mazumdar, M., Poirier, D., Wang, C., Azzi, A., and Zhou, M. (2010). Molecular therapy of breast cancer: progress and future directions. Nat Rev Endocrinol *6*, 485–493.

López-Bigas, N., Kisiel, T.A., DeWaal, D.C., Holmes, K.B., Volkert, T.L., Gupta, S., Love, J., Murray, H.L., Young, R.A., and Benevolenskaya, E.V. (2008). Genome-wide Analysis of the H3K4 Histone Demethylase RBP2 Reveals a Transcriptional Program Controlling Differentiation. Mol. Cell *31*, 520–530.

Mähönen, A.P., Bonke, M., Kauppinen, L., Riikonen, M., Benfey, P.N., and

Helariutta, Y. (2000). A novel two-component hybrid molecule regulates vascular morphogenesis of the Arabidopsis root. Genes Dev *14*, 2938–2943.

Miyazawa, Y., Nakajima, N., Abe, T., Sakai, A., Fujioka, S., Kawano, S., Kuroiwa, T., and Yoshida, S. (2003). Activation of cell proliferation by brassinolide application in tobacco BY-2 cells: effects of brassinolide on cell multiplication, cell-cycle-related gene expression, and organellar DNA contents. J Exp Bot *54*, 2669–2678.

Mora-García, S., Vert, G., Yin, Y., Caño-Delgado, A., Cheong, H., and Chory, J. (2004). Nuclear protein phosphatases with Kelch-repeat domains modulate the response to brassinosteroids in Arabidopsis. Genes Dev *18*, 448–460.

Moreno-Risueno, M.A., van Norman, J.M., Moreno, A., Zhang, J., Ahnert, S.E., and Benfey, P.N. (2010). Oscillating gene expression determines competence for periodic Arabidopsis root branching. Science *329*, 1306–1311.

Morohashi, K., and Grotewold, E. (2009). A systems approach reveals regulatory circuitry for Arabidopsis trichome initiation by the GL3 and GL1 selectors. PLoS Genet *5*, e1000396.

Morrison, S.J., and Spradling, A.C. (2008). Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. Cell *132*, 598–611.

Moubayidin, L., Di Mambro, R., Sozzani, R., Pacifici, E., Salvi, E., Terpstra, I., Bao, D., van Dijken, A., Ioio, dello, R., Perilli, S., et al. (2013). Spatial Coordination between Stem Cell Activity and Cell Differentiation in the Root Meristem. Dev Cell *26*, 405–415.

Moubayidin, L., Perilli, S., Ioio, dello, R., Mambro, R.D., Costantino, P., and Sabatini, S. (2010). The rate of cell differentiation controls the Arabidopsis root meristem growth phase. Curr Biol *20*, 1138–1143.

Mouchel, C.F., Osmont, K.S., and Hardtke, C.S. (2006). BRX mediates feedback between brassinosteroid levels and auxin signaling in root growth. Nature *443*, 458–461.

Müssig, C., Fischer, S., and Altmann, T. (2002). Brassinosteroid-regulated gene expression. Plant Physiol *129*, 1241–1251.

Müssig, C., Shin, G.-H., and Altmann, T. (2003). Brassinosteroids promote root growth in Arabidopsis. Plant Physiol *133*, 1261–1271.

Nakajima, K., Sena, G., Nawy, T., and Benfey, P.N. (2001). Intercellular movement of the putative transcription factor SHR in root patterning. Nature *413*, 307–311.

Nam, K.H., and Li, J. (2002). BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signaling. Cell *110*, 203–212.

Nawy, T., Lee, J.-Y., Colinas, J., Wang, J.Y., Thongrod, S.C., Malamy, J.E., Birnbaum, K., and Benfey, P.N. (2005). Transcriptional profile of the Arabidopsis root quiescent center. Plant Cell *17*, 1908–1925.

Nemhauser, J.L., Hong, F., and Chory, J. (2006). Different Plant Hormones Regulate Similar Processes through Largely Nonoverlapping Transcriptional Responses. Cell *126*, 467–475.

Nemhauser, J.L., Mockler, T.C., and Chory, J. (2004). Interdependency of brassinosteroid and auxin signaling in Arabidopsis. PLoS Biol *2*, E258.

Novak, B., Tyson, J.J., Gyorffy, B., and Csikász-Nagy, A. (2007). Irreversible cell-cycle transitions are due to systems-level feedback. Nat Cell Biol *9*, 724–728.

Oh, E., Zhu, J.-Y., and Wang, Z.-Y. (2012). Interaction between BZR1 and PIF4 integrates brassinosteroid and environmental responses. Nat Cell Biol *14*, 802–809.

Oh, M.-H., Sun, J., Oh, D.H., Zielinski, R.E., Clouse, S.D., and Huber, S.C. (2011). Enhancing Arabidopsis Leaf Growth by Engineering the BRASSINOSTEROID INSENSITIVE1 Receptor Kinase. Plant Physiol *157*, 120–131.

Ohashi-Ito, K., and Fukuda, H. (2003). HD-zip III homeobox genes that include a novel member, ZeHB-13 (Zinnia)/ATHB-15 (Arabidopsis), are involved in procambium and xylem cell differentiation. Plant Cell Physiol *44*, 1350–1358.

Ohashi-Ito, K., Demura, T., and Fukuda, H. (2002). Promotion of transcript accumulation of novel Zinnia immature xylem-specific HD-Zip III homeobox genes by brassinosteroids. Plant Cell Physiol *43*, 1146–1153.

Ortega-Martínez, O., Pernas, M., Carol, R.J., and Dolan, L. (2007). Ethylene modulates stem cell division in the Arabidopsis thaliana root. Science *317*, 507–510.

Overvoorde, P., Fukaki, H., and Beeckman, T. (2010). Auxin control of root development. Cold Spring Harb Perspect Biol *2*, a001537.

Palovaara, J., Saiga, S., and Weijers, D. (2013). Transcriptomics approaches in the early Arabidopsis embryo. Trends Plant Sci *18*, 514–521.

Peng, P., Yan, Z., Zhu, Y., and Li, J. (2008). Regulation of the Arabidopsis GSK3-like kinase BRASSINOSTEROID-INSENSITIVE 2 through proteasome-mediated protein degradation. Mol Plant *1*, 338–346.

Perez-Perez, J.M., Ponce, M.R., and Micol, J.L. (2004). The ULTRACURVATA2 gene of Arabidopsis encodes an FK506-binding protein involved in auxin and brassinosteroid signaling. Plant Physiol *134*, 101–117.

Petersson, S.V., Johansson, A.I., Kowalczyk, M., Makoveychuk, A., Wang, J.Y., Moritz, T., Grebe, M., Benfey, P.N., Sandberg, G., and Ljung, K. (2009). An Auxin Gradient and Maximum in the Arabidopsis Root Apex Shown by High-Resolution Cell-Specific Analysis of IAA Distribution and Synthesis. Plant Cell *21*, 1659–1668.

Rademacher, E.H., Möller, B., Lokerse, A.S., Llavata-Peris, C.I., van den Berg, W., and Weijers, D. (2011). A cellular expression map of the Arabidopsis AUXIN RESPONSE FACTOR gene family. Plant J *68*, 597–606.

Ramsköld, D., Luo, S., Wang, Y.-C., Li, R., Deng, Q., Faridani, O.R., Daniels, G.A., Khrebtukova, I., Loring, J.F., Laurent, L.C., et al. (2012). Full-length mRNA-Seq from single-cell levels of RNA and individual circulating tumor cells. Nature Publishing Group *30*, 777–782.

Riou-Khamlichi, C., Huntley, R., Jacqmard, A., and Murray, J.A. (1999). Cytokinin activation of Arabidopsis cell division through a D-type cyclin. Science *283*, 1541–1544.

Risbridger, G.P., Davis, I.D., Birrell, S.N., and Tilley, W.D. (2010). Breast and prostate cancer: more similar than different. Nat. Rev. Cancer *10*, 205–212.

Russinova, E., Borst, J.-W., Kwaaitaal, M., Caño-Delgado, A., Yin, Y., Chory, J., and de Vries, S.C. (2004). Heterodimerization and endocytosis of Arabidopsis brassinosteroid receptors BRI1 and AtSERK3 (BAK1). Plant Cell *16*, 3216–3229.

Ryu, H., Kim, K., Cho, H., Park, J., Choe, S., and Hwang, I. (2007). Nucleocytoplasmic shuttling of BZR1 mediated by phosphorylation is essential in Arabidopsis brassinosteroid signaling. Plant Cell *19*, 2749–2762.

Sabatini, S., Beis, D., Wolkenfelt, H., Murfett, J., Guilfoyle, T., Malamy, J., Benfey, P., Leyser, O., Bechtold, N., Weisbeek, P., et al. (1999). An auxin-dependent distal organizer of pattern and polarity in the Arabidopsis root. Cell *99*, 463–472.

Sabatini, S., Heidstra, R., Wildwater, M., and Scheres, B. (2003). SCARECROW is involved in positioning the stem cell niche in the Arabidopsis root meristem. Genes Dev *17*, 354–358.

Sarkar, A.K., Luijten, M., Miyashima, S., Lenhard, M., Hashimoto, T., Nakajima, K., Scheres, B., Heidstra, R., and Laux, T. (2007). Conserved factors regulate signaling in Arabidopsis thaliana shoot and root stem cell organizers. Nature *446*, 811–814.

Savaldi-Goldstein, S., Peto, C., and Chory, J. (2007). The epidermis both drives and restricts plant shoot growth. Nature *446*, 199–202.

Scacchi, E., Salinas, P., Gujas, B., Santuari, L., Krogan, N., Ragni, L., Berleth,

T., and Hardtke, C.S. (2010). Spatio-temporal sequence of cross-regulatory events in root meristem growth. Proc Natl Acad Sci USA *107*, 22734–22739.

Schena, M., Shalon, D., Davis, R.W., and Brown, P.O. (1995). Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science *270*, 467–470.

Scheres, B. (2007). Stem-cell niches: nursery rhymes across kingdoms. Nat Rev Mol Cell Biol *8*, 345–354.

Schmid, M., Davison, T.S., Henz, S.R., Pape, U.J., Demar, M., Vingron, M., Schölkopf, B., Weigel, D., and Lohmann, J.U. (2005). A gene expression map of Arabidopsis thaliana development. Nature Genetics *37*, 501–506.

Shani, E., Weinstain, R., Zhang, Y., Castillejo, C., Kaiserli, E., Chory, J., Tsien, R.Y., and Estelle, M. (2013). Gibberellins accumulate in the elongating endodermal cells of Arabidopsis root. Proc Natl Acad Sci USA *110*, 4834–4839.

Shishkova, S., Rost, T.L., and Dubrovsky, J.G. (2008). Determinate root growth and meristem maintenance in angiosperms. Ann Bot *101*, 319–340.

Shiu, S., Bleecker, AB (2001). Receptor-like kinases from Arabidopsis form a monophyletic gene family related to animal receptor kinases. Proc Natl Acad Sci USA *98*, 10763–10768.

Siegal-Gaskins, D., Mejia-Guerra, M.K., Smith, G.D., and Grotewold, E. (2011). Emergence of switch-like behavior in a large family of simple biochemical networks. PLoS Comput. Biol. *7*, e1002039.

Simões, B.M., and Vivanco, M.D. (2011). Cancer stem cells in the human mammary gland and regulation of their differentiation by estrogen. Future Oncol *7*, 995–1006.

Stahl, Y., Wink, R.H., Ingram, G.C., and Simon, R. (2009). A signaling module controlling the stem cell niche in Arabidopsis root meristems. Curr Biol *19*, 909–914.

Steber, C.M., and McCourt, P. (2001). A role for brassinosteroids in germination in Arabidopsis. Plant Physiol *125*, 763–769.

Stuart, J.A., and Brown, M.F. (2006). Energy, quiescence and the cellular basis of animal life spans. Comparative Biochemistry and Physiology Part a: Molecular & Integrative Physiology *143*, 12–23.

Sun, Y., Fan, X.-Y., Cao, D.-M., Tang, W., He, K., Zhu, J.-Y., He, J.-X., Bai, M.-Y., Zhu, S., Oh, E., et al. (2010). Integration of brassinosteroid signal transduction with the transcription network for plant growth regulation in Arabidopsis. Dev Cell *19*, 765–777.

Swarup, R., Kramer, E.M., Perry, P., Knox, K., Leyser, H.M.O., Haseloff, J.,

Beemster, G.T.S., Bhalerao, R., and Bennett, M.J. (2005). Root gravitropism requires lateral root cap and epidermal cells for transport and response to a mobile auxin signal. Nat Cell Biol *7*, 1057–1065.

Szekeres, M., Németh, K., Koncz-Kálmán, Z., Mathur, J., Kauschmann, A., Altmann, T., Rédei, G.P., Nagy, F., Schell, J., and Koncz, C. (1996). Brassinosteroids rescue the deficiency of CYP90, a cytochrome P450, controlling cell elongation and de-etiolation in Arabidopsis. Cell *85*, 171–182.

Tang, F., Barbacioru, C., Wang, Y., Nordman, E., Lee, C., Xu, N., Wang, X., Bodeau, J., Tuch, B.B., Siddiqui, A., et al. (2009). mRNA-Seq whole-transcriptome analysis of a single cell. Nat Methods *6*, 377–382.

Tang, W., Kim, T.-W., Oses-Prieto, J.A., Sun, Y., Deng, Z., Zhu, S., Wang, R., Burlingame, A.L., and Wang, Z.-Y. (2008). BSKs mediate signal transduction from the receptor kinase BRI1 in Arabidopsis. Science *321*, 557–560.

Thibaud-Nissen, F., Wu, H., Richmond, T., Redman, J.C., Johnson, C., Green, R., Arias, J., and Town, C.D. (2006). Development of Arabidopsis whole-genome microarrays and their application to the discovery of binding sites for the TGA2 transcription factor in salicylic acid-treated plants. The Plant Journal *47*, 152–162.

Thummel, C.S., and Chory, J. (2002). Steroid signaling in plants and insects--common themes, different pathways. Genes Dev *16*, 3113–3129.

Truernit, E., Bauby, H., Dubreucq, B., Grandjean, O., Runions, J., Barthélémy, J., and Palauqui, J.-C. (2008). High-resolution whole-mount imaging of three-dimensional tissue organization and gene expression enables the study of Phloem development and structure in Arabidopsis. Plant Cell *20*, 1494–1503.

Tsukagoshi, H., Busch, W., and Benfey, P.N. (2010). Transcriptional regulation of ROS controls transition from proliferation to differentiation in the root. Cell *143*, 606–616.

Ubeda-Tomas, S., Beemster, G.T.S., and Bennett, M.J. (2012). Hormonal regulation of root growth: integrating local activities into global behaviour. Trends Plant Sci 1–6.

Ubeda-Tomas, S., Federici, F., Casimiro, I., Beemster, G.T.S., Bhalerao, R., Swarup, R., Doerner, P., Haseloff, J., and Bennett, M.J. (2009). Gibberellin signaling in the endodermis controls Arabidopsis root meristem size. Curr Biol *19*, 1194–1199.

Ubeda-Tomás, S., Swarup, R., Coates, J., Swarup, K., Laplaze, L., Beemster, G.T.S., Hedden, P., Bhalerao, R., and Bennett, M.J. (2008). Root growth in Arabidopsis requires gibberellin/DELLA signaling in the endodermis. Nat Cell Biol *10*, 625–628.

van den Berg, C., Weisbeek, P., and Scheres, B. (1998). Cell fate and cell differentiation status in the Arabidopsis root. Planta *205*, 483–491.

van den Berg, C., Willemsen, V., and Hage, W. (1995). Cell fate in the Arabidopsis root meristem determined by directional signaling. Nature.

van den Berg, C., Willemsen, V., Hendriks, G., Weisbeek, P., and Scheres, B. (1997). Short-range control of cell differentiation in the Arabidopsis root meristem. Nature *390*, 287–289.

van Esse, G.W., Westphal, A.H., Surendran, R.P., Albrecht, C., van Veen, B., Borst, J.-W., and de Vries, S.C. (2011). Quantification of the BRI1 receptor in planta. Plant Physiol.

van Esse, W.G., Van Mourik, S., Stigter, H., Hove, Ten, C.C.A., Molenaar, J., and de Vries, S.C. (2012). A mathematical model for BRI1 mediated signaling in root growth and hypocotyl elongation. Plant Physiol.

Vatén, A., Dettmer, J., Wu, S., Stierhof, Y.-D., Miyashima, S., Yadav, S.R., Roberts, C.J., Campilho, A., Bulone, V., Lichtenberger, R., et al. (2011). Callose Biosynthesis Regulates Symplastic Trafficking during Root Development. Dev Cell *21*, 1144–1155.

Vert, G., Nemhauser, J.L., Geldner, N., Hong, F., and Chory, J. (2005). Molecular mechanisms of steroid hormone signaling in plants. Annu Rev Cell Dev Biol *21*, 177–201.

Viotti, C., Bubeck, J., Stierhof, Y.-D., Krebs, M., Langhans, M., van den Berg, W., van Dongen, W., Richter, S., Geldner, N., Takano, J., et al. (2010). Endocytic and secretory traffic in Arabidopsis merge in the trans-Golgi network/early endosome, an independent and highly dynamic organelle. Plant Cell *22*, 1344–1357.

Wachsman, G., Heidstra, R., and Scheres, B. (2011). Distinct Cell-Autonomous Functions of RETINOBLASTOMA-RELATED in Arabidopsis Stem Cells Revealed by the Brother of Brainbow Clonal Analysis System. Plant Cell.

Wang, X., Li, X., Meisenhelder, J., Hunter, T., Yoshida, S., Asami, T., and Chory, J. (2005). Autoregulation and homodimerization are involved in the activation of the plant steroid receptor BRI1. Dev Cell *8*, 855–865.

Wang, Z.Y., Seto, H., Fujioka, S., Yoshida, S., and Chory, J. (2001). BRI1 is a critical component of a plasma-membrane receptor for plant steroids. Nature *410*, 380–383.

Wang, Z.-Y., Nakano, T., Gendron, J., He, J., Chen, M., Vafeados, D., Yang, Y., Fujioka, S., Yoshida, S., Asami, T., et al. (2002). Nuclear-localized BZR1 mediates brassinosteroid-induced growth and feedback suppression of brassinosteroid biosynthesis. Dev Cell *2*, 505–513.

Wang, Z., Gerstein, M., and Snyder, M. (2009). RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet *10*, 57–63.

Weijers, D., Franke-van Dijk, M., Vencken, R.J., Quint, A., Hooykaas, P., and Offringa, R. (2001). An Arabidopsis Minute-like phenotype caused by a semi-dominant mutation in a RIBOSOMAL PROTEIN S5 gene. Development *128*, 4289–4299.

Wildwater, M., Campilho, A., Perez-Perez, J., Heidstra, R., Blilou, I., Korthout, H., Chatterjee, J., Mariconti, L., Gruissem, W., and Scheres, B. (2005). The RETINOBLASTOMA-RELATED gene regulates stem cell maintenance in Arabidopsis roots. Cell *123*, 1337–1349.

Wilson, A., and Trumpp, A. (2006). Bone-marrow haematopoietic-stem-cell niches. Nat. Rev. Immunol. *6*, 93–106.

Wisman, E., and Ohlrogge, J. (2000). Arabidopsis microarray service facilities. Plant Physiol *124*, 1468–1471.

Xie, L., Yang, C., and Wang, X. (2011). Brassinosteroids can regulate cellulose biosynthesis by controlling the expression of CESA genes in Arabidopsis. J Exp Bot.

Yadav, R.K., Girke, T., Pasala, S., Xie, M., and Reddy, G.V. (2009). Gene expression map of the Arabidopsis shoot apical meristem stem cell niche. Proc Natl Acad Sci USA *106*, 4941–4946.

Yamamuro, C., Ihara, Y., Wu, X., Noguchi, T., Fujioka, S., Takatsuto, S., Ashikari, M., Kitano, H., and Matsuoka, M. (2000). Loss of function of a rice brassinosteroid insensitive1 homolog prevents internode elongation and bending of the lamina joint. Plant Cell *12*, 1591–1606.

Ye, Q., Zhu, W., Li, L., Zhang, S., Yin, Y., Ma, H., and Wang, X. (2010). Brassinosteroids control male fertility by regulating the expression of key genes involved in Arabidopsis anther and pollen development. Proc Natl Acad Sci USA *107*, 6100–6105.

Yin, Y., Wang, Z.-Y., Mora-García, S., Li, J., Yoshida, S., Asami, T., and Chory, J. (2002). BES1 accumulates in the nucleus in response to brassinosteroids to regulate gene expression and promote stem elongation. Cell *109*, 181–191.

Yoshimitsu, Y., Tanaka, K., Fukuda, W., Asami, T., Yoshida, S., Hayashi, K.-I., Kamiya, Y., Jikumaru, Y., Shigeta, T., Nakamura, Y., et al. (2011). Transcription of DWARF4 plays a crucial role in auxin-regulated root elongation in addition to brassinosteroid homeostasis in Arabidopsis thaliana. PLoS ONE *6*, e23851.

Yu, X., Li, L., Zola, J., Aluru, M., Ye, H., Foudree, A., Guo, H., Anderson, S., Aluru, S., Liu, P., et al. (2011). A brassinosteroid transcriptional network revealed by genome-wide identification of BESI target genes in Arabidopsis

thaliana. Plant J *65*, 634–646.

Zanetti, M.E., Chang, I.-F., Gong, F., Galbraith, D.W., and Bailey-Serres, J. (2005). Immunopurification of polyribosomal complexes of Arabidopsis for global analysis of gene expression. Plant Physiol *138*, 624–635.

Zhang, H., Han, W., de Smet, I., Talboys, P., Loya, R., Hassan, A., Rong, H., Jürgens, G., Paul Knox, J., and Wang, M.-H. (2010). ABA promotes quiescence of the quiescent centre and suppresses stem cell differentiation in the Arabidopsis primary root meristem. Plant J *64*, 764–774.

Zhang, X., Henriques, R., Lin, S.-S., Niu, Q.-W., and Chua, N.-H. (2006). Agrobacterium-mediated transformation of Arabidopsis thaliana using the floral dip method. Nat Protoc *1*, 641–646.

Zhao, Y., Hull, A.K., Gupta, N.R., Goss, K.A., Alonso, J., Ecker, J.R., Normanly, J., Chory, J., and Celenza, J.L. (2002). Trp-dependent auxin biosynthesis in Arabidopsis: involvement of cytochrome P450s CYP79B2 and CYP79B3. Genes Dev *16*, 3100–3112.

Zheng, Y., Ren, N., Wang, H., Stromberg, A.J., and Perry, S.E. (2009). Global Identification of Targets of the Arabidopsis MADS Domain Protein AGAMOUS-Like15. Plant Cell *21*, 2563–2577.

Zhiponova, M.K., Vanhoutte, I., Boudolf, V., Betti, C., Dhondt, S., Coppens, F., Mylle, E., Maes, S., González-García, M.P., Caño-Delgado, A.I., et al. (2013). Brassinosteroid production and signaling differentially control cell division and expansion in the leaf. New Phytol *197*, 490–502.

Zhu, J.Y., Sae-Seaw, J., and Wang, Z.Y. (2013). Brassinosteroid signaling. Development *140*, 1615–1620.

Zhu, T., and Wang, X. (2000). Large-scale profiling of the Arabidopsis transcriptome. Plant Physiol *124*, 1472–1476.

Zurek, D.M., Rayle, D.L., McMorris, T.C., and Clouse, S.D. (1994). Investigation of Gene Expression, Growth Kinetics, and Wall Extensibility during Brassinosteroid-Regulated Stem Elongation. Plant Physiol *104*, 505–513.

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PUBLICATIONS AND COMUNICATIONS

Research articles;

1) Gustavo A. Maselli, Javier I. Bianchi, Claudio H. Slamovits, **Josep Vilarrasa-Blasi**, Ana I. Caño-Delgado and Santiago Mora-Garcia. Revisiting the evolutionary history and roles of protein phosphatases with Kelch-like domains in plants. Plant Physiology 2014.

2) Irani NG, Di Rubbo S, Mylle E, Van den Begin J, Schneider-Pizoń J, Hniliková J, Šíša M, Buyst D, **Vilarrasa-Blasi** J, Szatmári AM, Van Damme D, Mishev K, Codreanu MC, Kohout L, Strnad M, Caño-Delgado AI, Friml J, Madder A, Russinova E. Fluorescent castasterone reveals BRI1 signaling from the plasma membrane. Nat Chem Biol. 2012.

3) González-García MP, **Vilarrasa-Blasi** J, Zhiponova M, Divol F, Mora-García S, Russinova E, Caño-Delgado AI. Brassinosteroids control meristem size by promoting cell cycle progression in Arabidopsis roots. Development. 2011.

4) Mascarell-Creus A, Cañizares J, **Vilarrasa-Blasi** J, Mora-García S, Blanca J, Gonzalez-Ibeas D, Saladié M, Roig C, Deleu W, Picó-Silvent B, López-Bigas N, Aranda MA, Garcia-Mas J, Nuez F, Puigdomènech P, Caño-Delgado AI. Ano ligo-based microarray offers novel transcriptomic approaches for the analysis of pathogen resistance and fruit quality traits in melón (Cucumis melo L.). BMC Genomics. 2009.

Manuscript under revision or in preparation

1. Josep Vilarrasa-Blasi, Mary-Paz González-García, David Frigola, Norma Fàbregas, Konstantinos G. Alexiou, Nuria López-Bigas, Susana Rivas, Alain Jauneau, Jan U. Lohmann, Philip N. Benfey, Marta Ibañes and Ana I. Caño-

Delgado. Regulation of plant stem cell quiescence by a novel Brassinosteroid signaling module. (under revision)

2. Irina Pavelescu[&], **Josep Vilarrasa-Blasi**[&], Mary-Paz González-García Marta Ibañes and Ana I. Caño-Delgado. A cellular dynamics analysis of Brassinosteroid contribution to root growth in *Arabidopsis thaliana.*
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3. Josep Vilarrasa-Blasi[&], Albert Mascarell-Creus[&], Jordi Ruyra-Ripoll, Daniel González-Ibeas, Nuria López-Bigas, Pere Puigdomènech, Antonio J. Monforte, Miguel A. Aranda, and Ana Caño-Delgado. STAN and OLLIE: a genetic tool for the molecular dissection of fruit shape in melon. ([&] co-first authors)

Books chapters and other revisions:

1. Brassinosteroid signaling in root development. **Josep Vilarrasa-Blasi**, Mary-Paz González-García and Ana I Caño-Delgado. *Plant Roots: The Hidden Half*, 4th edition 2013.. ISBN:

Communications;

1. Josep Vilarrasa-Blasi, Mary-Paz González-García, Philip Benfey and Ana Caño-Delgado. BRAVO a novel Brassinosteroid signaling component controls BR-dependent divisions of the quiescent center cells in Arabidopsis thaliana primary roots. Poster. *DGZ/GFE meeting*, Heidelberg, Germany. 2013.

2. Pavelescu,I, **Josep Vilarrasa-Blasi,** MP González-García, Marta Ibañes and, Ana I. Caño-Delgado. Brassinosteroids control growth dynamics in Arabidospsis. Poster. *EMBO conference: Plant development and enviromental interaction*, Matera, Italy. 2012.

3. Pavelescu,I, **Josep Vilarrasa-Blasi,** MP González-García, Marta Ibañes and, Ana I. Caño-Delgado. Brassinosteroids control growth dynamics in Arabidospsis. Poster.
1st International Brassinosteroid Conference, Barcelona, Spain. 2012.

4. Mary-Paz González-García, **Josep Vilarrasa-Blasi,** Alexiou K.G., Lopez-Bigas N., Benfey P.N., Caño-Delgado A.I.. Los Brassinosteroides regulan el desarrollo de la raíz mediante el balance del ciclo celular y la diferenciación en tipos celulares específicos. *XII Phytohormones symposium*, Gran Canaria, Spain. 2012.

5. Mary-Paz González-García, **Josep Vilarrasa-Blasi** and, Ana I. Caño-Delgado. Plant steroid hormones control cell cycle and differentation in Arabidopsis root. Chapter & Poster. *Spanish society of cell biology*, Torremolinos, Spain, 2011

6. Josep Vilarrasa-Blasi, Pavelescu,I., MP González-García, Ana Confraria, Marta Ibañes and, Ana I. Caño-Delgado. Brassinosteroids control growth dynamics in Arabidospsis. Poster. *Plant growth biology and modeling*, Elche, Spain. 2011.

7. Josep Vilarrasa-Blasi, Mary-Paz González-García, Ana I. Caño-Delgado. Investigating the role of brassinosteroids in meristem activity and stem cell differentation in the Arabidopsis primary root. Poster. *IPGSA conference, 20th International conference on plant growth substances*, Tarragona, Spain. 2010.

8. Mary-Paz González-García, **Josep Vilarrasa-Blasi**, Zhiponova M, Russinova E, Caño-Delgado A. I., Brassinosteroid contribution to meristematic cell divisions in the primary root of a Arabidopsis thaliana. Poster. *IPGSA conference, 20th International conference on plant growth substances*, Tarragona, Spain. 2010

9. Albert Mascarell[&], **Josep Vilarrasa-Blasi**[&], Santiago Mora-García, Jordi Ruyra, Daniel G. Ibeas, Pere Puigdomènech, Miguel Aranda, Antonio J. Monforte and Ana I. Caño-Delgado. STAN and OLLIE: two fruit-shape QTLs reveal the importance of early ovary development in fruit shape morphogenesis

in melon (Cucumis melo). Poster. *IX Plant biology meeting*, Santiago de Compostela, Spain. 2008.

10. Josep Vilarrasa-Blasi. Molecular characteritzation of melon fruit shape. Oral presentation. *Catalan society of biology*, Barcelona, Spain. 2008.

11. Josep Vilarrasa-Blasi, Albert Mascarell-Creus, Santiago Mora-García, Jordi Ruyra, Gemma Roselló , Daniel G. Ibéas, Miguel Aranda, Pere Puigdomènech, Antonio Monforte, Ana I. Caño-Delgado. Molecular caracteritzation of melon fruit shape. Poster. *2ND Languedoc Roussillon-Catalogne meeting on plant integrative biology*. Roses, Girona, Spain. 2008.

12. Albert Mascarell, **Josep Vilarrasa-Blasi**, Jordi Ruyra, Daniel Gonzalez-Ibeas, Miguel A. Aranda, Pere Puigdomènech, Antonio J. Monforte i Ana I. Caño-Delgado. Genetic and genomic dissection of melon fleshy fruit morphogenesis. Poster. *Plant GEM,* Tenerife, Spain. 2007.

AWARDS and OTHER MERITS

- PhD fellowship from Education Department (Government of Catalan country).
- Short-term fellowship from Catalan government in Jan Lohmann lab.
- Support grant to attend the FESPB/EPSO conference, Ireland June 2014

LANGUAGES

Catalan (native)
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English, high verbal, reading and writing skills
French, medium verbal, reading and writing skills

PUBLICATIONS