Role of *SCHIZORIZA* in asymmetric cell division, cell fate segregation and specification in *Arabidopsis* root development

Vera Maria Angenesa Jansweijer

ISBN: 978-90-5335-688-3

Cover by: Beuk, Hans de Vries
Lay-out by: Nikki Vermeulen, Ridderprint BV, Ridderkerk, the Netherlands
Printed by: Ridderprint BV, Ridderkerk, the Netherlands

Printing of this thesis was financially supported by the J.E. Jurriaanse Stichting

This work was supported by a Horizon grant (050-71-054), which is part of the Netherlands Genomics Initiative/Netherlands Organization for Scientific Research. The research described was done in the section Molecular Genetics, Department of Biology, Utrecht University

Role of *SCHIZORIZA* in asymmetric cell division, cell fate segregation and specification in *Arabidopsis* root development

De rol van *SCHIZORIZA* in asymmetrische celdeling, scheiding en specificering van cel identiteiten tijdens de ontwikkeling van de wortel in *Arabidopsis*

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht
op gezag van de rector magnificus, prof. dr. G.J. van der Zwaan,
ingevolge het besluit van het college voor promoties
in het openbaar te verdedigen op
woensdag 12 juni 2013 des middags 12.45 uur

door

Vera Maria Angenesa Jansweijer

geboren op 23 september 1982
te Amsterdam

Promotor: Prof.dr. B.J.G. Scheres

Co-promotor: Dr. R. Heidstra

Voor mijn ouders en grootouders

"Ut sementem feceris ita metes"
- Cicero

CONTENTS

Chapter 1	General Introduction	9
Chapter 2	Downstream effectors highlight *SCHIZORIZA* function in tissue separation	25
Chapter 3	*SCHIZORIZA* controls stem cell niche specification in conjunction with *PLETHORA* and *SHORTROOT-SCARECROW* pathways	57
Chapter 4	Identification of proteins interacting with SCHIZORIZA	73
Chapter 5	Strategies for genetic dissection of *SCHIZORIZA* cell fate separation roles	101
Chapter 6	Summary and concluding remarks	111
Chapter 7	Nederlandse samenvatting	121
Chapter 8	References	127
Chapter 9	Dankwoord	141
Chapter 10	Curriculum Vitae	149

Chapter 1

General Introduction

Asymmetric cell division leads the way to cellular diversity

Multicellular organisms develop their large variety of cell types from just one single cell, the zygote. Both plants and animals use asymmetric cell division to establish a multicellular body plan (Horvitz, Herskowitz 1992). How different cell and tissue types are determined, how patterns are created and maintained, and which players are involved, are fundamental questions in developmental biology.

By definition, asymmetric cell division produces two daughter cells that are different from each other (Horvitz, Herskowitz 1992). Distinct fates are passed on to the daughter cells through two general mechanisms involving intrinsic and extrinsic cues. Intrinsic fate determinants are unevenly segregated, for example through asymmetry in cell shape or unequal distribution of molecular components (Horvitz, Herskowitz 1992). In extrinsically regulated asymmetric cell division, external cues from the microenvironment confer distinct fates. For example, surrounding cells can influence fate determination (Horvitz, Herskowitz 1992, Lawrence 2005). Often both intrinsic and extrinsic cues together are involved in asymmetric cell division (Scheres, Benfey 1999). A well-known example of intrinsic cues during *Drosophila* neuronal development, is the segregation of the membrane protein Numb and the homeobox transcription factor Prospero (Pros). The neuroblasts divide asymmetrically generating a new neuroblast and a ganglion mother cell (GMC). Subsequently, the GMC divides symmetrically into two neurons or glia cells (Wodarz, Huttner 2003). When the neuroblast conducts its asymmetric cell division, Numb and Pros intrinsically determine cell fate by localizing polarly to the basal cortex. After division is completed Numb and Pros segregate into the basal daughter cell (Jan, Jan 2001). There Pros regulates the transcription of GMC specific genes and additionally it suppresses the transcription of cell cycle regulating genes, so that, after one symmetric cell division of the GMC the cells differentiate into neurons and glia cells (Wodarz, Huttner 2003). Numb action entails antagonizing the activity of Notch. Notch is the transmembrane receptor which facilitates cell-cell communication. By antagonizing Notch, Numb ensures separation of distinct fates between two daughter cells (Jan, Jan 2001). In this example, Notch signaling illustrates an extrinsic cue leading to different cell fates.

As opposed to animal development, where all organs and tissues are already formed during embryogenesis, the mature plant embryo shows little resemblance to a postembryonic plant (Steeves, Sussex 1989). Unlike animals, plants must continuously develop completely new organs and tissues throughout their entire life. In addition, plants are sessile organisms, predestined to one place. Within a plant itself, immobility is a recurring theme. Plant cells are unable to move, because cell walls fix their position. In contrast to animal cells, which acquire a certain cell fate and then move to their destination, plants cells, contained by their cell wall, do not move. Instead plant cells have developed the potential to flexibly switch between cell fates if needed. Mixture of cell fates within one cell as a consequence of loss of function of fate determinants is exclusively seen in plant cells (Costa, Shaw 2007). The elegant application of asymmetric cell divisions and the potential to

reprogram asymmetric cell divisions, throughout their entire life, enables plants to obtain their impressive body plans and give them the ability to adjust cell fates if needed.

Plants grow from their meristems, groups of dividing cells residing at foci of development. Within a meristem, stem cell niches are vital for maintaining the balance between stem cell self-renewal and differentiation. Stem cell divisions in *Arabidopsis* roots are asymmetric. Each root stem cell division gives rise to a new stem cell and a daughter cell that embarks on a trajectory towards differentiation, even though a limited number of cell divisions may still occur. Here, I will introduce root meristem initiation and meristem maintenance in the model plant *Arabidopsis thaliana* to illustrate the implementation of asymmetric cell division to create pattern and diversity.

EMBRYOGENESIS

During embryogenesis, the first outline of the plant and later stem cell niches is initiated and specified. In all plants, only a limited amount of cell divisions lead a single-celled zygote to develop into a patterned embryo in which future shoot and root are defined. In *Arabidopsis* the order of cell divisions and the segregation of fates to daughter cell has been well studied (Jürgens, Mayer 1994, Jürgens 1992, Dolan et al. 1993, Yeung, Meinke 1993, Scheres et al. 1994). The zygote is the first cell that divides asymmetrically. Before asymmetric cell division, the zygote undergoes subcellular reorganization, having its nucleus and vacuole polarly localized (Mansfield, Briarty 1991). After cell division, a small apical daughter cell and a larger basal daughter cell are formed. The apical cell is the progenitor for the majority of the cells and tissues that form the embryo, whereas the basal daughter cell will form the suspensor of which only the upper hypophyseal cell contributes to the embryo itself (Jürgens, Mayer 1994, Jürgens 1992, Dolan et al. 1993, Yeung, Meinke 1993, Scheres et al. 1994). *WUSCHEL-RELATED HOMEODOMAIN* (*WOX*) transcription factors are putative intrinsically acting determinants during early embryonic divisions. After the initial asymmetric cell division, the apical cell is characterized by *WOX2* expression, whereas in the basal cell *WOX8* and *WOX9* are expressed (Haecker et al. 2004, Wu, Chory & Weigel 2007). *WOX8* stays expressed in all suspensor cells. Subsequently, *WOX9* expression broadens to include the central domain of the embryo, while *WOX9* expression in the uppermost suspensor cell fades (Haecker et al. 2004). The apical cell lineage development is defective in 30-50% of *wox2* mutant embryos (Haecker et al. 2004) and strong *wox9* mutants arrest at three divisions post zygotic elongation (Wu, Chory & Weigel 2007). *wox8* single mutant embryos do not show a phenotype (Haecker et al. 2004), but eradication of WOX8 in either *wox2* or *wox9* mutant background greatly enhances the corresponding phenotypes, leading to an earlier arrest in *wox8;wox9* embryos (at 2-cell stage) and defected separation of cotyledons in *wox2;wox8* embryos (Wu, Chory & Weigel 2007, Breuninger et al. 2008). Later, in globular stage embryos, *WOX5* becomes expressed in the hypophysis, specified from the upper suspensor cell (Haecker et al. 2004, Sarkar et al. 2007).

Origin of the embryonic root meristem

The origin of a root lies in the early stages of embryo development. The core for the quiescent center (QC) and later columella root cap cells resides in the hypophysis (Jürgens, Mayer 1994, Jürgens 1992, Dolan et al. 1993, Yeung, Meinke 1993, Scheres et al. 1994) and future root stem cells are formed from the adjacent cells. Signaling of the plant hormone auxin is essential for patterning in both plant embryos and mature roots. *AUXIN RESPONSE FACTOR5 (ARF5)/ MONOPTEROS* (*MP*) is responsible for proper embryo formation. Embryos without *MP* function display severe defects and develop into rootless seedlings. The first defects can be traced back to octant-stage embryos, where aberrant divisions of the embryo proper and the hypophysis result in failure to set up the future body plan (Berleth, Jurgens 1993, Przemeck et al. 1996). Dominant stabilizing mutants of *INDOLE-3-ACETIC ACID INDUCIBLE12* (*IAA12*)/ *BODENLOS* (*BDL*) show embryo phenotypes almost identical to *mp* (Weijers et al. 2005, Hamann et al. 2002, Hamann et al. 2002, Hamann, Mayer & Jurgens 1999). BDL protein binds MP, thereby keeping it inactive (Weijers et al. 2005, Hamann et al. 2002, Weijers et al. 2006). Auxin promotes degradation of BDL thus releasing MP (Dharmasiri et al. 2005). This enables MP to activate *PIN1* expression which can contribute to auxin efflux to the hypophysis. There, in the absence of MP that is not expressed in the hypophysis, an unknown ARF is proposed to be activated, which aids to the establishment of hypophysis fate (Weijers et al. 2006). Two basic helix–loop–helix (bHLH) transcription factors *TARGET OF MP5* (*TMO5*) and *TMO7* are induced in the cells adjacent to the hypophysis downstream of *MP* action. Both are needed for *MP*-dependent root initiation. TMO5 acts cell-autonomously, from the hypophysis-adjacent embryo cells. TMO7 is translated in provascular cells of the globular stage embryo and from there it moves to the hypophysis precursor to establish hypophyis and thus root initiaton (Schlereth et al. 2010).

However, not only transcription factors orchestrate pattern formation during embryogenesis. Along the radial axis of the embryo two receptor-like kinases, *RECEPTOR-LIKE PROTEIN KINASE1* (*RPK1*) and *TOADSTOOL2* (*TOAD2)* are redundantly required for pattern formation. Only double mutant *rpk1;toad2* embryos always all display the so called '*toadstool*' phenotype. Furthermore, the morphogenesis of *rpk1;toad2+/-* embryos is heavily affected and *rpk1* single mutant embryos exhibit the *toadstool* phenotype at low frequency. The gene dosage of *TOAD2* in an *rpk1* background is thus very important, suggesting that signaling mediated by *RPK1* and *TOAD2* must be above a critical threshold for proper embryo development. In *toadstool* embryos the cells at the position of the protoderm do not express protoderm markers *ARABIDOPSIS THALIANA MERISTEM LAYER1* (*ATML1)* and *KANADI* (*KAN*), instead they express vascular and subprotoderm markers *SHORTROOT (SHR)* and *PINHEAD/ZWILLE* (*PNH/ZLL*). Furthermore, cells in the position of the ground tissue initials of *toadstool* embryos do not express ground tissue initial markers, but vascular primordium markers (Nodine, Yadegari & Tax 2007), which reveals that outer-to-inner cell-fate transformations are controlled by receptor-like kinases.

From heart stage onwards a clear root and shoot meristem and corresponding stem cell niches can be distinguished and these niches start to display characteristic stem cell division patterns (Dolan et al. 1993, Scheres et al. 1997). Strictly oriented asymmetric cell divisions of different stem cells are fundamental to root patterning. Specification of the root stem cell niche is controlled by *PLETHORA* (*PLT*), *SHR* and *SCARECROW* (*SCR*) transcription factors (Aida et al. 2004, Blilou et al. 2005, Galinha et al. 2007, Galinha et al. 2007, Helariutta et al. 2000, Di Laurenzio et al. 1996, Wysocka-Diller et al. 2000). *PLT* transcription factors have been shown to specify embryonic root, through repression of CLASS III HOMEODOMAIN-LEUCINE ZIPPER (HD-ZIP III) transcription factors. *PLT1* and *PLT2* are necessary for the homeotic conversion of shoots to roots in *topless* (*tpl)* mutants. And *PLT1* and *PLT2* genes are direct targets of the transcriptional co-repressor *TPL* (Smith, Long 2010). This, indicates that *PLT1* and *PLT2* are essential determinants of basal fate. On the other hand, HD-ZIP III genes *PHABULOSA* (*PHB*) and *PHAVOLUTA* (*PHV*) determine embryonic apical fate. Ectopic expression of *PHB* or *PHV* disrupts embryo patterning, causing abolished root development and ultimately embryo lethality. This shows that it is essential that *PHB* and *PHV* need to be restricted to the apical part of the embryo to establish the root meristem (Grigg et al. 2009). Furthermore, induction of a miRNA resistant *PHB* cDNA expressed from the *PLT2* promoter causes a complete conversion of the embryonic root pole into a second shoot pole (Smith, Long 2010), further illustrating that *PLT* and the HD-ZIP III genes execute antagonistic functions in specifying the embryonic root and shoot poles.

STEM CELL DIVISIONS IN MATURE ROOT

Roots grow from the root tips. There, growth is made possible by a small group of stem cells surrounding QC. New cells are provided continuously by asymmetric cell divisions. These newly formed stem cell daughter cells undergo three subsequent stages of development in order to facilitate root growth. First, they undergo multiple divisions in the meristematic zone. Second, in the elongation zone, the cells gradually cease division, expand and elongate. Finally, cells acquire their specialized characteristics in the differentiation zone. Repeated sloughing off the outermost root cap layer maintains distal positioning of the stem cells. The root structure, which is patterned during embryogenesis, reaches adult dimensions in mature roots. For each meristem cell, the fate can be predicted well (Dolan et al. 1993). The root consists of separate cell files, which are concentrically organized. From inner to most outer cell type, there is stele (vasculature and pericycle), endodermis, cortex and epidermis. Cortex and endodermis, two ground tissue layers, share a cortex/endodermis stem cell. Also epidermis and lateral root cap develop from the same clonal origin. Distal to the QC, the columella stem cells reside. Their daughter cells differentiate into columella cells, which contain starch and are able to sense gravity (Dolan et al. 1993, Scheres et al. 1997, Berg et al. 1995).

Ground tissue

One of the most studied tissue types in *Arabidopsis* roots is the ground tissue, which consists of an endodermis and a cortex layer, surrounding the vasculature. Endodermis and cortex are formed by an asymmetric periclinal division of the ground tissue stem cell daughter starting in heart stage embryos (Dolan et al. 1993). *SHR* and *SCR* direct the asymmetric cell division of the cortex/endodermis initial daughter which is instructive for ground tissue patterning. In *shr* mutant, the single ground tissue layer shows cortex identity, suggesting *SHR* is also needed for endodermis specification. The single ground tissue layer in *scr* has a mixed endodermis-cortex fate (Helariutta et al. 2000, Di Laurenzio et al. 1996, Benfey et al. 1993). *SHR* is expressed in the stele but the protein moves outward a single tissue layer to QC, ground tissue initials and endodermis (Nakajima et al. 2001). There SHR binds the *SCR* promoter, thus directly regulating *SCR* expression (Levesque et al. 2006). Moreover, it was found that SHR and SCR physically interact and share many target genes (Cui et al. 2007, Sozzani et al. 2010). Thus, SCR together with SHR are required to induce the division separating cortex and endodermis layers. Clonal deletion of *SCR* from ground tissue stem cells, causes a phenotype similar to *scr* mutant. This shows *SCR* is specifically needed to promote ground tissue periclinal divisions. Clonal induction of *SCR* in *scr* mutant shows that in principle all ground tissue cells have the potential for periclinal division, showing the need for a feedback signalling to strictly position periclinal divisions for proper patterning (Heidstra, Welch & Scheres 2004).

SHR and *SCR* directly activate transcription of a D-type cyclin, *CYCD6;1,* which is expressed in the cortex/endodermis initial. It was suggested that *CYCD6;1* together with its redundantly acting partners *CDKB2;1* and *CDKB2;2* facilitates asymmetric cell divisions in cortex/endodermis initial daughter (Sozzani et al. 2010). However, recently it has become clear that there is no direct link to asymmetric cell division. Instead CYCD6;1 is involved in a feedback mechanism, being a potential mediator of RETINOBLASTOMA-RELATED (RBR) phosphorylation (Cruz-Ramírez et al. 2012). RBR binds SCR through its LxCxE motif and by phosphorylation of RBR, the CYCD6;1-CDK complex prevents SCR-RBR interaction. Upon increase of auxin concentration, *CYCD6;1* expression expands and extra periclinal divisions occur in the ground tissue. This coincides with movement of SHR to the extra formed inner cell layer, indicating that auxin induces asymmetric cell divisions. In addition, in a *SCR* LxCxE motif mutant, where RBR mediated repression of SCR activity is hampered, auxin elevation induced successive ectopic asymmetric cell divisions in the transit amplifying zone correlating with strong shootward-extended *CYCD6;1* expression (Cruz-Ramírez et al. 2012). Thus, the balance between SCR-RBR interaction on the one hand and RBR phosphorylation by CYCD6;1 on the other defines the exact position of asymmetric cell divisions. Mathematical modeling confirms that SCR-RBR interaction and RBR phosphorylation occurs in two feed forward loops, that closely rely on each other, creating a robust bistable system (Cruz-Ramírez et al. 2012). On top of that, SHR and auxin distribution restrict asymmetric division to the stem cell region. Auxin and SHR influx trigger the 'on' state of the bistable SHR-SCR-RBR circuit, as well as the radial confinement of SHR.

Protein degradation resets the bistable asymmetric cell division switch, bringing it back to an 'off' state (Cruz-Ramírez et al. 2012). These findings were recently supported by (Weimer et al. 2012) and provide a mechanistic foundation to when- and where the asymmetric cell division of cortex/endodermis initial daughter occurs. Furthermore, the observation that RBR binds basic helix-loop-helix (bHLH) transcription factor SPEECHLESS (SPCH) (Weimer et al. 2012), known for its role in stomata development (MacAlister, Ohashi-Ito & Bergmann 2006) hints towards a more general role for RBR in asymmetric cell divisions. A non-solved issue in this model is that high SHR and SCR levels (and thus asymmetric cell divisions) should also occur in the QC, whereas this in not the case in life roots. Possibly, an extra transcriptional program or complex formation with different partners within the QC suppresses the output or formation of the SHR-SCR-RBR complex.

Fully nuclear localized SHR does not move and does not rescue the *shr-2* mutant phenotype. When provided with a Nuclear Export Signal (NES), thereby promoting accumulation to the cytoplasm, SHR protein movement was allowed again. Mutations in the SHR sequence which result in reduced nuclear accumulation of SHR, also prevent cell-to-cell transport (Gallagher et al. 2004, Gallagher, Benfey 2008). These experiments show that both cytoplasmic and nuclear localization of SHR in the stele are indispensable for SHR movement. SHR movement is affected in *shr interacting embryonic lethal* (*siel*) mutants indicating SIEL promotes SHR movement out of the stele. SIEL, an endosome-associated protein, was found to physically interact with SHR and proposed to act as an intracellular shuttle for SHR movement (Koizumi et al. 2011). In addition, *SHR* and *SCR* regulate *SIEL* expression. *siel* homozygous roots are similar to *shr-2* heterozygotes, displaying ectopic cell divisions in the ground tissue that lead to extra cells and extra cell layers in each ground tissue layer. The phenotype of *siel* homozygotes is consistent with reduced SHR activity. *siel-4;scr-4* and *siel-4;shr-2* double mutants display a more severe phenotype than either of the single mutants alone as all radial cell layers were affected. The most severe *siel-4;shr-2* double mutants lacked all internal cell layers and form root hairs very close to the root tip, suggesting termination of root growth (Koizumi et al. 2011).

Besides their role in ground tissue patterning *SHR* and *SCR* act to pattern the vasculature. mRNA profiling of wild-type, *shr* and *scr* roots indicated that nearly half of the genes co-regulated by *SHR* and *SCR* display peak expression in the stele. And *scr* and *shr* mutant roots show differentiation of the metaxylem in the place of protoxylem. Expressing *SHR* strictly from the endodermis in *shr-2* mutant roots protoxylem formation was largely restored (Carlsbecker et al. 2010). These experiments reveal a crosstalk between the vasculature and endodermis facilitated by cell-to-cell movement of the transcription factor SHR from vasculature into endodermis and microRNAs *MIR165a* and *MIR166b* in opposite direction (Carlsbecker et al. 2010). Together, SHR and SCR activate *MIR165a/6b* in the endodermis in direct fashion, which move into the stele to restrict their target HD-ZIP III (e.g. *PHB*) mRNA domains. Indeed, a mutant harboring a point mutation in the *miR165/6* target site in the *PHB* gene, displays ectopic differentiation of metaxylem in place of protoxylem, showing that degradation of *PHB* by *miRNA165/6* is of ultimate importance for xylem patterning (Carlsbecker et al. 2010).

The fact that in *scr* mutant roots SHR movement is no longer restricted to endodermis (Heidstra, Welch & Scheres 2004, Sena, Jung & Benfey 2004) implies that SCR is needed to restrict SHR to the endodermis (Cui et al. 2007, Welch et al. 2007). The C2H2 zinc finger transcription factor *JACKDAW* (*JKD*) is expressed in QC, cortex/endodermis initial and in lower amounts in the endodermis. Loss of *JKD* results in reduced nuclear localized SHR and movement of SHR outside of the endodermis giving ectopic asymmetric divisions in the cortex indicating also JKD acts to inhibit SHR movement (Welch et al. 2007). Initiation of *JKD* expression in the embryo is independent of *SHR* or *SCR*, whereas postembryonic *JKD* expression does depend on *SCR*, suggesting a complex feedback between these two regulators (Welch et al. 2007). SHR-SCR nuclear complex also activates two paralogs of *JKD*, *MAGPIE* (*MPG*) and *NUTCRACKER* (*NUC*). MGP RNAi lines are shown to suppress ectopic cortex divisions in *jkd* mutant suggesting *MGP* promotes asymmetric cell divisions, whereas *JKD* inhibits. Protein binding studies prove that JKD and MGP form nuclear protein complexes with SHR and SCR (Welch et al. 2007), this way JKD and MGP together regulate the boundaries of the ground tissue layers and asymmetric cell division.

QC and columella

Laser ablation studies have shown that the QC is crucial for stem cell maintenance (Scheres et al. 1997, Berg et al. 1995). Differentiation of columella stem cell daughters below the QC can be blocked by RNAi-mediated down regulation of *RBR (rRBR)*. Since long, RB proteins are known to be implicated in cell cycle progression both in unicellular organisms, such as algae (Umen, Goodenough 2001) and in multicellular organisms like plants (Park et al. 2005) and animals, where it was first identified as tumor suppressor gene (Friend et al. 1986). In plants RB was first found in *Zea Mays* (Grafi et al. 1996). Here it was shown to be structurally related to the human version of Rb, furthermore functional resemblance to human Rb was established (Grafi et al. 1996). In *Arabidopsis,* rRBR roots display several layers of undifferentiated columella stem cells below the QC. After ablating the QC in rRBR roots, the extra layers of columella stem cells start to differentiate. This indicates that stem-cell-promoting signals from the QC have the potential to work over several cell layers, but normally are counteracted in cells which are not in direct contact with the QC (Haecker et al. 2004, Wildwater et al. 2005). *WOX5* is expressed in the QC, where it is necessary to prevent differentiation of the distal columella stem cells (Haecker et al. 2004, Sarkar et al. 2007). *wox5* mutant roots display enlarged QC cells and the columella stem cells differentiate. Root growth and meristem size seem unaffected in *wox5* mutant roots. Also QC markers are still expressed in *wox5* roots, although broader than in wild-type. This suggest *WOX5* is mainly needed to maintain columella stem cells (Sarkar et al. 2007). Overexpression of *WOX5* in the columella leads to increased layers of columella stem cells below the QC. However, ablating the QC does not suppress the *WOX5* overexpression phenotype

(Sarkar et al. 2007). Possibly, the abundant cell layers caused by *WOX5* overexpression, consist of cells which (partly) maintain QC identity as well, suggesting a mix of columella and QC fates. In this case, *WOX5* downstream signals may enable the ectopic stem cell pool to maintain itself (Sarkar et al. 2007). The character of these the QC-derived signals still remains unclear. Candidate components for signaling in the root niche are *CLAVATA3/ ESR RELATED40* (*CLE40*) and *ARABIDOPSIS CRINKLY4* (*ACR4*). CLE40 is expressed in the root differentiation zone and in differentiated columella cells and application of CLE40p results in loss of columella stem cells (Stahl et al. 2009). Both *cle40* and *acr4* mutant roots display increased columella stem cell layers and expanded *WOX5* expression (Stahl et al. 2009, De Smet et al. 2008). When treated with CLE40p, *acr4* roots still display additional columella stem cell layers, suggesting that *ACR4* is a CLE40 receptor (Stahl et al. 2009, De Smet et al. 2008). The proposed module is as follows: WOX5 signaling from the QC promotes columella stem cell fate, this signal decreases as it goes toward the root cap, therefore allowing the columella stem cell daughter cells to differentiate into columella cells, expressing CLE40. Increased CLE40 levels in turn negatively feedback on WOX5 expression domain via ACR4. Thus a robust system balancing stem cell proliferation would be established (Stahl et al. 2009). Recently, it was shown that the receptor kinase *CLAVATA1* (*CLV1*), known for its role in shoot meristem maintenance (Clark et al. 1996, Clark, Running & Meyerowitz 1993), executes a similar function in root meristems. Like *cle40* and *acr4*, *clv1* mutant roots show additional columella stem cell layers below the QC, suggesting a role for *CLV1* in distal stem cell regulation as well (Stahl et al. 2013). When *clv1* mutant roots were treated with CLE40p a loss of columella stem cells ('stemness') was caused, even stronger than in wild-type. Therefore, CLV1 was proposed to serve to moderate stemness as apparently it is not necessary in the presence of excess CLE40p, like ACR4 is. Since *ACR4* is transcriptionally up regulated by *CLE40,* whereas *CLV1* is not, it was proposed that *ACR4* might be responsible for this strong loss of stemness. Double-mutant combinations of *cle40*, *clv1*, or *acr4* are not additive, suggesting that these three genes act in the same pathway. The expression domains of CLV1 and ACR4 overlap in the distal root meristem and both proteins localize to the plasma membrane and plasmodesmata. Furthermore, CLV1 and ACR4 interact in planta and can form homo-and heteromeric complexes (Stahl et al. 2013). Altogether, it was proposed that CLV1 functions in root stemness control by moderating ACR4-dependent signaling through binding to ACR4 (Stahl et al. 2013).

The above mentioned *SHR* and *SCR* transcription factors also function in QC maintenance. Several QC markers are no longer expressed in *shr* and *scr* mutant background and stem cells are lost (Sabatini et al. 2003). Consequently, roots of both mutants are shorter than wild-type and root growth terminates as the meristem dissipates (Helariutta et al. 2000, Di Laurenzio et al. 1996, Sabatini et al. 2003). Complementation studies revealed that *SCR* acts cell autonomously in the QC and expression of *SCR* does not rescue the QC defects of *shr* mutants showing both SHR and SCR proteins are needed in the QC to maintain the stem cell niche (Sabatini et al. 2003). In addition to *SHR* and *SCR*, two AP2-type transcription factors, *PLT1* and *PLT2*, redundantly contribute to the

maintenance of the QC and stem cell niche (Aida et al. 2004). Niche activity in *plt1;plt2* double mutant roots is comprised and root grow is decreased and terminates. Defects occur from heart stage embryo onwards where the *plt1;plt2* lens-shaped and root cap progenitor cell exhibits aberrant cell divisions (Aida et al. 2004). *PLT* expression is unaltered in *shr* or *scr* mutant roots. Also, *SHR* and *SCR* expression is not affected in *plt1;plt2* double mutants. Triple mutant *plt1;plt2;shr* and *plt1;plt2;scr* combinations show additive phenotypes, indicating *PLT* and *SHR*/*SCR* represent parallel pathways in root stem cell niche specification (Aida et al. 2004). PLT proteins act in a dose dependent manner with single mutants showing only mild defects, double mutants *plt1;plt2* being affected in root niche maintenance and triple mutant *plt1;plt2;plt3* seedlings are rootless. If one copy of *BBM/PLT4* is removed in this background (*plt1;plt2;plt3;plt4+/-*), the seedlings completely lack both root and hypocotyl, resembling *mp* mutants. They are present in a gradient throughout the root meristem which is most abundant around the QC. High ectopic expression of PLT maintains root stem cells, intermediate levels facilitate transient amplifying cell divisions that make up the meristem region, and low levels or absence of PLT allows progression of differentiation (Aida et al. 2004, Galinha et al. 2007, Galinha et al. 2007). *PLT*s activate *PIN* expression in a positive feedback loop. Computational analysis of polar auxin transport by PIN proteins, shows a stable auxin gradient in the root meristem, having an auxin maximum situated around the QC (Blilou et al. 2005, Sabatini et al. 1999, Grieneisen et al. 2007). When the root tip is excised or the QC is ablated, a new stem cell niche is formed in the place where a new auxin maximum establishes. This shows that a local auxin maximum is instructive to position the stem cell niche (Sabatini et al. 1999, Sena et al. 2009, Xu et al. 2006). Modeling PIN facilitated auxin transport, based by experimental data, shows that a stable auxin maximum and a robust auxin gradient along the root longitunal axis, correlates with auxins role in cell division and cell expansion and is able to explain maintenance of meristematic and elongation zones during root growth (Grieneisen et al. 2007).

Lateral root cap

Oriented asymmetric cell divisions of a shared epidermis/lateral root cap initial give rise to epidermis and lateral root cap layers. Two plant specific NAM, ATAF1/2, and CUC2 (NAC) domain transcription factors *FEZ* and *SOMBRERO* (*SMB*) are instructive for patterning of the root cap (Willemsen et al. 2008). *FEZ* is expressed in root cap initials. Defective lateral root cap formative periclinal divisions are causing *fez* mutant root cap to display less columella and lateral root cap layers. On the other hand, *smb* mutant roots display additional columella and lateral root cap layers. More precisely, there is an extra layer of stem cell-like cells, below columella and epidermis/lateral root cap stem cells in *smb* mutant roots. Time-lapse tracking experiments suggest that periclinal stem cell divisions are shared between the two stem cell like layers in *smb* consistent with the single extra layer in *smb* roots. SMB is present in stem cell daughters and it accumulates in maturing root cap layers. *FEZ* therefore seems to specifically stimulate formative periclinal divisions in epidermis/lateral root cap and columella stem cells. In turn, *SMB* promotes daughter cell fate in root cap cells. In agreement,

FEZ and *SMB* appear to act in a feedback loop. *FEZ* mRNA is increased and broader distributed in *smb* mutant roots. *SMB* mRNA is also increased in *smb* roots and its expression is maintained in more mature cell layers. This indicates that *SMB* restricts *FEZ* mRNA to the stem cell region and at the same time represses its own expression. Conversely, in *fez* mutant roots, *SMB* transcript fails to accumulate in the stem cell area and remains expressed in more mature LRC cells, suggesting that FEZ specifically activates SMB in the stem cell daughters (Willemsen et al. 2008). Two additional NAC domain transcription factors, *BEARSKIN1* (*BRN1*) and *BRN2* are both expressed in the root cap. In *brn1-1;brn2-1* double mutants the columella root cap layers fail to detach. Triple mutants *smb-3;brn1-1;brn2-1* display dramatic, synergistic phenotypes, which are most obvious from a mass of root cap cells that stay attached to root tip. These results indicate that *SMB* also directs maturation of the root cap, redundantly with *BRN1* and *BRN2* (Bennett et al. 2010).

Epidermis sub-specification

Epidermal cells ultimately differentiate into root hair cells or non-hair cells. The position of epidermis cells relative to underlying cortex cells determines whether a cell will be specified as hair-or non-hair cell. Trichoblasts are formed in the cleft between two underlying cortex cells and differentiate into hair cells. Atrichoblasts are formed in epidermal cells overlying just a single cortex cell, these cells will become non-hair cells (Schiefelbein et al. 2009, Tominaga-Wada, Ishida & Wada 2011). WEREWOLF (WER) specifies non hair fate, via interaction with GLABRA3 (GL3) and TRANSPARENT TESTA GLABRA 1 (TTG1). On the other hand single-repeat R3 MYB-like CAPRICE (CPC) represses non-hair fate (Schiefelbein et al. 2009, Tominaga-Wada, Ishida & Wada 2011). *SCRAMBLED* (*SCM*) encodes a leucine-rich receptor-like kinase and in *scm* mutants root hair formation is randomized and *WER* expression is no longer restricted to non-hair cells. During early developmental stages SCM-GFP protein accumulates in both hair-and non-hair cells of the meristematic epidermis. Interestingly, at later-meristematic and early-elongation zones, the protein is mainly seen in differentiating hair cells, hinting to a specific role for SCM here (Schiefelbein et al. 2009, Kwak, Schiefelbein 2008). A mechanism was proposed whereby SCM signaling represses WER transcription in hair cells, which leads to differential transcription factor activities in the hair and non-hair cells. Consequently, in the hair cells SCM expression and accumulation increases. Conversely, in non-hair cells root hair fate was suppressed by the WER-GL3/EGL3-TTG1 complex, which at the same time inhibits SCM in the non-hair cells. Also in *jkd* mutant roots *WER* is expressed in all epidermal cells. And double mutant *scm;jkd* roots reveals epistasis of *scm*, indicating that *SCM* acts downstream of *JKD* (Hassan, Scheres & Blilou 2010). Rescue of *jkd* mutants by expressing *JKD* from the cortex is sufficient to complement the epidermal patterning defects, indicating that JKD action on epidermal patterning occurs non-cell autonomously (Hassan, Scheres & Blilou 2010). This hints towards a signaling molecule from the cortex to specify overlying epidermal cell fate via activation of SCM.

Role of *SCZ* in tissue patterning

Originally, the *schizoriza* (*scz*) mutant was identified by its remarkable root hair phenotype (Mylona et al. 2002). *scz* mutant roots display an irregular root hair pattern and root hairs are formed from cells underlying the epidermis (Mylona et al. 2002, ten Hove et al. 2010, Pernas, Ryan & Dolan 2010). *SCZ* encodes a B-type heat shock transcription factor and *SCZ* mRNA expression is first detected in the QC progenitor of triangular stage embryos. From heart stage onwards *SCZ* is expressed in the ground tissue stem cells and daughter cells and weakly in vasculature (ten Hove et al. 2010, Pernas, Ryan & Dolan 2010). QC markers, are all properly initiated in *scz* mutant embryos. However, postembryonically QC markers are expressed throughout the QC/columella region, indicating the QC is properly specified during embryogenesis, but QC and columella cell fate do not properly segregate in *scz* mutant roots (ten Hove et al. 2010, Pernas, Ryan & Dolan 2010). Also epidermal/lateral root cap layers show mixed fates. Since *SCZ* is not expressed outside the ground tissue it must act non cell-autonomously to segregate the surrounding epidermal and lateral root cap fates (Mylona et al. 2002, ten Hove et al. 2010, Pernas, Ryan & Dolan 2010). Additionally, postembryonic *scz* mutant roots appear to lack cortex fate. Instead, ground tissue encompasses a double endodermis layer and a mutant subepidermal layer which is able to generate root hairs, again suggesting improper fate segregation. The initial radial defect in *scz* can be traced back to heart stage embryos where the cortex/endodermis initial divides periclinally. Subsequent additional periclinal divisions give rise to the doubled endodermis. The lack of cortex fate and the absolute requirement of cortically expressed *SCZ* to fully complement the mutant phenotype indicate *SCZ* is needed cell-autonomously to specify cortex. From there it may either influence signaling to maintain proper fates in surrounding tissues or, in addition, move outward to exert this function together with factors emanating from the cortex. Surprisingly, double mutant *scz;scr* seedlings fail to set up a genuine ground tissue, revealing a genetic interaction between *SCZ* and *SCR* in the formation of ground tissue (Pernas, Ryan & Dolan 2010).

Summarizing, *SCZ* is relevant for specification of ground tissue and segregation of surrounding QC/columella and epidermal/lateral root cap fates (Mylona et al. 2002, ten Hove et al. 2010, Pernas, Ryan & Dolan 2010). This makes it an intriguing factor for studying asymmetric cell division and fate determination in *Arabidopsis* roots.

SCOPE OF THIS THESIS

The molecular foundation to *SCZs* mode of action is still unclear. Major questions towards further understanding *SCZ* function are: through which effectors *SCZ* exerts its cell-autonomous and non-cell autonomous actions; how *SCZ* relates to the root patterning factors *PLT* and *SHR*/*SCR*; what are the co-factors that SCZ employs for its function; and whether the complex mutant phenotype can be broken down in distinct functions of *SCZ* during development. This study aims to contribute to

a deeper understanding of asymmetric cell division and fate segregation, by studying the different aspects of *SCZ* function in root development.

To identify downstream effectors of *SCZ,* we employ a microarray profiling analysis (Chapter 2). The target genes found endorse our view on the role of *SCZ* in cortex specification and outer tissue fate segregation (Mylona et al. 2002, ten Hove et al. 2010, Pernas, Ryan & Dolan 2010). We investigate the relation between *SCZ* and the clade of root cap patterning NAC domain transcription factors *FEZ*, *SMB*, *BRN1* and *BRN2* (Willemsen et al. 2008, Bennett et al. 2010). We describe the function of three additional transcription factors, *ANTHOCYANINLESS2* (*ANL2*), *TUBBY LIKE PROTEIN 8* (*TLP8*) and a C2H2 type Zinc Finger, *ZN7571*, formerly unknown to play a role in patterning of the root meristem. *SCZ*s action in patterning the stem cell niche can be further analyzed by investigation of its interaction with the well characterized root patterning transcription factor genes *SHR*, *SCR*, *PLT1* and *PLT2* (Chapter 3). We confirm a genetic interaction between *SCZ* and *SHR/SCR* transcription factor genes and we perform double mutant analyses showing that both *SCZ* and *SHR*/*SCR* are needed to establish the root stem cell niche during embryogenesis.

Three protein interactors for SCZ are subsequently described (Chapter 4). A direct physical interaction of SCZ with the TEOSINTE BRANCHED1/ CYCLOIDEA/ PROLIFERATING CELL NUCLEAR ANTIGEN FACTOR (TCP) transcription factors: TCP19 and TCP20 is revealed. As well as SCZs direct physical link with the Leucine Zipper transcription factor FLC expressor (FLX). We propose a role for TCP20 to link SCZ and SCR interaction in the QC, to explain their genetic interaction described in Chapter 3. With FLX as a SCZ binding partner, we speculate at a degradation mechanism through which SCZ segregates cell fate. Furthermore, we attempt to dissect different aspects of the *scz-2* phenotype by a classical forward genetics approach (Chapter 5). Two distinct genetic foundations are shown to suppress different components of *scz-2* phenotype. Herewith our hypothesis that *SCZ* influences the many different asymmetric cell division events in the root meristem via distinct targets is reinforced. Finally, a summarizing discussion of preceding chapters is given and perspectives for future research are highlighted.

Chapter 2

Downstream effectors highlight *SCHIZORIZA* function in tissue separation

Jansweijer VMA[1,2], Sanchez Perez GF[1,3], Timmermans A[1], ten Hove CA[1,4], Scheres B[1,2], Heidstra R[1,2]

[1] Department of Biology, section Molecular Genetics, Utrecht University, 3584 CH Utrecht, The Netherlands.
[2] Current affiliation: Plant Developmental Biology, Wageningen University Research, 6708 PB Wageningen, The Netherlands.
[3] Current affiliation: Plant Research International, Wageningen University Research, 6708 PB Wageningen, The Netherlands.
[4] Current affiliation: Laboratory of Biochemistry, WU Agrotechnology & Food Sciences, 6708 PB Wageningen, The Netherlands.

"La Primavera"

ABSTRACT

Asymmetric cell division is a fundamental mechanism for generating cellular diversity and patterns in multicellular organisms. The *Arabidopsis* root meristem is laid down during embryogenesis. Its organization is derived from strict asymmetric cell divisions of different stem cells. The *SCHIZORIZA* (*SCZ*) transcription factor is involved in a novel mechanism of cell fate separation. *SCZ* acts cell-autonomously to specify cortical cell identity and non-cell-autonomously to separate cell fates in surrounding layers. In addition, *SCZ* is implicated in stem cell niche formation. Transcriptional profiling was undertaken to identify downstream effectors of *SCZ*. Consistent with its presumed function we find two major classes of differentially regulated targets. Genes up regulated by *SCZ* are predominantly expressed in cortex, while genes down regulated by *SCZ* are enriched in the root cap. The latter class contains a suite of transcriptional regulators for root cap fate and differentiation as well as factors with predominant expression in root cap and epidermis. The function of these target genes confirms a role for *SCZ* in the maintenance of transcription factor expression patterns, which contribute to cell fate separation and stem cell function.

INTRODUCTION

Through asymmetric cell division plants are able to position different tissues in precise spatial arrangements. The orientation of such divisions and the fate of daughter cells have to be tightly controlled because plant cells are fixed to their position by rigid cell walls. Growth occurs from stem cell populations that reside at the opposite ends of the apical-basal body axis. These stem cell niches find their origin in embryogenesis (Scheres et al. 1994). At the basal end, the root meristem is formed from strict asymmetric cell divisions of different stem cells (Scheres et al. 1994, Dolan et al. 1993). Root stem cell niche specification and maintenance is controlled by SHORTROOT (SHR)/ SCARECROW (SCR) and PLETHORA (PLT) transcription factors (Aida et al. 2004, Galinha et al. 2007, Sabatini et al. 2003). In addition, SHR/SCR are required in the niche for infrequent asymmetric QC divisions that create new root cap stem cells (Cruz-Ramirez et al, submitted). SHR and SCR are also required for ground tissue patterning (Di Laurenzio et al. 1996, Helariutta et al. 2000, Nakajima et al. 2001). Endodermis and cortex are formed by periclinal asymmetric division of the ground tissue stem cell daughter (Dolan et al. 1993). This division is induced upon activation of *SCR* by SHR moving in from the vascular tissue and strongly regulated by additional binding partners (Nakajima et al. 2001, Cui et al. 2007, Heidstra, Welch & Scheres 2004, Welch et al. 2007, Sozzani et al. 2010, Cruz-Ramírez et al. 2012). Recently, a novel mechanism of cell fate separation in plants that involves the *SCHIZORIZA* (*SCZ*) transcription factor was described (Pernas, Ryan & Dolan 2010, ten Hove et al. 2010). *SCZ* is required for cortical cell identity in a cell-autonomous fashion and directs cell fates in surrounding layers non-cell-autonomously. In addition, *SCZ* is implicated in stem cell formation (Pernas, Ryan & Dolan 2010). *SCZ* acts with *SHR*/*SCR* to specify ground tissue and a functional stem cell niche (Pernas, Ryan & Dolan 2010)(Chapter 3).

Here we describe a transcriptional profiling approach to identify downstream effectors of *SCZ* by comparing wild-type with *scz* mutant roots. We highlight important aspects of *SCZ* target regulation. Genes elevated in *scz* mutants show a predominant expression pattern in peripheral tissues such as columella, lateral root cap and epidermis whereas genes with lowered expression in *scz* mutants are mostly expressed in cortex. We analyze function and regulation of several downstream transcription factor genes expressed in peripheral tissues.

RESULTS

Identification of *SCZ* target genes

scz mutants display a variety of root developmental defects mainly characterized by defective segregation of cell fates in the outer tissues of the root and an incomplete specification of cortex. *scz* roots lag behind in length compared to wild-type, but growth does not terminate (Pernas, Ryan & Dolan 2010, ten Hove et al. 2010, Mylona et al. 2002).

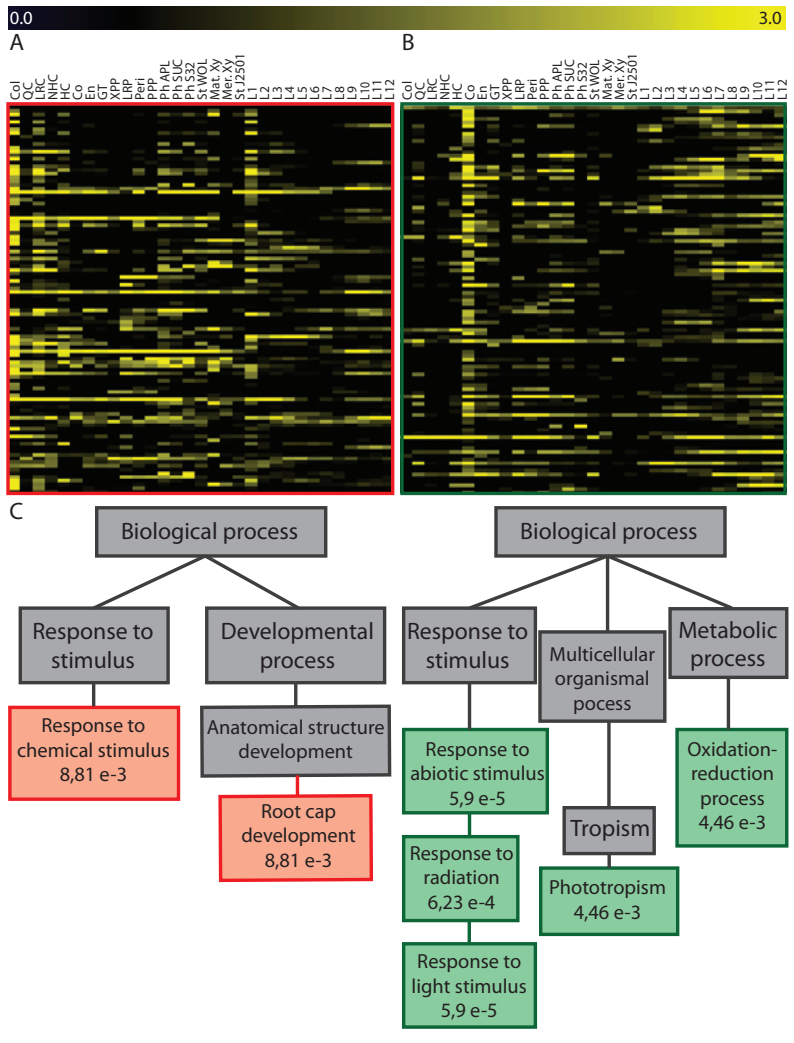

Figure 1. Identification of *SCZ* targets.

(A-B) Expression profile of genes differentially regulated comparing 4-day old *scz-2* mutant versus Col-0 roots by ATH1 microarray analysis (B>2). (A) Genes up regulated in *scz-2* roots (red box, FC>1,5). (B) Genes down regulated in *scz-2* roots (green box, FC<-1,5). Root expression data according to the high resolution root spatiotemporal map (Brady et al. 2007). Columella (Col), QC, Lateral Root Cap (LRC), non hair cells (NHC), hair cells (HC), cortex (Co), endodermis (En), ground tissue (GT), lateral root primordium (LRP), pericycle (XPP, Peri, PPP), phloem (Phl APL/SUC/S32), stele (St WOL), xylem (Mat.Xy, Mer.Xy, StJ2501). (C) GO analysis of differentially expressed genes reveals over representation of 7 categories of Biological Process (Virtual Plant 1.3, New York University, p<0,01). P-value per category is indicated in the box.

To identify genes affected by *SCZ* activity we performed differential gene expression analysis employing *Arabidopsis* whole genome ATH1 microarrays (Affymetrics). We compared the

transcription profile of 4-day old *scz-2* mutant roots with those of Columbia-0 (Col-0) wild-type. We used Limma and Affy package for Affymetrics data in R (www.r-project.org) to evaluate differentially expressed genes based on criteria of significance (B>2) and fold change (FC>1,5/ FC<-1,5). We found that 217 genes are significantly up regulated (Supplementary Table 1) and 380 genes are down regulated in *scz-2* roots (Supplementary Table 1). We analyzed the expression of differentially regulated genes using the whole root spatiotemporal gene expression dataset (Brady et al. 2007). Interestingly, up regulated genes (Figure 1A, red box) seem to be expressed in columella, lateral root cap and epidermis mostly and correlate with expression in the lowest longitudinal section L1, suggesting *SCZ* acts to repress peripherally expressed genes in the meristem. Genes down regulated in *scz-2* roots (Figure 1B, green box) are mostly expressed in cortex towards the maturing parts of the root L3 and upward (meristem and differentiation zone), suggesting a role for *SCZ* regulating cortex cell differentiation. Both observations support earlier findings that *SCZ* is needed for cortex specification (ten Hove et al. 2010).

To get a first indication if the differentially regulated gene list can provide specific clues about processes involving *SCZ* function, we assessed functional Gene Ontology (GO) Biological Process categories (Virtual Plant 1.3). Compared to wild-type, 7 categories are over-represented for the *SCZ* putative target list (Figure 1C). For the down regulated genes five categories stand out, "response to light stimulus" with an observed (O) percentage of genes of 9,3%, instead of the expected (E) 3,2% based on random distribution; "response to abiotic stimulus" (O=18,1%, E=9,1%), "response to radiation" (O=9,1% E=3,3%), "phototropism"(O=1,6%, E=0,1%) and "oxidation-reduction process" (O=11,3%, E=5,3%). These five categories contain 90 genes in total and are consistent with involvement of *SCZ* in cortex differentiation and/or stress responses, which may relate to its membership of a protein family annotated as Heat Shock Transcription Factors. The category "root cap development" catches the eye in the up regulated target genes list (O=2,4%, E=0,1%), and contains four NAC domain transcription factors *FEZ*, *SOMBRERO* (*SMB*), *BEARSKIN1* (*BRN1*) and *BRN2* all described to be involved in patterning of the root cap (Bennett et al. 2010, Willemsen et al. 2008). Encouraged by the identification of such a precise root cap development gene ontology class we focused on (putative) transcription factor genes targeted by *SCZ*.

In total, 45 putative transcription factors are found differentially expressed in *scz-2* roots (B>2) with 24 transcription factors down regulated (FC<-1,5) and 21 up regulated (FC>1,5), respectively (Table 1). For example, the zinc finger transcription factor *JACKDAW* (*JKD*), known to act from the cortex to specify epidermal cell fate (Hassan, Scheres & Blilou 2010), is down regulated in *scz-2* roots. Also, *TEOSINTE BRANCHED, CYCLOIDEA AND PCF 21 (TCP21)*, belonging to a family that is considered to promote plant growth and proliferation (Li et al. 2005, Hervé et al. 2009) is down regulated.

Table 1. Transcription factors regulated by *SCZ*.

Up in *scz-2*					Down in *scz-2*				
LOCUS	**Name**	**TF family**	FC	B	**LOCUS**	**Name**	**TF family**	FC	B
AT5G57420	IAA33	AUX/IAA	2,60257	10,14	AT2G40260	NN	MYB	-2,4996	10,63
AT3G22760	SOL1	CPP	1,9097	9,95	AT2G38300	NN	MYB	-3,4999	10,23
AT1G33280	**BRN1**	NAC	2,8218	7,89	AT2G25000	WRKY60	WRKY	-2,136	8,37
AT1G79580	**SMB**	NAC	2,59335	7,41	AT5G03150	JKD	ZnF C2H2	-2,5304	8,35
AT4G10350	**BRN2**	NAC	3,45424	7,41	AT1G64380	NN	AP2-EREBP	-2,3897	7,59
AT1G17310	NN	MADS	3,33469	7,34	AT3G50870	MNP/HAN	C2C2-Gata	-1,665	6,02
AT4G17490	NN	AP2-EREBP	2,1847	7,08	AT3G05800	bHLH150	bHLH	-1,7267	5,71
AT1G16070	**TLP8**	TUB	1,62647	6,47	AT2G02070	IDD5	C2H2	-1,6614	5,24
AT5G10510	AIL6	AP2-EREBP	1,82624	5,84	AT5G15150	ATHB3/HAT7	Homeobox	-1,5705	5,2
AT1G26870	**FEZ**	NAC	1,97645	5,58	AT4G05100	MYB74	MYB	-1,8527	5,12
AT5G13910	LEP	AP2-EREBP	1,70106	5,35	AT1G04240	IAA3/SHY2	AUX/IAA	-1,6091	5,05
AT1G59530	BZIP4	BZIP	1,82911	5,2	AT5G61430	ANAC100	NAC	-1,6835	4,99
AT1G46264	HSFB4	HSF	1,66745	4,85	AT1G13300	NN	Myb/G2-like	-1,9316	4,44
AT1G75710	**ZN7571**	C2H2	1,6112	4,62	AT2G35940	BLH1/EDA29	Homeobox	-1,9325	3,96
AT3G16770	NN	AP2-EREBP	2,00848	4,32	AT3G25790	NN	Myb/G2-like	-1,5958	3,79
AT1G21910	NN	AP2-EREBP	1,55941	3,64	AT1G68880	NN	bZIP	-1,985	3,71
AT3G13000	NN	NN	1,68822	3,32	AT2G46830	CCA1	NN	-1,8701	3,51
AT4G18450	NN	AP2-EREBP	1,9427	2,33	AT3G49760	bZIP5	bZIP	-1,6819	3,45
AT5G03510	NN	ZnF C2H2	1,70375	2,22	AT1G31050	NN	bHLH	-1,5735	3,23
AT4G00730	**ANL2**	Homeobox	1,68239	2,12	AT5G24120	SIG5/SIGE	NN	-1,5403	2,68
AT5G13330	Rap2.6L	AP2-EREBP	1,68454	2,06	AT2G01430	ATHB17	Homeobox	-1,5185	2,43
					AT4G38620	MYB4	MYB	-1,5855	2,24
					AT5G44190	ATGLK2/ GLK2/GPRI2	Myb/G2-like	-1,5948	2,21
					AT5G08330	TCP21	TCP	-1,5476	2,11

Differentially expressed transcription factor genes comparing 4-day old *scz-2* mutant versus Col-0 roots by ATH1 microarray analysis (B>2, FC>1,5/ FC<-1,5). In bold transcription factors analyzed in this study.

NAC domain transcription factors are repressed by *SCZ*

Amongst the transcription factors up regulated in *scz-2* roots are the above mentioned NAC domain transcription factors. To validate *FEZ*, *SMB*, *BRN1* and *BRN2* expression changes, we performed qRT-PCR experiments on wild-type and *scz-2* mutant roots. We found that each of the four genes indeed is up regulated in *scz-2* roots (Figure 3A). *BRN1* and *BRN2* show the highest relative up regulated expression in *scz-2* (~7-fold), whereas *SMB* is 5-fold up regulated and *FEZ* approximately 4-fold in *scz-2* roots.

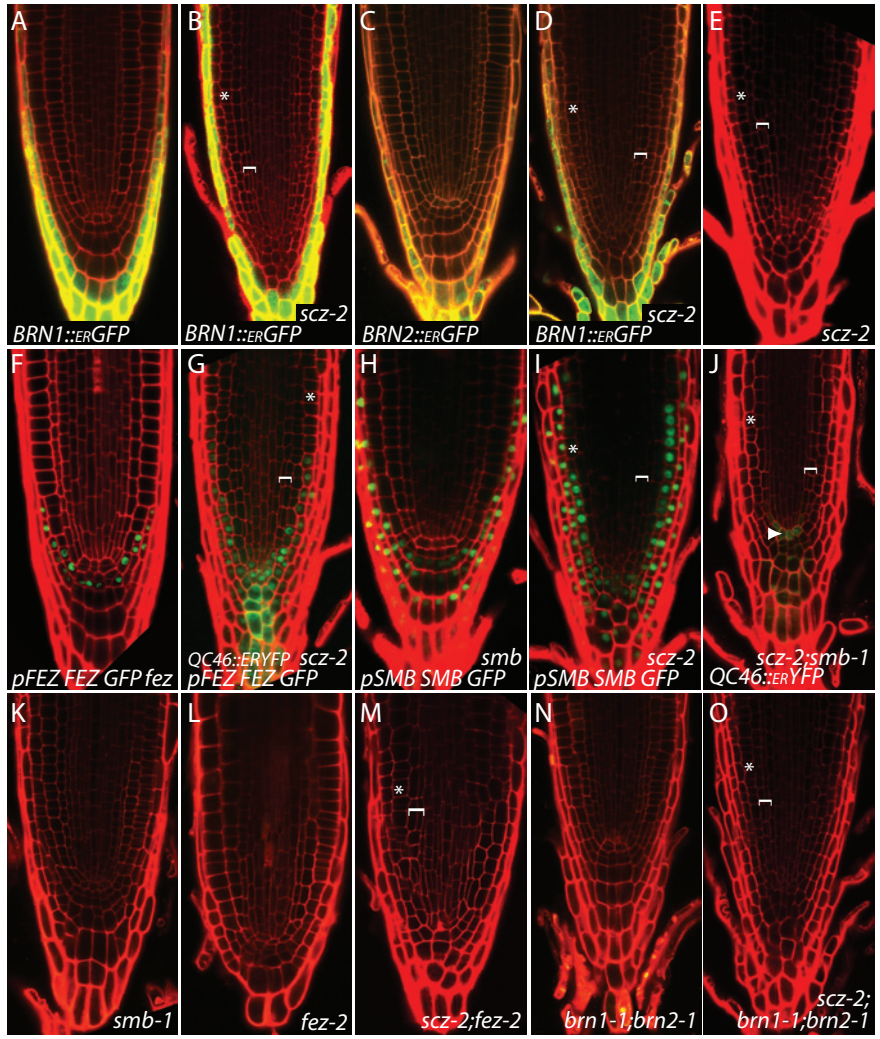

Figure 2. NAC domain transcription factors are regulated by *SCZ*.

(A,B) *BRN1* expression in wild-type (A) and *scz-2* (B) roots. (C,D) *BRN2* expression in wild-type (C) and *scz-2* (D) roots. (E) *scz-2* mutant root. (F,G) *pFEZ::FEZ:GFP* expression in *fez-2* (F) and *QC45::$_{ER}$YFP;scz-2* (G) roots. (H,I) *pSMB::SMB:GFP* expression in *smb-3* (H) and *scz-2* (I) roots. (J) *scz-2;smb-1* double mutant root. (K-O) *smb-1* (K), *fez-2* (L), *scz-2;fez-2* (M), *brn1-1;brn2-1* (N), and *scz-2;brn1-1;brn2-1* (O) mutant roots. White arrow head marks QC. Square bracket marks *scz-2* ectopic endodermal layers, asterisk marks *scz-2* derived mutant subepidermal layer. Roots depicted at 5dpg.

We reasoned that up regulation of the four NAC transcription factors might be caused by more root cap tissue in *scz-2* roots in which the putative target genes are expressed. Alternatively, expression may invade additional tissues as observed for a number of markers/genes including SMB:GFP (ten Hove et al. 2010). To study the effect of *SCZ* on expression of *FEZ*, *SMB*, *BRN1* and *BRN2* we used

protein-fusion lines of FEZ and SMB (Willemsen et al. 2008) and constructed promoter-reporter fusion lines for *BRN1* and *BRN2*. In *scz-2* mutant roots pFEZ::FEZ:GFP is expressed broader in the stem cell niche compared to wild-type (Figure 2F,G). Its expression even extends to epidermal and mutant subepidermal layers and to the vasculature. A similar effect is seen with expression of pSMB::SMB:GFP, which extends to epidermis (Figure 2H,I (ten Hove et al. 2010)). These results suggest *SCZ* is needed to repress FEZ and SMB outside of their normal expression domain. Expression of *pBRN1::$_{ER}$GFP* and *pBRN2::$_{ER}$GFP* in *scz-2* extends shoot wards in the lateral root cap (Figure 2A-D) suggesting these cells do not properly mature. Alternatively, positive regulators of these proteins are stabilized in *scz* mutants, which allows them to be expressed over a longer time span and hence occupy a larger domain. Previously, we have shown that the *scz-2;smb-3* double mutant combination restores QC morphology and positioning (ten Hove et al. 2010)). Therefore we tested whether *fez*, *brn1* and *brn2* could also suppress (part of) the *scz-2* phenotype. As a control we use the weaker *smb-1* allele (Figure 2K) (Willemsen et al. 2008) which in *scz-2;smb-1* double mutant combination is also able to specifically suppress the QC/columella defects of *scz-2* (Figure 2J). Double mutant *scz-2;fez* and *scz-2;brn1-1;brn2-2* triple mutant roots display additive phenotypes (Figure 2M,O), suggesting *SCZ* acts independent of *FEZ and BRN* genes.

To be able to distinguish between direct or indirect regulation by *SCZ*, we constructed a glucocorticoid hormone inducible version of *SCZ* fused to the rat Glucocorticoid Receptor (GR). Initial effects of GR:SCZ activation in roots are seen in QC of *35S::GR:SCZ;scz-2* roots after 16 hours dexamethasone (dex) induction. The expression of the *QC46::$_{ER}$YFP* marker, which is broadened in *scz-2* mutant roots, is focused to the QC, where it is originally expressed (ten Hove et al. 2010)(Figure 3D). After 30 hours dex induction the QC defect is restored and below QC a layer of columella stem cells (devoid of starch) is maintained. This shows that *35S::GR:SCZ* complements *scz-2*. Based on the rescue experiments and considering the adverse effects of long cyclohexamide (chx, a protein synthesis inhibitor) incubation times we decided to test regulation of *FEZ*, *SMB*, *BRN1* and *BRN2* after 4 hours induction of *GR:SCZ* by qRT-PCR (Figure 3G). *FEZ* and *SMB* expression is not significantly changed by *GR:SCZ* induction, indicating these are indirectly regulated. Surprisingly, both *BRN1* and *BRN2* are activated by *35S::GR:SCZ* (dex) and we find that *BRN2* is being directly activated (chx/dex). This observation conflicts with our expectations based on expression of *BRN* genes in *scz-2* roots. However differences may be caused due to the short induction time, or use of 35S promoter.

New factors for niche patterning and maintenance

Amongst the 45 transcription factors regulated by *SCZ* there were 35 genes to which no function in the *Arabidopsis* root niche was ascribed yet (Table 1, Supplementary Table 3). Therefore, we decided to analyze these in further detail. First, for each of these transcription factor genes, their corresponding knock out mutants (if available from public seed stocks) were analyzed focusing on root growth and meristem organization (Supplementary Table 3). Three of the analyzed mutants displayed an evident phenotype.

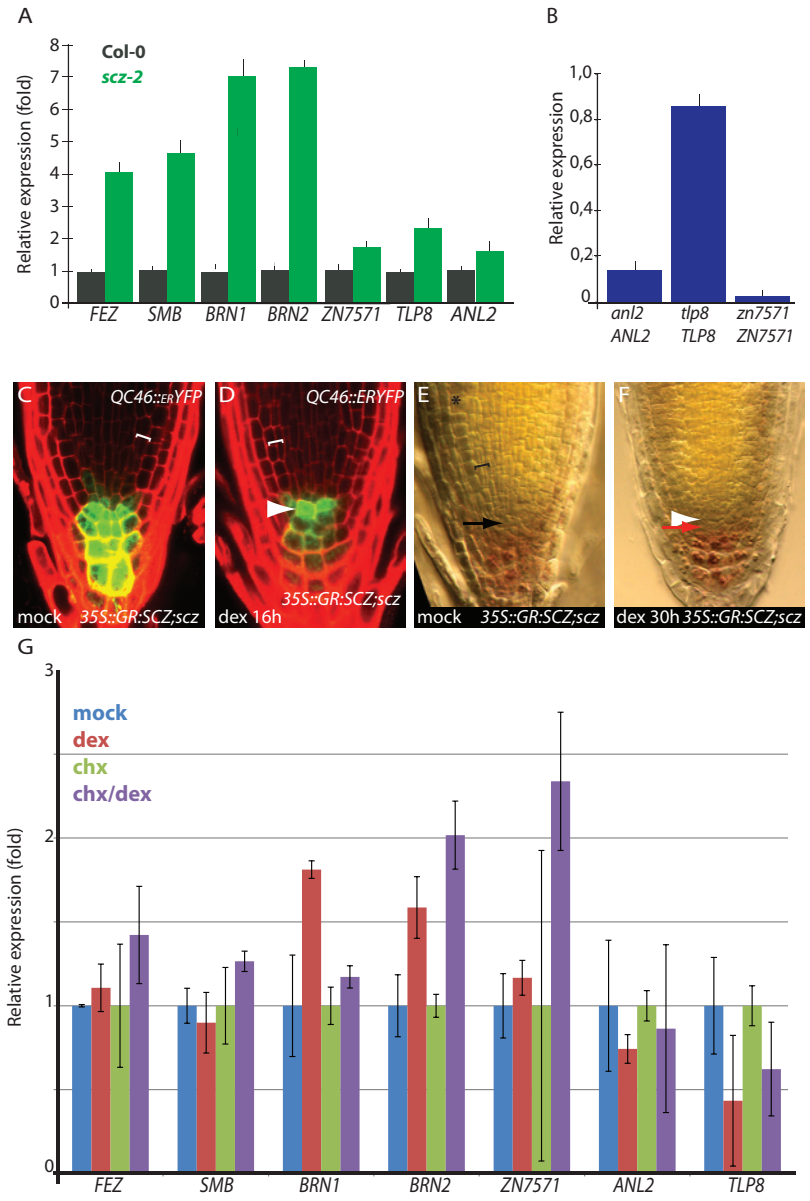

Figure 3. *SCZ* target validation.

(A) qRT-PCR showing relative mRNA levels of *FEZ*, *SMB*, *BRN1*, *BRN2*, *ZN7571*, *ANL2* and *TLP8* in Col-0 (grey bars) versus *scz-2* (green bars) roots. (B) Remaining relative mRNA abundance of *ANL2*, *TLP8* and *ZN7571* in their corresponding mutant compared to wild-type roots. (C,F) *35S::GR:SCZ;scz-2* mock (C,E), 16h (D) and 30h (F) dex treated roots (5-6dpg). Square bracket marks *scz-2* ectopic endodermal layers, asterisk marks *scz-2* derived mutant subepidermal layer. (G) qRT-PCR showing relative mRNA accumulation of *FEZ*, *SMB*, *BRN1*, *BRN2*, *ZN7571*, *ANL2* and *TLP8* in *35S::GR:SCZ;scz-2* roots after 4 hour mock (blue), dex (red), chx (green) and dex/chx (purple) treatment.

The corresponding genes were: *ANTHOCYANINLESS2* (*ANL2*, *At4G00730*) belonging to the HD-GLABRA2 homeodomain leucine zipper IV class of transcription factors; *TUBBY LIKE PROTEIN 8* (*TLP8*, *AT1G16070*); and *ZN7571* (*AT1G75710*), a C2H2 type Zinc Finger transcription factor. To validate *ANL2*, *TLP8* and *ZN7571* as putative *SCZ* targets identified by microarray we performed qRT-PCR experiments on wild-type and *scz-2* mutant roots. We confirmed that each gene is up regulated around 2-fold in *scz-2* roots (Figure 3A). These results suggest that *ANL2*, *TLP8* and *ZN7571* genes may be repressed by *SCZ*.

ANL2

ANL2 was originally found to be involved in accumulation of anthocyanin (Kubo et al. 1999). More recently, *ANL2* was also shown to play a role in root development. In the *anl2* mutant meristem, ectopic cells between epidermis and cortex are formed that develop from epidermal cells and acquire cortex fate (Kubo, Hayashi 2011). We studied the expression patterns of *ANL2* in wild-type and *scz-2* mutant background by promoter-reporter fusion lines. *pANL2::GUS* is expressed in QC, epidermis and lateral root cap (Figure 4Q). In *scz-2,* initial experiments suggest *ANL2* expression is maintained although at lower levels. It seems expression does not extend outside epidermis and lateral root cap cells (Figure 4W). Prolonged GUS treatment should confirm that *ANL2* expression indeed does not invade other tissue types. We detected extremely reduced transcription levels by qRT-PCR in mutant roots of an *ANL2* knockout line harboring an *I* element insertion in intron 6 (Kubo et al. 1999) (Figure 3B), suggesting this is a null allele. Analysis of *anl2* mutants roots revealed impaired root growth (Figure 4A). Root growth lags behind compared to wild-type (Figure 4B). Shoot development and seed set appear unaffected in *anl2* plants (not shown). To determine the cause underlying the impaired root growth, we counted the number of small meristematic cortex cells (Sabatini et al. 2003). Root meristem size of *anl2* roots at 6 days post germination (6dpg) is reduced (~32 cells) (Figure 4C), suggesting *anl2* decreased root length is caused at least in part by a lower number of meristematic cells.

Detailed examination of the *anl2* root meristem revealed disturbed organization of the root stem cell niche and columella (Figure 4F). The *anl2* root cap consists of irregularly shaped cells, however a functional QC can be distinguished overlying a layer of columella stem cells devoid of starch granules which mark differentiated columella cells (Figure 4K). To find out if the abnormalities in *anl2* are due to defects in initiation or maintenance of the stem cell niche, we analyzed *anl2* mutant embryos from heart stage onward where QC and stem cell niche are set up (Scheres et al. 1994). We found that 8 out of 15 *anl2* embryos show ectopic division of the QC in torpedo- and mature stage embryos (Figure 4U, green arrowhead) indicating embryonic origin of patterning defects. This leads to an average of 3,75 columella layers amongst *anl2* torpedo stage embryos (n=25), whereas wild-type typically has 3 at this stage. Initiation and maintenance of the stem cell niche is controlled by the *SHR, SCR* and *PLT* transcription factor genes in parallel fashion (Aida et al. 2004, Galinha et al. 2007, Sabatini et al. 2003).

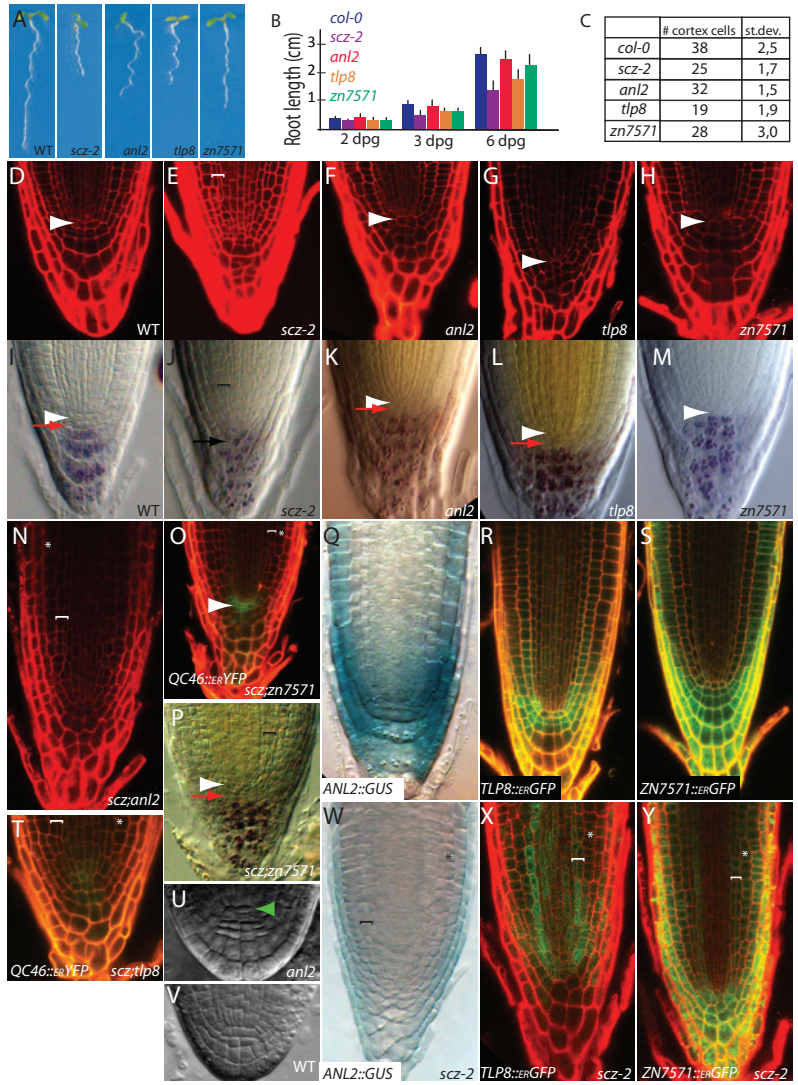

Figure 4. Functional analysis of *SCZ* regulated transcription factors.

(A) 6-Day-old wild-type, *scz-2*, *anl2*, *tlp8* and *zn7571* seedlings. (B) Root length (in cm) of wild-type (blue), *scz-2* (purple), *anl2* (red), *tlp8* (orange) and *zn7571* (green bars) seedlings at indicated days post germination (dpg). For each data point n>50; error bars, standard error of the mean. (C) Number of meristematic cortex cells of wild-type, *scz-2*, *anl2*, *tlp8* and *zn7571* roots (6dpg). (D-H) Confocal images of wild-type (D), *scz-2* (E), *anl2* (F), *tlp8* (G) and *zn7571* (H) roots (5dpg). (I-M) Lugol stained roots of wild-type (I), *scz-2* (J), *anl2* (K), *tlp8* (L) and *zn7571* (M) (6dpg). (N-O) *scz-2;anl2* (N) and *QC46::$_{ER}$YFP,scz-2;zn7571* roots (5dpg). (P) Lugol stained *scz-2;zn7571* root (5dpg). (Q,W) *ANL2::GUS* expression in wild-type (Q) and *scz-2* (W) roots (5dpg). (R,X) *TLP8::$_{ER}$GFP* expression in wild-type (R) and *scz-2* (X) roots (4dpg). (S-Y) *ZN7571::$_{ER}$GFP* expression in wild-type (S) roots (Y) (6dpg). (U,V) torpedo stage *anl2* (U) and wild-type (V) embryo. Green arrowhead marks aberrant QC division in U. White arrowhead marks QC. Red arrow marks columella stem cells devoid of purple starch granules. Black arrow marks accumulation of starch granules in mutant QC region. Asterisk marks *scz-2* derived mutant subepidermal layer. Bracket indicates *scz-2* derived ectopic endodermal layers.

To find out if *ANL2* interacts with the *SHR/SCR* or *PLT* pathway, we crossed *anl2* to *shr*, *scr* single and *plt1;plt2* double mutants. We find that *anl2;shr-2* and *anl2;scr-4* double mutants resemble *shr-2* and *scr-4* single mutant. *anl2;plt1-4;plt2-2* grow shorter (~10%) roots than *anl2* single or *plt1-4;plt2-2* double mutants (data not shown). This indicates that *ANL2* acts genetically downstream of *SHR/SCR* and in parallel to the *PLT* pathway. *ANL2* functions downstream of *SCZ* and is up regulated in *scz-2* mutant roots according to mRNA expression data. To assess whether increased expression of *ANL2* might be responsible for (parts of) the *scz-2* phenotype we created double mutant lines. Double mutant *scz-2;anl2* grow shorter (~20%) than the corresponding single mutant roots (not shown) showing that *anl2* mutation has an additive effect on the *scz-2* root growth defect. Root meristem organization of *scz-2;anl2* double mutants resembles *scz-2* indicating the *anl2* phenotype may be masked by the pleiotropic *scz* defects (Figure 4N). Finally, to distinguish between direct or indirect regulation by *SCZ* we determined the expression of *ANL2* by qRT-PCR, using *35S::GR:SCZ;scz-2* roots upon 4 hours dex and dex/chx induction. Although there is trend of *ANL2* being down regulated by *SCZ*, at the current time point the expression levels were not significantly altered in either condition (Figure 3G).

TLP8

TUBBY proteins in mammals are required for maintenance and function of neuronal cells (Boggon et al. 1999). In plants, *TLP*s are reported to be involved in stress signaling (Yang et al. 2008). Of the 11 *TLP*s in Arabidopsis, only *TLP8* lacks the F-box domain that is characteristic for plant *TLP*s (Cai et al. 2007; Yang et al. 2008). A phenotype for *tlp8* mutant roots has not been reported. The *tlp8* mutant used in our study is a TILLING line (Torii et al. 1996). In agreement with the nature of the mutation we still amplified mutant *TLP8* transcripts in qRT-PCR experiments (Figure 3B). We investigated the expression patterns of *TLP8::$_{ER}$GFP* in wild-type and found it broadly expressed in the stem cell niche extending into young ground tissue, epidermis and root cap cells and fading as the cells mature (Figure 4R). In *scz-2, TLP8* expression is stabilized in ground tissue, particularly endodermis and extends throughout the meristem (Figure 4X). Thus, *SCZ* activity is needed to suppress ectopic *TLP8* expression.

Root length measurements show that *tlp8* roots stay shorter compared to wild-type roots and suggest they do not terminate (Figure 4A,B). At 6 dpg the *tlp8* root meristem consists of fewer cells (~19) compared to wild-type (~38) (Figure 4C). Therefore, *tlp8* decreased root length could be caused by a lower number of meristematic cells. Analyzing *tlp8* root meristem we see an apparently functional QC and a layer of columella stem cells devoid of starch granules below QC (Figure 4G,L). The *tlp8* root cap displays an irregular morphology and appears shorter. Indeed, *tlp8* roots possess 3,2 columella layers on average, whereas wild-type typically has 5 layers (n=15). Heart-, torpedo- and mature stage *tlp8* embryos resemble wild-type (not shown) indicating niche defects occur post-embryonic.

We crossed *tlp8* to *shr*, *scr* single and *plt1;plt2* double mutants to find out if *TLP8* interacts with the *SHR/SCR* or *PLT* pathway. Both *tlp8;shr-2* and *tlp8;scr-4* double mutant roots are shorter (~10%) than either *shr-2*, *scr-4* or *tlp8* single mutant roots whereas *tlp8;plt1-4;plt2-2* triple mutant roots resemble *plt1-4;plt2-2* double mutant (not shown). Thus, *TLP8* appears to acts downstream of the *PLT* genes and in parallel to *SHR/SCR* pathway. To find out if *TLP8* increased expression is responsible for (parts of) the *scz-2* phenotype we analyzed *scz-2;tlp8* double mutant lines. Double mutant *scz-2;tlp8* roots are shorter (~25%) than *scz-2* or *tlp8* single mutant roots (not shown), indicating *tlp8* mutation has an additive effect on the *scz-2* root growth defect. In *scz-2;tlp8* root meristems, aspects typical of both *scz-2* and *tlp8* are seen (Figure 4T). *scz-2* QC maintenance defects and a reduced/pointed root cap typical for *tlp8* indicates additive effects of *scz-2* and *tlp8* in *scz-2;tlp8* root meristem organization. We analyzed *TLP8* expression in *35S::GR:SCZ;scz-2* roots after 4 hours dex and dex/chx incubation by qRT-PCR. Although *TLP8* expression tends be down regulated upon induction of GR:SCZ (Figure 3G) in agreement with observed elevated expression in *scz* mutant roots, the time of induction appears too short for statistical relevance.

ZN7571

To *ZN7571* no specific role in plant biology has been ascribed. We analyzed expression of *ZN7571::$_{ER}$GFP* in wild-type and found it expressed weakly in QC and ground tissue stem cells and fading in their daughter cells. Expression is stronger in all cells of meristematic epidermis, lateral root cap and columella (Figure 4S). In *scz-2*, *ZN7571* is stabilized in the ground tissue and even appears to extend to the outer vasculature whereas expression in epidermis and root cap layers remains (Figure 4Y). This shows that *SCZ* activity is required for suppression of ectopic *ZN7571* expression. We did not detect significant transcript levels by qRT-PCR in a *zn7571* mutant with a T-DNA insertion located in the first exon (Figure 3B), suggesting this is a null allele. *zn7571* mutant roots grow shorter compared to wild-type, although growth appears indeterminate (Figure 4A,B). Shoot development and seed set is not affected in *zn7571* plants (not shown). To determine the cause underlying the impaired root growth, we counted the number of small meristematic cortex cells (Sabatini et al. 2003). The root meristem of *zn7571* roots consist of fewer cells (~28) compared to wild-type roots (Figure 4C), indicating the lower number of meristematic cells is (at least partly) responsible for the decreased root length. When we examined the *zn7571* root meristem more closely we were able to distinguish the QC based on morphological criteria (Figure 4H,M arrowhead). However, presence of starch granules in cells directly below indicates QC function is disturbed (Figure 4M). Heart-, torpedo- and mature stage *zn7571* embryos resemble wild-type (not shown), indicating defects observed occur post-embryonically. *zn7571* was crossed to *shr*, *scr* single and *plt1;plt2* double mutants to find out if *ZN7571* interacts with the *SHR/SCR* or *PLT* pathway. *zn7571;shr-2* double mutant root length resembles that of *shr-2* single mutants whereas triple mutant *zn7571;plt1-4;plt2-2* roots are shorter (~10%) than *zn7571* single or *plt1-4;plt2-2* double mutant roots (not shown), indicating that *ZN7571* act downstream of *SHR/SCR* and in parallel to the *PLT* pathway.

Interestingly, when analyzing *scz-2;zn7571* double mutant roots a QC can be distinguished and the *QC46::$_{ER}$YFP* marker is restored to the QC (Figure 4O). In addition, starch staining shows that there is a cell layer devoid of starch granules, indicating *scz-2* QC function is restored with respect to columella stem cell maintenance (Figure 4P). However, the *scz-2* radial patterning and root growth phenotype (Figure 4O,P, not shown) remain. Thus, *zn7571* represents a suppressor of the *scz*-2 QC/columella phenotype. In qRT-PCR using *35S::GR:SCZ;scz-2* roots upon 4 hours dex and dex/chx induction we detected a tendency towards up regulation of *ZN7571* expression in dex/chx treated roots (Figure 3G). This observation conflicts with our expectations based on elevated expression of *ZN7571* in *scz-2* roots. However, differences may be caused due to the short induction time or use of the 35S promoter.

DISCUSSION

We describe a transcriptional profiling strategy to find downstream target genes of *SCZ*. Based on published profiling data (Brady et al. 2007), the clear distinction in expression pattern of the majority of up- and down regulated genes in the *scz-2* roots nicely confirms the observed phenotypic defects. Up regulated genes correspond with expression in the lateral root cap and columella, whereas cortex expressed genes are down regulated. In (Brady et al. 2007) two different marker lines for cortex profiling are used, one is expressed in ground tissue from the tip upwards (J0571/N9094) and one is expressed from elongation zone upward (CORTEX). The majority of the down regulated genes in *scz* mutant root falls in the CORTEX category. This indicates not only a role in cortex specification but also a role for *SCZ* in cortex differentiation.

With the analysis of transcription profiles of *scz-2* versus wild-type roots, an indication of the amount of differential gene expression is obtained. However, to identify the immediate targets of *SCZ* it is required to profile early after gene induction. Since such an approach is not taken here, genes in the *SCZ* target list represent both direct and indirect transcriptional targets. The SCZ sequence harbors two putative repression sites in the C-terminal domain of the protein, a R/KLFGV motif (Ikeda, Ohme-Takagi 2009) and a LxLxL type EAR motif (Kagale, Links & Rozwadowski 2010). Repressive activity of SCZ could not be experimentally proven in repressor assays (Guan et al. 2012). Nevertheless, if we expect SCZ to act as a repressor solely, only genes repressed can be direct target genes. Furthermore, it is also possible *SCZ* has a dual repressor and activator function, potentially even in distinct tissues. Such a scenario is reminiscent to the way *WUSCHEL* (*WUS*) acts. Homeodomain transcription factor *WUS* is crucial for stem cell maintenance in the shoot apical meristem (Schoof et al. 2000, Brand et al. 2000). *WUS* is shown to activate its direct target gene *AGAMOUS* (*AG*) (Lohmann et al. 2001) and repress *A-TYPE ARABIDOPSIS RESPONSE REGULATOR7* (*ARR7*) (Leibfried et al. 2005, To et al. 2004). In early flowers, *WUS* activates *AG* expression (Lohmann et al. 2001), thereby terminating stem cell maintenance and initiating floral patterning (Bowman, Smyth & Meyerowitz 1989). On the other hand, repression of *ARR7* by *WUS* is necessary for proper meristem function (Leibfried et al. 2005, To et al. 2004, Kiba et al. 2003).

SCZ belongs to the family of heat shock transcription factors (HSF). To execute their task, HSFs bind to the promoter of Heat Shock Proteins (HSP) via conserved cis-acting elements, the heat shock elements (HSEs) (Schöffl, Prändl & Reindl 1998). However, none of the HSPs on the ATH1 array was found to be differentially regulated by *SCZ* in the microarray experiments described here, suggesting that *SCZ* is not mediating stress response. Nevertheless, the GO annotation of putative *SCZ* target genes leaves open the possibility that *SCZ* may be implicated in certain stress responses.

Four putative *SCZ* down regulated genes *FEZ*, *SMB*, *BRN1* and *BRN2* stood out because they all encode NAC domain transcription factors involved in root cap development. Expression analysis shows that increased gene expression of these genes follows two different scenarios. First, expression can be broadened but remain restricted in the same tissue. This is the case for *BRN1* and *BRN2* gene expression, which broadens into the additional root cap tissues displayed in the *scz-2* roots. This could hint to a role for *SCZ* for in time degradation or destabilization of positive regulators of *BRN1* and *BRN2*. Consequently, in *scz-2* mutant roots, these positive regulators remain stabilized and a prolonged activity of *BRN1* and *BRN2* is allowed resulting in gene expression in a larger domain. Surprisingly, in qRT-PCR experiments on *35S::GR:SCZ* roots, *BRN2* appears directly up regulated by *SCZ* and *BRN1* is up regulated indirectly. Given the up regulation of *BRN* genes in *scz-2* mutant roots, we were expecting to find a down regulation of gene expression. At least, this shows that ectopically expressed *SCZ* has the potential to directly activate *BRN2*. Possibly, ectopic expression of *35S* driving *GR:SCZ* giving rise to the formation of ectopic root cap layers, as seen in *35S::SCZ* roots (ten Hove et al. 2010), can explain up regulation of *BRN1* and *BRN2* after *SCZ* induction. In the second scenario, apparent elevated expression is caused due to extended expression to other tissue types. This is observed for FEZ and SMB. FEZ expression invades epidermal and mutant subepidermal layers. Additionally, FEZ broadens to the vasculature and SMB expression broadens to include epidermis. This suggests *SCZ* is needed to repress FEZ and SMB expression outside their expression domains. This is in agreement with *SCZ* function to promote the segregation of fates outside the stele.

The three transcription factors *ANL2*, *TLP8* and *ZN7571* are important for columella/ root cap patterning, judging from their mutant phenotypes. The question is raised if cell fates in the root cap are correctly separated in each mutant root. In this light it is interesting to note, that *ZN7571* is predominantly expressed in those cell types where *SHR* and *SCR* do not function anymore (epidermis and root cap layers). In *scz-2* mutant roots *ZN7571* expression extends, invading ground tissue and outer vasculature. *SCZ* activity is thus required for suppression of ectopic *ZN7571* expression in the cell types where *SHR* and *SCR* act. Possibly, *SCZ* is needed keep *ZN7571* out of the *SHR-SCR* domain, in order to properly separate radial- and root cap tissue fates. Judging from its wild-type

expression pattern and mutant phenotype, we opt for a role for *ZN7571* to function to sustain QC activity towards maintenance of underlying columella stem cells. The *zn7571* mutant phenotype resembles that of the *wuschel related homeobox 5 (wox5)* mutant and introduction of QC markers and analysis of *WOX5* expression should reveal the role of *ZN7571* in QC patterning/ maintenance. Genetic interaction studies indicate *ZN7571* functions in parallel with *PLT* and downstream of *SHR/ SCR* genes which were also shown to affect *WOX5* expression in the QC (Sarkar et al. 2007). However, at this stage, we cannot rule out the defects seen in *zn7571* are a direct consequence of columella differentiation. Interestingly, *scz-2;zn7571* double mutant roots show restored QC function. This result reinforces the idea that *ZN7571* may have to be restricted to outer layers and failure of this may lead to the QC defect in *scz*. Analysis of different QC markers and lugol staining in *scz-2;zn7571* double mutants roots will aid to see if *zn7571* fully rescues the *scz-2* QC phenotype. GR:SCZ induction experiments suggest *ZN7571* is up regulated by *SCZ* in direct fashion. Possibly, *SCZ* repressing action on *ZN7571*, as observed from the *scz-2* versus wild-type microarray experiment, may only become visible after longer induction, eg. due to a feedback mechanism.

Also *ANL2* is likely to be involved in fate separation, since (Kubo, Hayashi 2011) found that in *anl2* roots ectopic cells between the epidermis and cortex layer are formed. These cells origin from epidermis but adapt cortex fate later. We find ectopic QC divisions in *anl2* mutant torpedo- and mature embryos, suggesting that the postembryonic columella phenotype originates from patterning defects. In future experiments, analysis of fate markers should strengthen this observation. From the 11 *TUBBY*-like proteins described in *Arabidopsis* (Yang et al. 2008), *TLP8* is the only member lacking a typical F-box domain and is therefore assumed not to function in protein degradation (Reitz et al. 2012). In the root, *TLP8* mRNA levels were found to be enriched in the transcriptional profile of QC (Nawy et al. 2005). We find *TLP8* broadly expressed in and around the QC region. Although root growth and meristem size is decreased, the *tlp8* mutant QC and columella stem cells are unaffected even though columella seems incorrectly specified. Possibly, columella cells mature sooner and therefore detach earlier, resulting in *tlp8* root tip phenotype. On the other hand, cell divisions could happen at slower rate in *tlp8*, hinting at a role for *TLP8* as a cell division rate determinant. Interestingly, *TLP8* expression extends upwards in the ground tissue in *scz-2*. *SCZ* could therefore act to repress *TLP8* in ground tissue specifically, ensuring correct patterning of the ground tissue.

In our mutant versus wild-type transcriptomics approach we identified three genes involved in root growth and niche patterning (*ANL2*, *TLP8*, *ZN7571*) of which one represents a new suppressor of the *scz* mutant phenotype (*ZN7571*). To identify communalities of the processes regulated by these genes additional transcriptomics experiments may reveal common target genes. To identify effectors more directly regulated by *SCZ*, transcriptomics using the inducible GR:SCZ protein will present a major improvement. To further separate the niche and radial pattern related target genes, tissue specific profiling upon GR:SCZ induced mutant complementation may be attempted. Together,

these approaches are expected to further uncover the mechanism of *SCZ* action. We conclude by suggesting that the role of *SCZ* is to support the distinct asymmetric cell division events in the root meristem. Possibly it does so by repressing activity of downstream effectors in certain cell types, resulting in different cell identities at different regions in the root meristem.

EXPERIMENTAL PROCEDURES

Microarray analysis

4-Day-old Columbia-0 (Col-0) and *scz-2* roots were dissected at root meristem height and frozen in liquid nitrogen. We included three biological replicates for each sample. Total RNA was then isolated from frozen material, using RNeasy minikit columns (Qiagen, http://www.qiagen.com) and sent to Service XS (http://www.servicexs.com) for hybridization on Affymetrix ATH1 microarray chips.

Normalization of the raw microarray dataset was done, using the Affy package in the statistical environment R (http://www.r-project.org). To convert raw data to log expression values the robust multi-array average (RMA) expression measure was used (Irizarry et al. 2003). Linear models were applied in the R package Limma (Linear Models for Microarray Data;(Smyth 2004, Smyth 2005)). Corresponding p-values were calculated by t-test and corrected for multiple testing errors according to (Benjamini, Hochberg 1995). A list of differentially expressed genes was obtained by selecting genes with a change in expression (up or down) over 1.5-fold (FC) and a False Discovery Rate (FDR) of 5%, which corresponds to a Q-value of ≤0.05 (Storey, Tibshirani 2003).

Plant materials and growth conditions

Origins of mutant and transgenic lines: *scz-2* (ten Hove et al. 2010), *fez-2 & smb-1* (Willemsen et al. 2008), *brn1-1;brn2-1* (Bennett et al. 2010), *anl2* (Kubo et al. 1999). *tlp8 (*N92030) and *zn7571* (N548268) were obtained from the Nottingham *Arabidopsis* Stock Centre (NASC). *tlp8* is a TILLING allele harboring a point mutation in the *TLP8* coding sequence (C>T in final exon). pFEZ::FEZ:GFP and pSMB::SMB:GFP are described in (Willemsen et al. 2008). For *ANL2* a β-glucuronidase (GUS) reporter fusion line was used (Kubo, Hayashi 2011). For additional promoter-$_{ER}$GFP constructs, promoter sequences upstream of the start codon (*BRN1* (2,2 kb), *BRN2* (2 kb), *TLP8* (1 kb) and *ZN7571* (2,2 kb)) were amplified from Col-0 genomic DNA and fused to the GFP coding sequence in a pGII0227 binary vector (Hellens et al. 2000) using Gateway technology (Invitrogen, Life Technologies). The constructs were transformed in Columbia-0 (Col-0) wild-type and *scz-2*. pFEZ::FEZ:GFP was crossed to *scz-2*. The *35S::SCZ* construct from (ten Hove et al. 2010) and was used as a template for overlap extension PCR cloning according to (Bryksin, Matsumura 2010) to create *35S::GR:SCZ*. All primers used are listed in Supplementary Table 2. Transgenic plants were created by *Agrobacterium tumefaciens* floral dipping using these constructs into the appropriate genetic background (Clough, Bent 1998). In T2, T3 and F3 generation we analyzed expression for all constructs. Seedlings and embryos were sterilized, plated and grown as described in (Scheres et al. 1994, Sabatini et al. 2003).

Genotyping

Genotyping *anl2* mutation was done according to (Kubo et al. 1999), genotyping *fez* and *smb* mutations was done according to (Willemsen et al. 2008), and *brn1;brn2* was done according to (Bennett et al. 2010). *zn7571* mutation was genotyped using primers ZN7571LP, ZN7571RP and LBb1.3. *tlp8* was genotyped with primers TLP8gF and TLP8gR. All primer sequences are in Supplementary Table 2.

Microscopy

Light microscopy, starch granule staining, measurement of root length and aniline blue staining was performed as described in (Welch et al. 2007, Willemsen et al. 2008, Bougourd, Marrison & Haseloff 2000). For confocal microscopy, roots and dissected embryos were mounted in propidium iodide (PI; 20 µg/mL in distilled water) or 4% glucose (+/- PI), respectively.

Q-PCR gene expression analysis

Induction

Dexamethasone (dex) was used as inducing agent and simultaneous application of cycloheximide (chx) as protein synthesis inhibitor allows for distinction between direct and indirect target genes (reviewed in (Gorte et al. 2011)). A 10mM dex stock was prepared in DMSO and added to the medium to a final concentration of 10µM. A 100mM chx stock was prepared in ethanol and added to the medium to a final concentration of 10µM. Equal amounts of DMSO and/or ethanol was added to controls as appropriate.

For induction, seedling were grown on plates with nylon membrane. At 4 dpg membranes with seedlings were transferred to mock, dex, or chx. The chx/dex samples were pretreated with chx for 15 minutes, before transfer to chx/dex plates. 4 Hours after induction, root tips were dissected and frozen in liquid nitrogen.

Expression analysis

RNeasy minikit columns (Qiagen, http://www.qiagen.com) were used to isolate RNA for quantitative PCR analysis (Q-PCR). To remove genomic DNA, totalRNA was DNase treated (RNase, free; Fermentas GmbBH, Germany, http://www.fermentas.com). cDNA was synthesized using SuperScript III reverse transcriptase (Invitrogen, Life Technologies) according to the instructions of the manufacturer. Q-PCR was done using ABIprim7700 Sequence detector and SYBRgreen™ chemistry (ABI, Foster City, CA, USA). Expression levels were calculated relative to ACTIN2 (AT3G18780) levels according to (Muller et al. 2002). Primers were designed according to the recommendations of q-PCR Master-mix manufacturer (ABI). Primers sequences are in Supplementary Table 2.

CHAPTER 2 SUPPLEMENTARY INFORMATION

Supplemental Table 1. Genes differentially regulated in *scz-2* roots.

Up in *scz-2*					**Down in *scz-2***				
ID	**LOCUS**	**Symbol**	FC	B	ID	**LOCUS**	**Symbol**	FC	B
257409_at	At2g17470	NA	13,77	21,07	263590_at	At2g01820	NA	32,03	22,44
246228_at	At4g36430	NA	11,80	17,90	264672_at	At1g09750	NA	4,97	20,95
261266_at	At1g26770	EXP10	4,23	15,30	254828_at	At4g12550	AIR1	4,40	19,57
252320_at	At3g48580	NA	5,00	15,18	256870_at	At3g26300	CYP71B34	6,80	19,31
248732_at	At5g48070	ATXTH20	2,86	13,18	264371_at	At1g12090	ELP	3,58	19,15
256601_s_at	At3g28290	AT14A	2,61	13,16	249516_s_at	At5g38550	NA	4,48	18,12
250475_at	At5g10180	AST68/ SULTR2;1	2,55	12,79	266500_at	At2g06925	PLA2-ALPHA	3,31	17,66
245642_at	At1g25275	NA	2,21	12,70	253309_at	At4g33790	NA	3,22	17,37
267083_at	At2g41100	ATCAL4 /TCH3	3,60	12,13	249729_at	At5g24410	NA	4,56	16,86
250798_at	At5g05340	NA	3,92	11,76	246530_at	At5g15725	NA	4,67	16,85
247925_at	At5g57560	TCH4/XTH22	3,55	11,32	266123_at	At2g45180	NA	3,91	16,84
265674_at	At2g32190	NA	2,83	11,09	254816_at	At4g12440	APT4	4,30	16,52
263063_s_at	At2g18140	NA	5,08	11,00	264100_at	At1g78970	LUP1	4,75	16,50
251612_at	At3g57950	NA	3,57	10,90	256751_at	At3g27170	CLC-B	3,60	16,25
250302_at	At5g11920	AtcwINV6	2,53	10,86	256022_at	At1g58360	AAP1/NAT2	2,80	15,74
266548_at	At2g35210	AGD10/RPA	2,29	10,82	259351_at	At3g05150	NA	3,45	15,46
266391_at	At2g41290	NA	2,07	10,41	266172_at	At2g39010	PIP2;6/PIP2E	3,68	15,43
263096_at	At2g16060	AHB1/NSHB1	2,40	10,35	256309_at	At1g30380	PSAK	4,90	15,32
253956_at	At4g26700	ATFIM1	2,06	10,23	266745_at	At2g02950	PKS1	4,92	15,23
247906_at	At5g57420	IAA33	2,60	10,14	247882_at	At5g57785	NA	4,01	14,63
257026_at	At3g19200	NA	3,13	10,04	251368_at	At3g61380	NA	3,18	14,47
255658_at	At4g00770	NA	2,44	10,00	249242_at	At5g42250	NA	3,15	14,19
258326_at	At3g22760	SOL1	1,91	9,95	262304_at	At1g70890	MLP43	4,47	13,88
263161_at	At1g54020	NA	2,58	9,79	248790_at	At5g47450	DELTA-TIP3	3,55	13,88
252989_at	At4g38420	sks9	3,60	9,79	248886_at	At5g46110	APE2/TPT	4,06	13,54
251293_at	At3g61930	NA	3,43	9,64	251438_s_at	At3g59930	NA	7,71	13,45
266893_at	At2g26070	RTE1	2,26	9,57	257628_at	At3g26290	CYP71B26	3,92	13,25
250062_at	At5g17760	NA	2,46	9,38	248263_at	At5g53370	PMEPCRF	2,39	13,22
246576_at	At1g31650	ROPGEF14	1,98	9,19	265111_at	At1g62510	NA	9,50	13,20
257859_at	At3g12955	NA	1,93	9,05	267457_at	At2g33790	NA	2,69	12,96
253936_at	At4g26880	NA	4,24	8,92	262643_at	At1g62770	NA	3,24	12,89
257450_at	At1g10530	NA	2,82	8,79	256359_at	At1g66460	NA	2,62	12,70
252103_at	At3g51410	NA	2,17	8,75	252711_at	At3g43720	NA	3,70	12,67
258287_at	At3g15990	SULTR3;4	2,11	8,66	255732_at	At1g25450	CER60/KCS5	2,59	12,52
249817_at	At5g23820	NA	1,94	8,63	257203_at	At3g23730	NA	2,88	12,52
246884_at	At5g26220	NA	3,09	8,61	249112_at	At5g43780	APS4	2,59	12,43
255863_s_at	At2g30310	NA	2,92	8,56	257297_at	At3g28040	NA	2,32	12,41

Differentially expressed genes comparing 4-day old *scz-2* mutant versus Col-0 roots by ATH1 microarray analysis (B>2, FC>1,5/ FC<-1,5).

Supplemental Table 1. Genes differentially regulated in *scz-2* roots.

Up in *scz-2*					Down in *scz-2*				
ID	**LOCUS**	**Symbol**	FC	B	ID	**LOCUS**	**Symbol**	FC	B
261023_at	At1g12200	NA	2,20	8,25	256600_at	At3g14850	NA	2,85	12,36
252131_at	At3g50930	BCS1	1,90	8,13	265246_at	At2g43050	ATPMEPCRD	2,77	12,31
261323_at	At1g44760	NA	1,79	8,09	264315_at	At1g70370	NA	2,29	12,26
254876_at	At4g11610	NA	1,96	8,02	254313_at	At4g22460	NA	3,24	12,22
252468_at	At3g46970	PHS2	1,76	7,99	254818_at	At4g12470	NA	6,59	12,10
252439_at	At3g47400	NA	2,43	7,93	263034_at	At1g24020	MLP423	2,35	11,99
256535_at	At1g33280	BRN1	2,82	7,89	245885_at	At5g09440	NA	2,08	11,94
255726_at	At1g25530	NA	1,63	7,88	255630_at	At4g00700	NA	3,66	11,85
252115_at	At3g51600	LTP5	2,47	7,67	252872_at	At4g40010	SRK2F	2,86	11,70
265544_at	At2g28260	CNGC15	2,25	7,66	262557_at	At1g31330	PSAF	3,04	11,60
259743_at	At1g71140	NA	2,66	7,65	245318_at	At4g16985	NA	2,12	11,55
266849_at	At2g25940	ALPHAVPE	1,83	7,53	247813_at	At5g58330	NA	2,24	11,52
261393_at	At1g79580	SMB	2,59	7,41	250868_at	At5g03860	MLS	4,37	11,48
260845_at	At1g17310	NA	3,33	7,34	262637_at	At1g06640	NA	2,35	11,47
260386_at	At1g74010	NA	2,49	7,29	250558_at	At5g07990	CYP75B1/D501	3,39	11,43
253880_at	At4g27590	NA	2,40	7,29	266992_at	At2g39200	MLO12	2,62	11,42
245250_at	At4g17490	ERF-6-6	2,18	7,08	262609_at	At1g13930	NA	2,16	11,18
260539_at	At2g43480	NA	2,32	7,03	256423_at	At1g33540	scpl18	2,92	11,13
254702_at	At4g17940	NA	1,71	7,01	249942_at	At5g22300	NIT4	2,81	11,02
259897_at	At1g71380	CEL3/GH9B3	6,88	6,93	247073_at	At5g66570	MSP-1/PSBO1	2,76	10,95
252189_at	At3g50070	CYCD3;3	1,65	6,91	260969_at	At1g12240	ATBETAFRUCT4	1,84	10,92
254090_at	At4g25010	NA	2,16	6,87	247246_at	At5g64620	C/VIF2	2,96	10,91
265494_at	At2g15680	NA	1,78	6,85	246992_at	At5g67430	NA	2,40	10,76
262369_at	At1g73010	NA	1,80	6,83	250620_at	At5g07190	ATS3	2,64	10,72
258678_at	At3g08690	UBC11	1,69	6,79	254240_at	At4g23496	SP1L5	2,64	10,64
259507_at	At1g43910	NA	1,86	6,72	263830_at	At2g40260	NA	2,50	10,63
254188_at	At4g23920	UGE2	1,75	6,65	248703_at	At5g48430	NA	2,24	10,54
261298_at	At1g48510	NA	1,66	6,65	257129_at	At3g20100	CYP705A19	1,98	10,48
254331_s_at	At4g22710	CYP706A1/A2	2,52	6,62	254239_at	At4g23400	PIP1;5/PIP1D	2,00	10,39
262666_at	At1g14080	FUT6	1,76	6,62	258629_at	At3g02850	SKOR	3,73	10,28
261840_at	At1g16070	AtTLP8	1,63	6,47	249097_at	At5g43520	NA	4,22	10,28
267287_at	At2g23630	sks16	2,83	6,42	257761_at	At3g23090	NA	1,88	10,24
266150_s_at	At2g12290	NA	1,74	6,37	267089_at	At2g38300	NA	3,50	10,23
256299_at	At1g69530	EXP1	1,96	6,35	260846_at	At1g17300	NA	2,99	10,23
247884_at	At5g57800	FLP1/WAX2	2,19	6,30	245546_at	At4g15290	CSLB05	1,86	10,22
256818_at	At3g21420	NA	1,71	6,29	266851_at	At2g26820	ATPP2-A3	2,14	10,08
263543_at	At2g21610	NA	2,91	6,27	255545_at	At4g01890	NA	2,63	10,06
267622_at	At2g39690	NA	2,25	6,22	250366_at	At5g11420	NA	3,04	10,05
248777_at	At5g47920	NA	1,97	6,18	265710_at	At2g03370	NA	2,41	10,05
263156_at	At1g54030	NA	1,99	6,15	250157_at	At5g15180	NA	2,60	10,02

Differentially expressed genes comparing 4-day old *scz-2* mutant versus Col-0 roots by ATH1 microarray analysis (B>2, FC>1,5/ FC<-1,5).

Supplemental Table 1. Genes differentially regulated in *scz-2* roots.

Up in *scz-2*					Down in *scz-2*				
ID	**LOCUS**	**Symbol**	FC	B	ID	**LOCUS**	**Symbol**	FC	B
255284_at	At4g04610	APR1/PRH19	1,98	6,03	256891_at	At3g19030	NA	2,19	10,00
253041_at	At4g37870	PCK1/PEPCK	1,94	6,02	252543_at	At3g45780	NPH1/PHOT1	2,47	9,92
267276_at	At2g30130	LBD12/PCK1	2,44	5,97	246368_at	At1g51890	NA	2,32	9,80
263153_s_at	At1g54010	NA	1,79	5,96	262444_at	At1g47480	NA	1,99	9,78
257481_at	At1g08430	ALMT1	2,10	5,94	251231_at	At3g62760	ATGSTF13	3,17	9,77
266276_at	At2g29330	TRI	2,77	5,92	262730_at	At1g16390	ATOCT3	2,80	9,71
258857_at	At3g02110	scpl25	1,71	5,90	248961_at	At5g45650	NA	1,98	9,70
245361_at	At4g17790	NA	1,59	5,89	250083_at	At5g17220	GST26/TT19	2,79	9,69
252956_at	At4g38580	ATFP6	1,78	5,86	265405_at	At2g16750	NA	1,88	9,52
250426_at	At5g10510	AIL6	1,83	5,84	267093_at	At2g38170	CAX1/RCI4	1,91	9,52
263653_at	At1g04310	ERS2	1,64	5,80	250474_at	At5g10230	ANN7	2,13	9,50
250662_at	At5g07010	NA	2,10	5,78	252365_at	At3g48350	NA	2,31	9,47
266561_at	At2g23960	NA	1,88	5,67	266168_at	At2g38870	NA	1,93	9,41
263406_at	At2g04160	AIR3	1,60	5,66	261203_at	At1g12845	NA	2,64	9,29
263683_at	At1g26870	FEZ	1,98	5,58	258719_at	At3g09540	NA	1,74	9,14
257947_at	At3g21720	NA	1,62	5,57	257143_at	At3g20110	CYP705A20	3,00	9,10
260558_at	At2g43600	NA	2,30	5,52	248572_at	At5g49800	NA	2,00	9,02
264565_at	At1g05280	NA	1,66	5,48	264092_at	At1g79040	PSBR	3,77	8,99
249312_at	At5g41550	NA	1,70	5,42	264078_at	At2g28470	BGAL8	2,05	8,93
245338_at	At4g16442	NA	1,79	5,41	263207_at	At1g10550	XET/XTH33	2,10	8,91
252387_at	At3g47800	NA	1,78	5,40	255842_at	At2g33530	scpl46	2,18	8,89
250231_at	At5g13910	LEP	1,70	5,35	251642_at	At3g57520	AtSIP2	2,17	8,74
252312_at	At3g49380	iqd15	1,72	5,28	261480_at	At1g14280	PKS2	2,90	8,71
257582_at	At1g50720	NA	2,62	5,26	247288_at	At5g64330	JK218/NPH3/ RPT3	1,87	8,69
250515_at	At5g09570	NA	1,86	5,25	247954_at	At5g56870	BGAL4	2,09	8,68
255564_s_at	At4g01750	RGXT1	1,68	5,22	257876_at	At3g17130	NA	2,26	8,65
257471_at	At1g59530	BZIP4	1,83	5,20	263765_at	At2g21540	NA	2,15	8,55
245728_at	At1g73340	NA	2,06	5,17	256759_at	At3g25640	NA	2,11	8,53
261187_at	At1g32860	NA	1,59	5,16	256781_at	At3g13650	NA	2,15	8,52
249719_at	At5g35735	NA	2,05	5,13	249894_at	At5g22580	NA	3,17	8,52
255022_at	At4g10350	BRN2	3,45	5,05	250243_at	At5g13630	CCH1/CHLH/ GUN5	2,64	8,51
254453_at	At4g21120	AAT1/CAT1	2,19	5,01	246238_at	At4g36670	NA	2,89	8,46
262784_at	At1g10760	GWD1	1,55	5,00	263560_s_at	At2g15370	FUT10	1,84	8,42
249983_at	At5g18470	NA	2,67	4,95	266996_at	At2g34490	CYP710A2	2,80	8,40
245800_at	At1g46264	HSFB4	1,67	4,85	263536_at	At2g25000	WRKY60	2,14	8,37
252415_at	At3g47340	ASN1/DIN6	2,21	4,83	250982_at	At5g03150	JKD	2,53	8,35
266124_at	At2g45080	cycp3;1	1,66	4,83	257272_at	At3g28130	NA	2,48	8,26
267164_at	At2g37700	NA	3,22	4,81	259358_at	At1g13250	GATL3	1,75	8,21

Differentially expressed genes comparing 4-day old *scz-2* mutant versus Col-0 roots by ATH1 microarray analysis (B>2, FC>1,5/ FC<-1,5).

Supplemental Table 1. Genes differentially regulated in *scz-2* roots.

Up in *scz-2*					Down in *scz-2*				
ID	**LOCUS**	**Symbol**	FC	B	ID	**LOCUS**	**Symbol**	FC	B
248118_at	At5g55050	NA	1,66	4,80	252997_at	At4g38400	EXPL2	1,80	8,14
256927_at	At3g22550	NA	1,54	4,69	249203_at	At5g42590	CYP71A16	2,15	7,96
255612_at	At4g01240	NA	2,43	4,67	256725_at	At2g34070	NA	1,96	7,85
254575_at	At4g19460	NA	2,43	4,63	245061_at	At2g39730	RCA	2,73	7,83
262969_at	At1g75710	NA	1,61	4,62	259388_at	At1g13420	NA	2,04	7,78
248541_at	At5g50180	NA	1,50	4,61	258145_at	At3g18200	NA	1,92	7,75
248623_at	At5g49170	NA	2,13	4,52	261768_at	At1g15550	GA3OX1/GA4	2,68	7,70
256714_at	At2g34080	NA	2,34	4,49	264209_at	At1g22740	RAB75/RABG3B	2,60	7,66
262325_at	At1g64160	NA	1,54	4,41	252458_at	At3g47210	NA	1,86	7,60
262254_at	At1g53920	GLIP5	1,70	4,39	259793_at	At1g64380	NA	2,39	7,59
258434_at	At3g16770	ERF72/RAP2.3	2,01	4,32	255294_at	At4g04750	NA	1,97	7,59
260557_at	At2g43610	NA	1,80	4,23	261907_at	At1g65060	4CL3	1,80	7,40
267003_at	At2g34340	NA	1,81	4,23	245213_at	At1g44575	NPQ4/PSBS	2,68	7,35
249522_at	At5g38700	NA	2,09	4,22	256736_at	At3g29410	NA	2,96	7,30
261957_at	At1g64660	ATMGL	2,03	4,22	264071_at	At2g27920	SCPL51	2,07	7,25
266895_at	At2g26040	NA	1,72	4,19	254630_at	At4g18360	NA	1,55	7,24
252611_at	At3g45130	LAS1	1,73	4,15	255298_at	At4g04840	NA	3,10	7,22
246532_at	At5g15870	NA	2,01	4,13	250471_at	At5g10170	NA	1,94	7,20
246498_at	At5g16230	NA	1,96	4,06	261746_at	At1g08380	PSAO	3,45	7,18
258299_at	At3g23410	NA	2,11	4,03	249037_at	At5g44130	FLA13	2,81	7,12
252740_at	At3g43270	NA	1,58	4,02	250882_at	At5g04000	NA	1,80	7,11
245545_at	At4g15280	NA	1,53	4,02	255860_at	At5g34940	AtGUS3	1,77	7,09
259142_at	At3g10200	NA	1,69	4,00	255502_at	At4g02410	NA	2,26	7,07
255676_at	At4g00490	BAM2/BMY9	1,60	3,95	245399_at	At4g17340	TIP2;2	1,72	7,05
255637_at	At4g00750	NA	1,66	3,94	264403_at	At2g25150	NA	3,11	7,05
253794_at	At4g28720	YUC8	1,59	3,93	254697_at	At4g17970	NA	1,98	7,03
249794_at	At5g23530	CXE18	1,93	3,93	261826_at	At1g11580	PMEPCRA	1,63	7,00
262785_at	At1g10750	NA	1,57	3,90	252341_at	At3g48940	NA	1,91	6,98
248741_at	At5g48170	SLY2	1,62	3,90	265075_at	At1g55450	NA	1,91	6,87
255345_at	At4g04460	NA	2,28	3,88	262099_s_at	At1g59500	GH3.4/AUR3/ BRU6/YDK1	2,98	6,86
258586_s_at	At3g04320	NA	3,06	3,83	262827_at	At1g13100	CYP71B29	1,84	6,81
261368_at	At1g53070	NA	1,65	3,80	249533_at	At5g38790	NA	2,37	6,76
248178_at	At5g54370	NA	2,65	3,79	265117_at	At1g62500	Co2	2,58	6,74
247431_at	At5g62520	SRO5	2,51	3,79	266330_at	At2g01530	MLP329	4,03	6,72
257715_at	At3g12750	ZIP1	1,55	3,78	262831_at	At1g14730	NA	2,36	6,69
265341_at	At2g18360	NA	3,22	3,74	249955_at	At5g18840	NA	3,33	6,64
250610_at	At5g07550	GRP19	1,97	3,74	257176_s_at	At3g23510	NA	1,59	6,63
260919_at	At1g21520	NA	2,89	3,72	266640_at	At2g35585	NA	2,24	6,63
265339_at	At2g18230	AtPPa2	1,62	3,69	246814_at	At5g27200	ACP5	1,95	6,53

Differentially expressed genes comparing 4-day old *scz-2* mutant versus Col-0 roots by ATH1 microarray analysis (B>2, FC>1,5/ FC<-1,5).

Supplemental Table 1. Genes differentially regulated in *scz-2* roots.

Up in *scz-2*					Down in *scz-2*				
ID	**LOCUS**	**Symbol**	FC	B	ID	**LOCUS**	**Symbol**	FC	B
261814_at	At1g08310	NA	2,07	3,68	259839_at	At1g52190	NA	2,22	6,53
260856_at	At1g21910	NA	1,56	3,64	265990_at	At2g24280	NA	1,97	6,52
254791_at	At4g12910	scpl20	1,75	3,62	266828_at	At2g22930	NA	2,41	6,48
254396_at	At4g21680	NA	1,82	3,62	254041_at	At4g25830	NA	1,70	6,45
264527_at	At1g30760	NA	1,96	3,59	263183_at	At1g05570	GSL6	1,69	6,44
245200_at	At1g67850	NA	2,47	3,59	251517_at	At3g59370	NA	2,33	6,44
257021_at	At3g19710	BCAT4	4,43	3,57	246481_s_at	At5g15960	KIN1/COR6.6/ KIN2	2,12	6,42
258029_at	At3g27580	ATPK7	1,64	3,56	252589_s_at	At3g45650	NAXT1	1,90	6,39
246229_at	At4g37160	sks15	2,27	3,55	245980_at	At5g13140	NA	2,06	6,36
260335_at	At1g74000	SS3	1,73	3,55	249686_at	At5g36140	CYP716A2	2,39	6,26
261518_at	At1g71695	NA	1,68	3,54	255261_s_at	At4g05110	ENT3/FUR1	2,06	6,26
267389_at	At2g44460	NA	1,97	3,53	256589_at	At3g28740	CYP81D1	2,22	6,17
262482_at	At1g17020	SRG1	2,05	3,48	257618_at	At3g24720	NA	1,83	6,12
253914_at	At4g27400	NA	2,86	3,36	249918_at	At5g19240	NA	1,93	6,04
246502_at	At5g16240	NA	1,50	3,33	248104_at	At5g55250	IAMT1	2,80	6,04
257547_at	At3g13000	NA	1,69	3,32	266215_at	At2g06850	EXT	1,70	6,04
262539_at	At1g17200	NA	1,78	3,30	252128_at	At3g50870	MNP	1,67	6,02
245768_at	At1g33590	NA	2,00	3,30	247193_at	At5g65380	NA	2,32	6,01
255413_at	At4g03140	NA	1,88	3,30	250549_at	At5g07860	NA	1,74	6,01
266756_at	At2g46950	CYP709B2	2,13	3,16	247483_at	At5g62420	NA	3,03	5,98
255524_at	At4g02330	ATPMEPCRB	2,26	3,10	245123_at	At2g47450	CAO	2,36	5,92
245208_at	At5g12330	LRP1	2,11	3,06	253052_at	At4g37310	CYP81H1	1,81	5,92
249346_at	At5g40780	LTH1	1,55	3,03	250611_at	At5g07200	YAP169	2,44	5,90
261985_at	At1g33750	NA	1,52	2,98	252976_s_at	At4g38550	NA	1,71	5,88
247136_at	At5g66170	NA	1,87	2,98	260771_at	At1g49160	WNK7	1,83	5,85
262244_at	At1g48260	CIPK17	1,54	2,97	267096_at	At2g38180	NA	1,59	5,85
247634_at	At5g60520	NA	1,73	2,92	258736_at	At3g05900	NA	1,85	5,81
246825_at	At5g26260	NA	1,53	2,92	249626_at	At5g37540	NA	1,81	5,79
254349_at	At4g22250	NA	1,58	2,88	264433_at	At1g61810	BGLU45/ BGLU45	2,54	5,78
254200_at	At4g24110	NA	1,59	2,85	265471_at	At2g37130	NA	1,87	5,77
246490_at	At5g15950	NA	1,69	2,76	266596_at	At2g46150	NA	1,72	5,77
247922_at	At5g57500	NA	1,72	2,74	253667_at	At4g30170	NA	1,54	5,75
258323_at	At3g22750	NA	1,71	2,72	258742_at	At3g05800	bHLH150	1,73	5,71
252097_at	At3g51090	NA	1,60	2,62	265884_at	At2g42320	NA	2,16	5,68
253522_at	At4g31290	NA	1,54	2,50	256252_at	At3g11340	NA	1,85	5,68
246537_at	At5g15940	NA	2,51	2,48	261226_at	At1g20190	EXP11	1,67	5,65
264682_at	At1g65570	NA	5,41	2,47	248371_at	At5g51810	GA20OX2	2,18	5,65
250992_at	At5g02260	EXP9	2,06	2,45	264247_at	At1g60160	NA	1,72	5,64

Differentially expressed genes comparing 4-day old *scz-2* mutant versus Col-0 roots by ATH1 microarray analysis (B>2, FC>1,5/ FC<-1,5).

Supplemental Table 1. Genes differentially regulated in *scz-2* roots.

Up in *scz-2*					Down in *scz-2*				
ID	**LOCUS**	**Symbol**	FC	B	ID	**LOCUS**	**Symbol**	FC	B
257288_at	At3g29670	NA	1,50	2,44	251664_at	At3g56940	CRD1	1,84	5,60
260608_at	At2g43870	NA	2,04	2,37	261230_at	At1g20010	TUB5	1,53	5,58
248642_at	At5g49120	NA	1,82	2,36	259118_at	At3g01310	NA	1,85	5,54
254674_at	At4g18450	NA	1,94	2,33	259606_at	At1g27920	NA	1,60	5,53
250770_at	At5g05390	LAC12	1,64	2,25	248622_at	At5g49360	BXL1	1,96	5,50
250949_at	At5g03510	NA	1,70	2,22	264923_s_at	At1g60740	TPX2	2,74	5,43
261691_at	At1g50060	NA	2,48	2,22	247729_at	At5g59530	NA	1,89	5,42
264907_at	At2g17280	NA	1,63	2,16	262863_at	At1g64910	NA	2,17	5,42
245141_at	At2g45400	BEN1	1,58	2,14	255962_at	At1g22335	NA	2,08	5,41
259653_at	At1g55240	NA	2,17	2,14	253016_at	At4g37960	NA	1,53	5,36
255636_at	At4g00730	AHDP/ANL2	1,68	2,12	255290_at	At4g04640	ATPC1	2,11	5,34
255823_at	At2g40470	LBD15	2,00	2,12	257650_at	At3g16800	NA	1,74	5,26
253999_at	At4g26200	ACS7	1,58	2,12	256170_at	At1g51790	NA	1,76	5,26
254213_at	At4g23590	NA	2,02	2,11	250968_at	At5g02890	NA	1,83	5,25
262658_at	At1g14220	NA	1,82	2,11	255957_at	At1g22160	NA	1,98	5,25
247554_at	At5g61010	ATEXO70E2	1,50	2,10	266120_at	At2g02070	AtIDD5	1,66	5,24
248408_at	At5g51520	NA	2,88	2,09	252882_at	At4g39675	NA	2,75	5,23
255543_at	At4g01870	NA	1,51	2,09	250108_at	At5g15150	ATHB3/HAT7	1,57	5,20
250287_at	At5g13330	Rap2.6L	1,68	2,06	259553_x_at	At1g21310	ATEXT3/RSH	1,83	5,16
247905_at	At5g57400	NA	1,67	2,05	245196_at	At1g67750	NA	1,75	5,15
248964_at	At5g45340	CYP707A3	1,56	2,03	255250_at	At4g05100	AtMYB74	1,85	5,12
265670_s_at	At2g32210	NA	2,11	2,03	256150_at	At1g55120	ATFRUCT5	2,20	5,12
254781_at	At4g12840	NA	1,83	2,01	267439_at	At2g19060	NA	1,74	5,08
					263656_at	At1g04240	IAA3/SHY2	1,61	5,05
					262118_at	At1g02850	NA	1,98	5,04
					264424_at	At1g61740	NA	1,80	5,04
					267612_at	At2g26690	NA	1,91	5,02
					262882_at	At1g64900	CYP89A2	1,57	5,01
					247519_at	At5g61430	ANAC100	1,68	4,99
					248625_at	At5g48880	KAT5/PKT2	1,95	4,99
					254914_at	At4g11290	NA	1,56	4,98
					258143_at	At3g18170	NA	1,69	4,96
					262935_at	At1g79410	AtOCT5	1,60	4,94
					259592_at	At1g27950	NA	1,81	4,92
					256097_at	At1g13670	NA	1,67	4,89
					257651_at	At3g16850	NA	1,56	4,88
					249271_at	At5g41790	CIP1	2,37	4,88
					248908_at	At5g45800	MEE62	1,60	4,86
					265116_at	At1g62480	NA	1,55	4,85
					248335_at	At5g52450	NA	1,62	4,81

Differentially expressed genes comparing 4-day old *scz-2* mutant versus Col-0 roots by ATH1 microarray analysis (B>2, FC>1,5/ FC<-1,5).

Supplemental Table 1. Genes differentially regulated in *scz-2* roots.

Up in *scz-2*					Down in *scz-2*				
ID	**LOCUS**	**Symbol**	FC	B	ID	**LOCUS**	**Symbol**	FC	B
					245560_at	At4g15480	UGT84A1	2,66	4,81
					261728_at	At1g76160	sks5	1,56	4,71
					248393_at	At5g52060	ATBAG1	1,51	4,68
					253886_at	At4g27710	CYP709B3	1,84	4,57
					264404_at	At2g25160	CYP82F1	1,77	4,57
					261825_at	At1g11545	NA	1,54	4,57
					262655_s_at	At1g14190	NA	1,99	4,54
					253957_at	At4g26320	AGP13	1,52	4,53
					251962_at	At3g53420	PIP2/PIP2A	1,50	4,50
					259841_at	At1g52200	NA	2,13	4,49
					257645_at	At3g25790	NA	1,60	4,44
					252377_at	At3g47960	NA	1,55	4,40
					265957_at	At2g37300	NA	2,23	4,40
					245096_at	At2g40880	FL3-27	1,78	4,36
					264091_at	At1g79110	NA	1,71	4,33
					263915_at	At2g36430	NA	1,72	4,33
					265986_at	At2g24230	NA	1,52	4,31
					266066_at	At2g18800	NA	1,76	4,30
					253720_at	At4g29270	NA	1,59	4,30
					248916_at	At5g45840	NA	1,73	4,30
					252437_at	At3g47380	NA	1,53	4,28
					262072_at	At1g59590	ZCF37	1,66	4,26
					253856_at	At4g28100	NA	1,61	4,23
					264223_s_at	At1g67520	CES101	1,87	4,20
					251072_at	At5g01740	NA	2,69	4,14
					255969_at	At1g22330	NA	1,65	4,10
					245076_at	At2g23170	GH3.3	2,11	4,09
					245410_at	At4g17220	ATMAP70-5	1,79	4,04
					263956_at	At2g35940	BLH1/EDA29	1,93	3,96
					250690_at	At5g06530	NA	1,64	3,95
					254225_at	At4g23670	NA	3,17	3,91
					249684_s_at	At5g36110	CYP716A1	2,02	3,91
					261958_at	At1g64500	NA	2,07	3,89
					259854_at	At1g72200	NA	1,57	3,89
					267002_s_at	At2g34430	LHB1B2/ LHCB1.5	2,43	3,89
					262645_at	At1g62750	SCO1	1,58	3,85
					264342_at	At1g12080	NA	1,89	3,85
					257573_at	At2g33990	iqd9	1,84	3,81
					259365_at	At1g13300	NA	1,93	3,79

Differentially expressed genes comparing 4-day old *scz-2* mutant versus Col-0 roots by ATH1 microarray analysis (B>2, FC>1,5/ FC<-1,5).

Supplemental Table 1. Genes differentially regulated in *scz-2* roots.

Up in *scz-2*					Down in *scz-2*				
ID	**LOCUS**	**Symbol**	FC	B	ID	**LOCUS**	**Symbol**	FC	B
					248932_at	At5g46050	PTR3	2,10	3,78
					262826_at	At1g13080	CYP71B2	1,83	3,78
					256542_at	At1g42550	PMI1	2,22	3,77
					257314_at	At3g26590	NA	1,55	3,76
					257197_at	At3g23800	SBP3	1,60	3,73
					266140_at	At2g28120	NA	2,39	3,72
					260030_at	At1g68880	AtbZIP	1,98	3,71
					249807_at	At5g23870	NA	1,66	3,67
					259353_at	At3g05190	NA	1,64	3,66
					252459_s_at	At3g47220	NA	1,71	3,65
					264647_at	At1g09090	ATRBOHB	1,91	3,65
					264511_at	At1g09350	AtGolS3	1,68	3,62
					249778_at	At5g24165	NA	1,85	3,61
					254361_at	At4g22212	NA	1,90	3,61
					266976_at	At2g39410	NA	1,88	3,61
					253088_at	At4g36220	CYP84A1/FAH1	2,03	3,60
					254430_at	At4g20820	NA	1,67	3,56
					266785_at	At2g28970	NA	1,90	3,55
					258080_at	At3g25930	NA	1,79	3,54
					267036_at	At2g38465	NA	1,60	3,54
					266719_at	At2g46830	CCA1	1,87	3,51
					245610_at	At4g14380	NA	1,53	3,47
					252232_at	At3g49760	AtbZIP5	1,68	3,45
					246197_at	At4g37010	NA	2,31	3,44
					264314_at	At1g70420	NA	1,66	3,39
					252040_at	At3g52060	NA	1,66	3,38
					264157_at	At1g65310	ATXTH17	1,55	3,36
					260236_at	At1g74470	NA	1,63	3,34
					253829_at	At4g28040	NA	1,96	3,34
					262875_at	At1g64970	TMT1/VTE4	1,53	3,32
					259801_at	At1g72230	NA	1,61	3,30
					260300_at	At1g80340	GA3OX2/GA4H	1,94	3,27
					249378_at	At5g40450	NA	1,83	3,27
					256008_s_at	At1g34040	NA	1,53	3,27
					264271_at	At1g60270	NA	1,88	3,24
					265160_at	At1g31050	NA	1,57	3,23
					257253_at	At3g24190	NA	1,50	3,22
					262399_at	At1g49500	NA	1,80	3,22
					257162_s_at	At3g24290	AMT1;5/ AMT1;3	1,82	3,22

Differentially expressed genes comparing 4-day old *scz-2* mutant versus Col-0 roots by ATH1 microarray analysis (B>2, FC>1,5/ FC<-1,5).

Supplemental Table 1. Genes differentially regulated in *scz-2* roots.

Up in *scz-2*					Down in *scz-2*				
ID	**LOCUS**	**Symbol**	FC	B	ID	**LOCUS**	**Symbol**	FC	B
					261241_at	At1g32950	NA	1,51	3,21
					252944_at	At4g39320	NA	1,67	3,19
					249484_at	At5g38970	BR6OX1/ CYP85A1	1,81	3,18
					266578_at	At2g23910	NA	2,11	3,17
					267080_at	At2g41190	NA	1,64	3,16
					259794_at	At1g64330	NA	1,63	3,16
					259445_at	At1g02400	GA2OX6/DTA1	1,75	3,14
					247450_at	At5g62350	NA	1,53	3,11
					251195_at	At3g62930	NA	1,73	3,09
					246373_at	At1g51860	NA	1,63	3,08
					251084_at	At5g01520	NA	1,75	3,04
					247030_at	At5g67210	NA	1,54	3,01
					255266_at	At4g05200	NA	1,79	3,01
					253379_at	At4g33330	PGSIP3	1,72	2,99
					251644_at	At3g57540	NA	2,37	2,99
					250467_at	At5g10100	NA	1,51	2,99
					259753_at	At1g71050	NA	1,55	2,97
					250576_at	At5g08250	NA	1,83	2,96
					266584_s_at	At2g14920	NA	1,70	2,92
					261428_at	At1g18870	ICS2	2,06	2,79
					245524_at	At4g15920	NA	1,78	2,78
					246601_at	At1g31710	NA	1,68	2,76
					248989_at	At5g45200	NA	1,76	2,75
					245947_at	At5g19530	ACL5	1,56	2,74
					249051_at	At5g44390	NA	1,58	2,73
					261027_at	At1g01340	ACBK1/ CNGC10	1,81	2,73
					264545_at	At1g55670	PSAG	1,51	2,71
					245262_at	At4g16563	NA	1,54	2,71
					249769_at	At5g24120	SIG5/SIGE	1,54	2,68
					254193_at	At4g23870	NA	1,56	2,64
					248337_at	At5g52310	COR78/RD29A	1,97	2,64
					256766_at	At3g22231	PCC1	1,61	2,63
					245575_at	At4g14760	NA	1,55	2,63
					245439_at	At4g16670	NA	1,68	2,62
					245554_at	At4g15380	CYP705A4	1,68	2,60
					247435_at	At5g62480	ATGSTU9/ GST14B	1,62	2,55
					247577_at	At5g61290	NA	1,58	2,54
					263596_at	At2g01900	NA	2,21	2,52

Differentially expressed genes comparing 4-day old *scz-2* mutant versus Col-0 roots by ATH1 microarray analysis (B>2, FC>1,5/ FC<-1,5).

Supplemental Table 1. Genes differentially regulated in *scz-2* roots.

Up in *scz-2*					Down in *scz-2*				
ID	LOCUS	Symbol	FC	B	ID	LOCUS	Symbol	FC	B
					252950_at	At4g38690	NA	2,10	2,52
					255248_at	At4g05180	PSBQ/PSII-Q	2,62	2,51
					253073_at	At4g37410	CYP81F4	1,82	2,51
					266346_at	At2g01430	ATHB-17/ ATHB17	1,52	2,43
					246216_at	At4g36380	ROT3	1,68	2,43
					253697_at	At4g29700	NA	1,58	2,42
					250842_at	At5g04490	VTE5	1,72	2,40
					253373_at	At4g33150	LKR/SDH	1,77	2,39
					262164_at	At1g78070	NA	1,53	2,38
					258257_at	At3g26770	NA	1,62	2,38
					251519_at	At3g59400	GUN4	1,80	2,37
					252197_at	At3g50230	NA	1,63	2,34
					252296_at	At3g48970	NA	1,71	2,33
					247570_at	At5g61250	AtGUS1	2,34	2,33
					253172_at	At4g35060	NA	1,60	2,27
					264590_at	At2g17710	NA	1,54	2,27
					254187_at	At4g23890	NA	2,00	2,27
					252958_at	At4g38620	MYB4	1,59	2,24
					249035_at	At5g44190	GLK2/GPRI2	1,59	2,21
					255457_at	At4g02770	PSAD-1	2,01	2,17
					262349_at	At2g48130	NA	1,52	2,17
					256062_at	At1g07090	NA	1,72	2,14
					260531_at	At2g47240	NA	1,54	2,13
					253718_at	At4g29450	NA	1,66	2,13
					250095_at	At5g17230	PSY	1,72	2,13
					246011_at	At5g08330	TCP21	1,55	2,11
					246969_at	At5g24880	NA	1,71	2,09
					254738_at	At4g13860	NA	1,69	2,07
					265265_at	At2g42900	NA	1,90	2,05
					245701_at	At5g04140	GLS1/GLU1	1,66	2,02
					262947_at	At1g75750	GASA1	1,53	2,01
					263841_at	At2g36870	NA	1,71	2,00

Differentially expressed genes comparing 4-day old *scz-2* mutant versus Col-0 roots by ATH1 microarray analysis (B>2, FC>1,5/ FC<-1,5).

Supplemental Table 2. Primer sequences

Primer	Sequence
cloning	
35SGRF	atataaggaagttcatttcatttggagaggacagcccaagATGattcagcaagccactgcagg
GRSCZR	taaccaccgtagctattctcgaccatcatcgccatGGACCTGATAGCGGCGTTtttttgatgaaacagaagctttttga
pTLP8F	TAGTTGGAATGGGTTCGAAggcttatcggttttggatga
pTLP8R	TTATGGAGTTGGGTTCGAAtctggatggattgctttcaa
pZN7571F	CAACTTTGTATAGAAAAGTTGccgttttgccaacttttcat
pZN7571R	GGGGACTGCTTTTTTGTACAAACTTGAGGTGTTGAAATCGAAgatgga
pBRN1F	CAACTTTGTATAGAAAAGTTGacaagaaatgtcggcttcca
pBRN1R	GGGGACTGCTTTTTTGTACAAACTTGAtcggttaataaatgatcaatgtttgt
pBRN2F	CAACTTTGTATAGAAAAGTTGtcacccttgaacccaagttt
pBRN2R	GGGGACTGCTTTTTTGTACAAACTTGAcgttttccttctcttgtctcca
genotyping	
LBb1.3	ATTTTGCCGATTTCGGAAC
Zn7571LP	CCGCCTTTAAGAAGGTCAAAC
Zn7571RP	CGTTAGATCTCGTTGAGCCAG
TLP8gF	TGGTGGTCGAACAGCTCTGAAGCA
TLP8gR	TTGGCTCATTCTGGTTTGAGCAAGT
qRTPCR	
Anl2F	CCCATATCACCAAAGGTCAA
Anl2R	ACTCTGATTCGCGTTCATTG
Tlp8F	TGTGCAGTTCGGATGAAAGT
Tlp8R	AGCTCCCTGTCCAAGTTGTT
Zn7571F	GTGGGAGTTACACGTCATCG
Zn7571R	CATTGGGAACAAGCACAAAC
FezF	CTGTCTCTCCCAACGATTCA
FezR	TGGTGGTAACGAGGAAACAA
SmbF	TGTGTCGCCAATCAACACTA
SmbR	GAACCACCATTGTTGTTACCC
qBrn1F2	CATCCAACGGACGAAGAAC
qBrn1R2	TCCTATCCTTGTGGCTGAAGA
Brn2F	CTTGAAGACGCTGATGATCC
Brn2R	ACATACCACCCATCCATCTTC

2

Supplemental Table 3. T-DNA insertion lines

Up in *scz-2*				Down in *scz-2*			
LOCUS	**Name**	**TF family**	**T-DNA line**	**LOCUS**	**Name**	**TF family**	**T-DNA line**
AT1G16070	TLP8	TUB	N92030	AT1G13300	NN	Myb/G2-Like	NA
AT1G17310	MADS	MADS	N571896	AT1G31050	NN	bHLH	N545538
AT1G21910	NN	AP2-EREBP	NA	AT1G64380	NN	AP2-EREBP	N448616
AT1G59530	BZIP4	BZIP	NA	AT1G68880	NN	bZIP	NA
AT1G75710	ZN7571	ZnF C2H2	N548268	AT2G02070	IDD5	ZnF C2H2	NA
AT3G13000	NN	NN	N532557	AT2G25000	WRKY60	WRKY	N620706
AT3G16770	NN	AP2-EREBP	NA	AT2G35940	BLH1/EDA29	Homeobox	N589095
AT3G22760	SOL1	CPP	N513678	AT2G38300	NN	Myb	N612072
AT4G00730	ANL2	Homeobox	Kubo ea 2011	AT2G40260	NN	MYB	NA
AT4G17490	NN	AP2-EREBP	N530723	AT2G46830	CCA1		N93800
AT4G18450	NN	AP2-EREBP	NA	AT3G05800	bHLH150	bHLH	NA
AT5G03510	NN	C2H2	GK389G05	AT3G25790	NN	Myb/G2-Like	NA
AT5G13330	Rap2.6L	AP2-EREBP	N532496	AT3G49760	AtbZIP5	bZIP	N70569
AT5G13910	LEP	AP2-EREBP	N560863	AT4G05100	MYB74	MYB	NA
AT5G57420	IAA33	AUX/IAA	GK524H11	AT4G38620	MYB4	MYB	N26404
				AT5G15150	ATHB3/HAT7	Homeobox	N70468/ N445074
				AT5G24120	SIG5/SIGE	NN	N549020
				AT5G44190	ATGLK2/ GLK2/GPRI2	Myb/G2-Like	N542286
				AT5G61430	ANAC100	NAC	NA

Not Analyzed (NA).

Chapter 3

SCHIZORIZA controls stem cell niche specification in conjunction with *PLETHORA* and *SHORTROOT-SCARECROW* pathways

Jansweijer VMA[1,2], Ten Hove CA[1,3], Terpstra I[1],
Aida M[1,4], Scheres B[1,2], Heidstra R[1,2]

[1] Department of Biology, section Molecular Genetics, Utrecht University, 3584 CH Utrecht, The Netherlands.
[2] Current affiliation: Plant Developmental Biology, Wageningen University Research, 6708 PB Wageningen, The Netherlands.
[3] Current affiliation: Laboratory of Biochemistry, WU Agrotechnology & Food Sciences, 6708 PB Wageningen, The Netherlands.
[4] Current affiliation: Graduate School of Biological Sciences, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 6300192, Japan.

"L'Estate"

ABSTRACT

Arabidopsis root growth is maintained by the activity of the stem cell niche in the heart of the root meristem. The stem cell niche is formed during embryogenesis. The Quiescent Center (QC) is then specified and the cells surrounding the QC are maintained as stem cells. QC fate and stem cell maintenance are controlled by the combinatorial action of *SHORTROOT* (*SHR*), *SCARECROW* (*SCR*) and *PLETHORA* (*PLT*) transcription factor genes. Here, we report specific functions of the transcription factor gene *SCHIZORIZA* (*SCZ*) in the stem cell niche. We provide evidence that *SCZ* is connected to both *PLT* and *SHR*/*SCR* pathway activities for stem cell niche specification in the embryo.

INTRODUCTION

The *Arabidopsis* root meristem is laid down during embryogenesis. In the heart stage embryo the QC is assumed to recruit the immediate neighboring cells as root stem cells, first discerned at late heart stage. The organization of the tissues that make up the root is completed at torpedo stage and maintained in the post-embryonic root by stereotyped asymmetric cell divisions of distinct stem cells. The QC continues to function as an organizing center for maintenance of the post-embryonic root stem cell niche (Jürgens, Mayer 1994, Scheres et al. 1995, Berg et al. 1995, Scheres et al. 1997). Acquisition of QC fate and maintenance of the stem cell niche is controlled by parallel activities of the *SHORTROOT* (*SHR*), *SCARECROW* (*SCR*) and *PLETHORA* (*PLT*) transcription factor genes (Sabatini et al. 2003, Aida et al. 2004, Galinha et al. 2007).

SHR and *SCR* encode GRAS-type transcription factors. *SHR* is expressed in the stele but the protein moves one tissue layer outward and activates transcription of its interacting partner *SCR* in the endodermis, ground tissue stem cells and QC. Unlike SHR, SCR protein appears not to move. Apart from their role in QC specification, both *SHR* and *SCR* are required for vascular and ground tissue patterning (Scheres et al. 1995, Di Laurenzio et al. 1996, Helariutta et al. 2000, Nakajima et al. 2001, Levesque et al. 2006, Benfey et al. 1993, Heidstra, Welch & Scheres 2004, Cruz-Ramírez et al. 2012, Carlsbecker et al. 2010, Sozzani et al. 2010). PLT proteins belong to the double AP2 domain transcription factor family (Krizek, Prost & Macias 2000). PLT1, PLT2, PLT3 and PLT4/BABYBOOM are expressed in an overlapping domain and graded fashion in the root meristem and act in a redundant and dose dependent way (Galinha et al. 2007). *PLT1*, *PLT2* and *PLT3* together are needed for the maintenance of the stem cell niche in embryogenesis, where they act to determine basal fate (Aida et al. 2004, Galinha et al. 2007, Smith, Long 2010). *plt1;plt2;plt3* triple mutants display rootless seedlings. If one copy of *PLT4* is removed in this background (*plt1;plt2;plt3;plt4+/-*) seedlings completely lack root and hypocotyl, resembling *monopteros* (*mp*). In the root, high ectopic expression of PLT maintains stem cells, intermediate levels facilitate transient amplifying cell divisions that make up the meristem region, and low levels or absence of PLT allows progression of differentiation (Galinha et al. 2007). *PLT* expression is not changed in *shr* nor in *scr* mutant roots. Vice versa, *SHR* and *SCR* are normally expressed in *plt1;plt2* double mutants. Together with the observation that *plt1;plt2;shr* and *plt1;plt2;scr* triple mutant combinations display additive phenotypes, these results indicate *PLT* and *SHR*/*SCR* represent parallel pathways in root stem cell niche specification (Aida et al. 2004).

SCHIZORIZA (*SCZ*) encodes a B-type Heat Shock transcription factor that was shown to play a role in patterning of the root stem cell niche. In the root, *SCZ* is expressed in the QC, ground tissue and vasculature progenitors and at lower levels in their daughters (ten Hove et al. 2010, Pernas, Ryan & Dolan 2010). Specifically, *SCZ* controls asymmetry of root stem cell divisions. The origin of the *scz* mutant defects can be traced back to heart stage embryo (ten Hove et al. 2010, Pernas, Ryan & Dolan

2010) when stem cells are first specified and the onset of root meristem activity occurs (Jürgens, Mayer 1994, Scheres et al. 1995). However, *SCZ* expression initiates earlier, in QC progenitor cells of triangular stage embryos (ten Hove et al. 2010, Pernas, Ryan & Dolan 2010). Expression remains high in QC from this stage onwards and shows overlap with that of *SHR, SCR* and *PLT* root patterning genes (Aida et al. 2004, Wysocka-Diller et al. 2000). Previous studies indicated a requirement of the *SHR/SCR* pathway for *scz* ectopic divisions (Mylona et al. 2002), and it has been suggested that embryos lacking both *SCZ* and *SCR* functions do not form a ground tissue because they do not develop ground tissue stem cells (Pernas, Ryan & Dolan 2010). Here, we endeavor to get a closer understanding of *SCZ* function in root niche specification during embryogenesis in relation to known niche specification factors. We identify the *PLT* genes as transcriptional regulators and the *SHR-SCR* pathway as functional interactors of *SCZ*. Thus, *SCZ* connects two main niche specification pathways.

RESULTS

Interaction between *SCZ*, *PLT1* and *PLT2* genes

Combining *scz* and *scr* mutations has a synergystic effect, resulting in seedlings with a strongly affected root (Pernas, Ryan & Dolan 2010). Also *plt1-4;plt2-2* double mutant roots are defective and niche activity is compromised visibly already in the heart stage embryo where the lens-shaped and root cap progenitor cell exhibit aberrant cell divisions (Aida et al. 2004). We investigated the transcriptional and genetic interaction between *SCZ* and *PLT1* and *PLT2* genes. Auxin distribution visualized by $DR5_{rev}$::*GFP* as well as *PLT1* and *PLT2* promoter and protein expression is not affected in postembryonic *scz-2* roots (Figure 1B,C,E-H, data not shown). In contrast, *SCZ*::$_{ER}$*CFP* peak expression in the niche is lost as *plt1-4;plt2-2* double mutant roots differentiate (Figure 1J,K). This may indicate a secondary effect due to the cessation of meristem activity or, alternatively, that *PLT* activity is required for the maintenance of high *SCZ* transcription levels in the QC. Overexpression and mutant complementation microarray experiments indicate that *SCZ* is up regulated within 6 hours upon PLT induction backing up the latter statement and placing *SCZ* transcriptionally downstream of the *PLT* genes (RH pers comm).

We introduced *scz-2* into the *plt1-4;plt2-2* double mutant to test how *SCZ* acts in the *PLT1/PLT2* niche maintenance pathway. In *scz-2;plt1-4;plt2-2* triple mutant roots, the *scz* mediated radial patterning defects still occur, indicating that these aspects are *PLT* independent (Figure 1M, bracket). Already, in early heart stage *scz-2;plt1-4;plt2-2* triple mutant embryos both defects are seen typical for *scz-2* (Figure 1P, dividing ground tissue, yellow bracket) and *plt1-4;plt2-2* (Figure 1N-P, enlarged QC progenitors, red arrowhead). Common defects remain in mature embryos, with *plt* related defects consisting of dividing QC and columella, whereas *scz-2* defects result in an additional ground tissue layer (Figure 1Q,R, blue arrowhead, bracket). These results indicate *SCZ* and *PLT* genes act

independent. Growth and meristem dissipation resembles that of *plt1-4;plt2-2* double mutants up to 3 days after germination suggesting epistasis until the early phase of post-embryonic development (Figure 1L, 2O). The enhanced growth defect at later post-embryonic stages may be explained by the compromised *SCR* expression in *scz* background (see below).

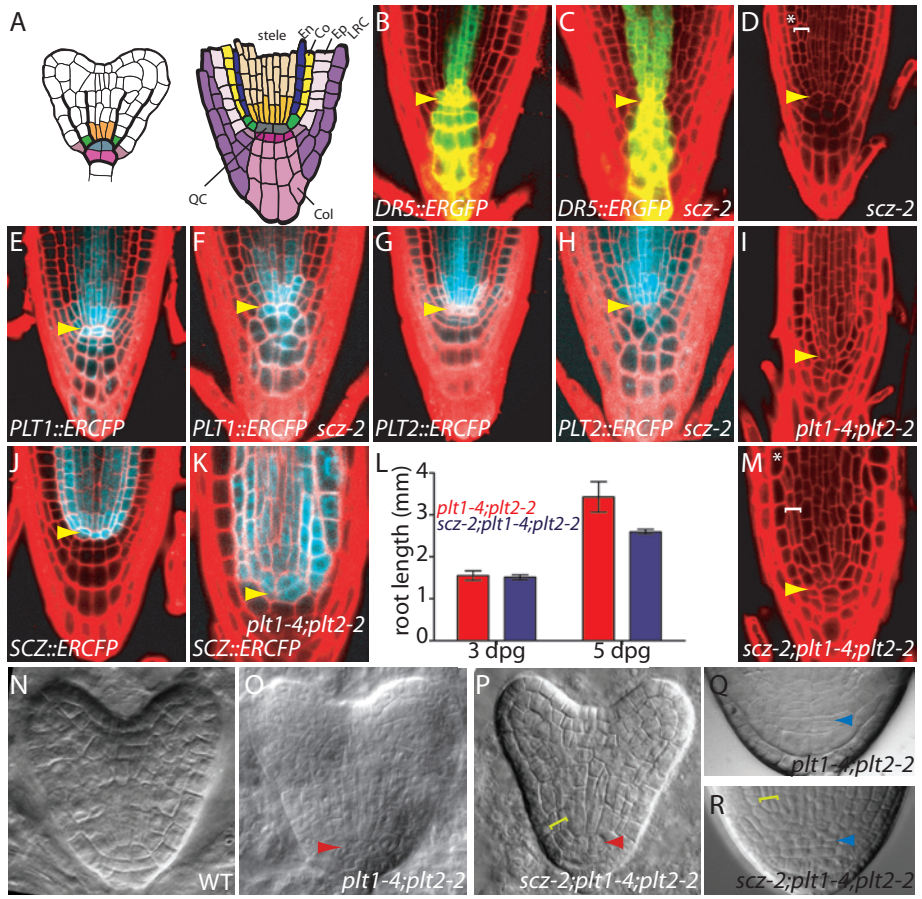

Figure 1. Interaction between *SCZ* and *PLT1/PLT2* genes.

(A) Schematic representation of the developing *Arabidopsis* root meristem. Left, heart stage embryo; right, seedling root meristem. (En) endodermis; (Co) cortex; (Ep) epidermis; (LRC) lateral root cap; (Col) columella; (QC) quiescent center. (B,C,E-H) 4-Day-old wild-type (B,E,G) and *scz-2* (C,F,H) roots expressing *DR5*::$_{ER}$*GFP* (B,C), *PLT1*::$_{ER}$*CFP* (E,F) and *PLT2*::$_{ER}$*CFP* (G,H). (D,I,M) 3-Day-old *scz-2* (D), *plt1-4;plt2-2* (I), *scz-2;plt1-4;plt2-2* (M) roots. (J,K) *SCZ*::$_{ER}$*CFP* expression in 3-day-old wild-type (J) and *plt1-4;plt2-2* (K) roots. (L) Root length measurements (in mm) of *plt1-4;plt2-2* and *scz-2;plt1-4;plt2-2* seedlings. A minimum of 10 seedling roots were measured for each time point. Error bars represent standard error of the mean. (N,P) Heart stage embryo of wild-type (N), *plt1-4;plt2-2* (O) and *scz-2;plt1-4;plt2-2* (P). (Q,R) mature embryo of *plt1-4;plt2-2* (Q) and *scz-2;plt1-4;plt2-2* (R). Yellow arrowhead indicates QC area. Square bracket marks *scz* ectopic endodermal layers, asterisk indicates *scz* mutant subepidermal layer. *plt1;plt2* characteristic expanded QC progenitors (red arrowhead), *plt1;plt2* characteristic ectopic columella division (blue arrowhead)

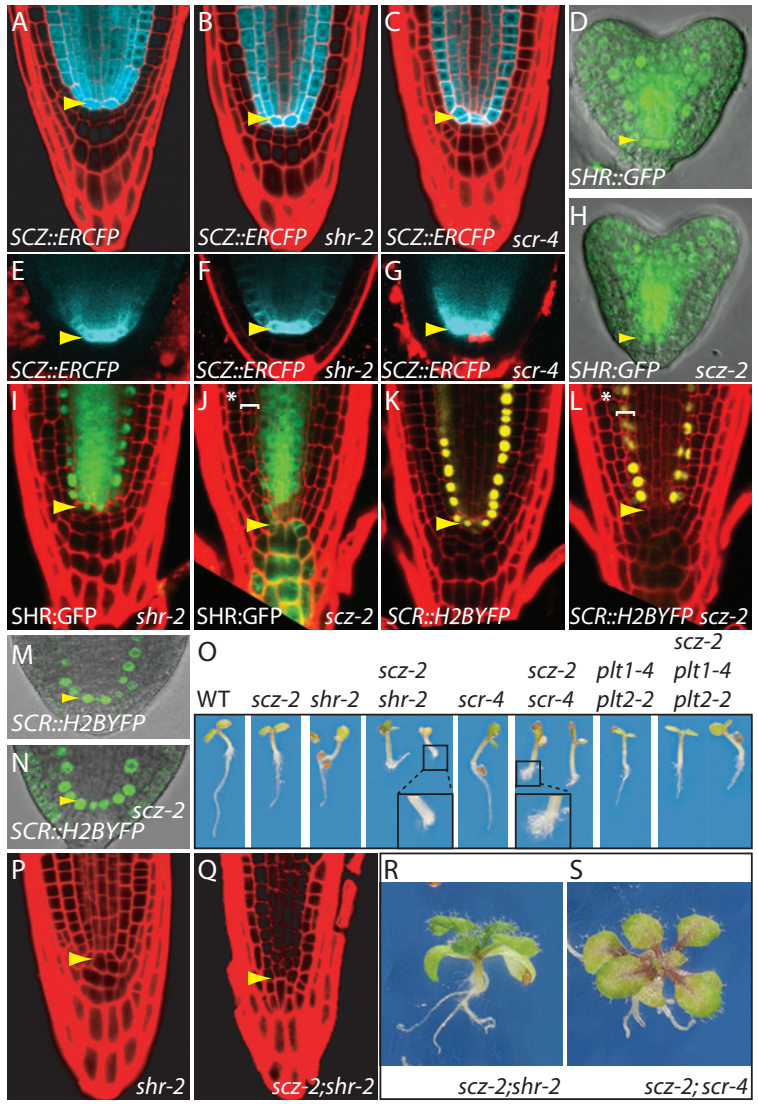

Figure 2. *SCZ* acts with *SHR/SCR* for root meristem initiation.

(A-C, E-G) *SCZ::$_{ER}$CFP* expression in 3-day-old wild-type (A,E), *shr-2* (B,F) and *scr-4* (C,G) roots and late torpedo stage embryos. (D,H,I,J) SHR:GFP expression in wild-type (D) and *scz-2* (H) heart stage embryos and in 5-day-old wild-type (I) and *scz-2 QC46::$_{ER}$YFP* (J) roots. (K-N) *SCR::$_{H2B}$YFP* expression in 5-day-old wild-type (K) and *scz-2* (L) roots and in late torpedo stage wild-type (M) and *scz-2* (N) embryos. (O) 3-Day-old wild-type, *scz-2*, *shr-2*, *scz-2;shr-2*, *scr-4*, *scz-2;scr-4*, *plt1-4;plt2-2* and *scz-2;plt1-4;plt2-2* seedlings. Insets show magnification of *scz-2;shr-2*, *scz-2;scr-4* seedlings lacking a primary root. (P,Q) 3-Day-old *shr-2* (P) and *scz-2;shr-2* roots (Q). (R,S) 19-Day-old originally rootless *scz-2;shr-2* (R) and *scz-2;scr-4* (S) seedlings develop adventitious roots from which lateral roots initiate. Yellow arrowhead indicates QC area. Square bracket marks *scz* ectopic endodermal layers, asterisk indicates *scz* mutant subepidermal layer.

SCZ and the *SHR*/*SCR* pathway specify QC and the stem cell niche

From early embryogenesis onwards *SCZ* gene expression overlaps with *SHR* and *SCR* gene and protein expression patterns. In addition, root patterning defects occur in QC and ground tissue of *scz*, *shr* and *scr* mutants (ten Hove et al. 2010, Wysocka-Diller et al. 2000). The reported genetic interaction between *SCZ* and *SCR* (Pernas, Ryan & Dolan 2010) prompted us to investigate the influence of *SCZ* on *SHR* and *SCR* expression and function and vice versa. *SCZ::$_{ER}$CFP* expression in *shr-2* and *scr-4* mutants is comparable to wild-type from embryogenesis onward and stays unaffected in mature roots, indicating that *SHR* and *SCR* are not required for *SCZ* transcription (Figure 2A-C,E-G). Expression analyses in *scz-2* embryos and seedling roots indicated that initiation of *SCR::$_{H2B}$YFP* and *SHR::SHR:GFP* expression are *SCZ* independent (Figure 2D,H,M,N). However, postembryonic SHR:GFP and *SCR::$_{H2B}$YFP* niche expression do depend on *SCZ* ((ten Hove et al. 2010), Figure 2I-L).

scz-2, *shr-2* and *scr-4* single mutants all display shorter roots and meristems, compared to wild-type (Figure 2O and data not shown). However, whereas *scz-2* roots grow indeterminate (ten Hove et al. 2010), root growth in *shr* and *scr* terminates as cells in the meristem differentiate (Sabatini et al. 2003). Surprisingly, we find around 75% of the *scz-2;shr-2* and *scz-2;scr-4* double mutants entirely lacking primary roots (Figure 2O) (n>50 for both double mutants). The remainder displayed fully differentiated root poles upon germination (Figure 2O,Q). Seedlings of both classes develop adventitious roots from which lateral roots initiate, indicating that *SCZ* is not required for lateral root formation (Figure 2R,S). *scz-2;shr-2* and *scz-2;scr-4* double mutant shoot growth is decreased compared to *shr-2* or *scr-4* single mutants, respectively, possibly due to their smaller root systems.

As both *scz-2;shr-2* and *scz-2;scr-4* double mutant combinations displayed identical phenotypes we chose to analyze embryonic development of *scz-2;shr-2* mutants in detail. The first visible defect in *scz-2* is an aberrant periclinal division of ground tissue initials at heart stage ((ten Hove et al. 2010); Figure 3B, green arrowhead). *scz-2;shr-2* double mutant embryos resemble *shr-2* up to this stage with a single layer of ground tissue due to defective periclinal ground tissue divisions (Figure 3C,D), confirming previous findings that *SHR/SCR* is required for *scz* ectopic divisions (Mylona et al. 2002). Importantly, additional phenotypes of the *scz-2;shr-2* double mutant become apparent at this stage. Delayed formative horizontal root cap divisions are replaced by vertical divisions at late heart stage (Figure 3D,H). Further horizontal root cap divisions are absent in later stage embryos. Most striking in phenotypically strong double mutants (n/total = 16/22) is the vertical division and subsequent longitudinal expansion of QC cells from torpedo stage onward (Figure 3L,P,T). These elongated cells end up resembling root cap cells according to morphological criteria at the mature embryo stage (Figure 3T). As a result a reduced columella and only few or no lateral root cap-like cells are observed (Figure 3T). The *scz-2;shr-2* epidermal layer is long and flattened, resembling the mixed epidermis-lateral root cap layer of *scz-2* single mutants. Surprisingly, mature embryonic root vascular tissue appears to be surrounded by two ground tissue-like layers that continue from hypocotyl all the way down to the distal root cap tissues (Figure 3T). The typical root-hypocotyl

junction can therefore not be distinguished. The apparent lack of QC and stem cell activity in *scz-2;shr-2* double mutants suggests that *SCZ* and *SHR* function together in specifying root niche cell types during embryogenesis.

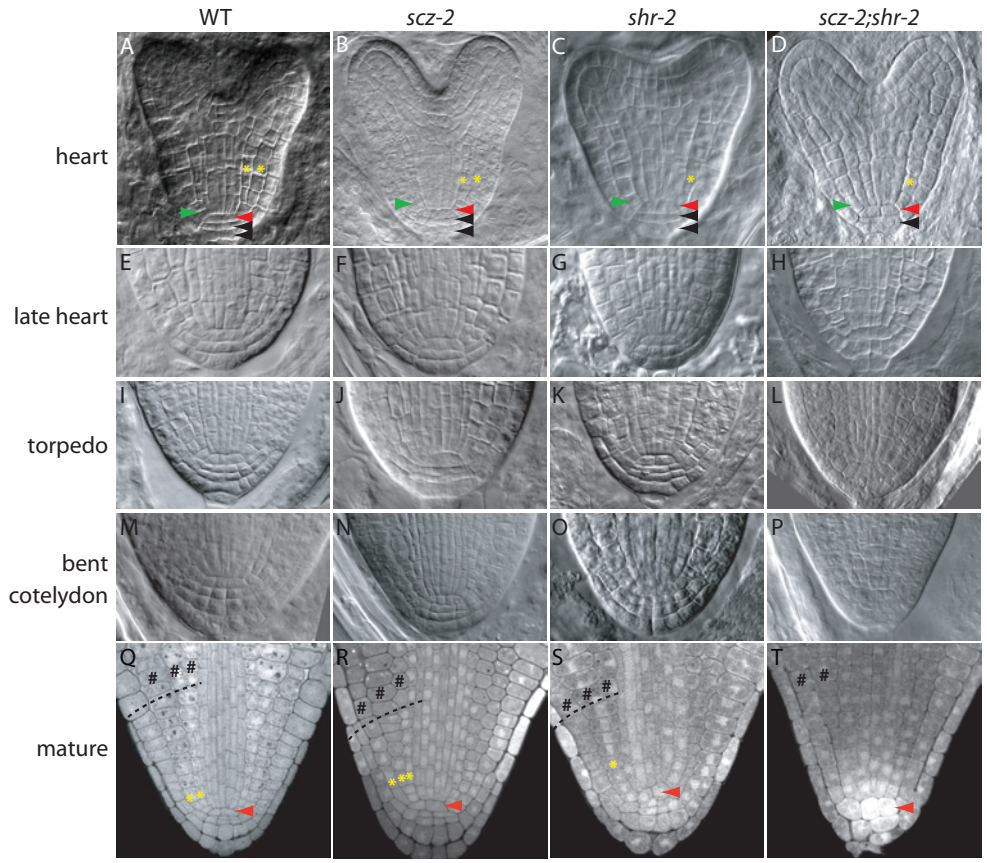

Figure 3. Both *SCZ* and *SHR* are required to set up the root stem cell niche.

(A-P) Early heart stage (A-D), late heart stage (E-H), torpedo stage (I-L) and bent cotyledon stage (M-P) wild-type (A,E,I,M), *scz-2* (B,F,J,N), *shr-2* (C,G,K,O) and *scz-2;shr-2* (D,H,L,P) embryos. Green arrowhead marks ground tissue initial; red arrowhead marks QC; black arrowhead marks root cap; yellow asterisk marks ground tissue. (Q-T) Aniline blue stained wild-type (Q), *scz-2* (R), *shr-2* (S) and *scz-2;shr-2* (T) mature embryos. Root/hypocotyl boundary (dashed line), ground tissue layers in hypocotyl (hash).

Protein interaction studies in yeast

Despite the strong phenotypes of the double mutants, embryonic expression of SHR and *SCR* is independent of *SCZ* activity and vice versa. This leaves open the possibility that the encoded proteins might interact. SHR and SCR proteins have been shown to functionally interact with each other and members of the JACKDAW family of zinc-finger proteins (Cui et al. 2007, Welch et al. 2007) and a

SHR-SCR complex binds *SCR* and *MGP* promoters (Cui et al. 2007). We therefore assessed whether SCZ could influence SHR and/or SCR activity through direct protein-protein interaction. Because the *scz-2;plt1-4;plt2-2* triple mutant phenotype is enhanced over time we also included PLT1 and PLT2 in the interaction assay. However, initial yeast two hybrid interaction studies indicate that there is no direct interaction between SCZ and SHR, SCR or PLT proteins (Table 1).

Table 1. SCZ does not physically interact with PLT, SHR or SCR.

bait\ prey	PLT1	PLT2	SHR	SCR	empty
SCZ	-	-	-	-	-
SCR	NA	NA	+	NA	-

Yeast two hybrid interactions. SCZ and SCR serve as a bait. PLT1, PLT2, SHR and SCR serve as a prey. SCR-SHR interaction is tested as a positive control and empty vector as a negative control. (+) interaction; (-) no interaction; (NA) not analyzed.

Cell fates are set up adequately in *scz-2;shr-2* double mutants

The aberrant development of *scz-2;shr-2* double mutant embryos suggest that tissue specification may be compromised. Therefore we analyzed tissue specific markers in double mutant embryos. Since first defects are seen in heart stage embryos, and the QC is initiated in this stage, we used heart stage embryos as the initial stage in our analysis. As a marker for vascular cell fate we chose the *WOODENLEG* (*WOL*) promoter driving GFP_{ER} (Pischke et al. 2006). The *N9094*(*J0571*) enhancer trap was chosen as a ground tissue marker (http://www.plantsci.cam.ac.uk/Haseloff) and *WEREWOLF* (*WER*) promoter driving CFP_{ER} (Galinha et al. 2007) was used as a marker for epidermis. *WOL::$_{ER}$GFP* is normally expressed in *scz-2*, *shr-2* single and *scz-2;shr-2* double mutant heart stage embryos compared to wild-type (Figure 4D) indicating that vascular fate is correctly set up in *scz-2;shr-2* double mutants. *N9094* is expressed in ground tissue in root and hypocotyl of wild-type embryos (Figure 4E). Interestingly, in *scz-2* embryo *N9094* expression is initiated in ground tissue including the future mutant subepidermal layer from which expression is excluded in postembryonic roots (Figure 4F, (Mylona et al. 2002, Pernas, Ryan & Dolan 2010)). In *shr-2* heart stage embryos, *N9094* is expressed in its single ground tissue layer (Figure 4G). Similarly, *scz-2;shr-2* heart stage embryos display *N9094* expression (Figure 4H) albeit at reduced levels. *WER::$_{ER}$CFP* is expressed in epidermis in wild-type, *scz-2* and *shr-2 (*Figure 4I-K). In *scz-2;shr-2* double mutant embryos expression is not changed (Figure 4L). Together, these results show that all radial markers are expressed at heart stage indicating that specification and patterning of the three main tissues is correctly initiated. As a marker for root (meristem) identity we used the *RCH1>>GFP_{ER}* transactivation line (Casamitjana-Martinez 2003). *RCH1>>GFP* expression initiates in epidermis of future root cells in heart stage embryos of wild-type, *scz-2* and *shr-2* (Figure 4M-O). In *scz-2;shr-2* double mutants *RCH1>>GFP* is expressed albeit in fewer cells (WT, *scz-2, shr-2*: n=6, *scz-2;shr*: n=2) and weaker (Figure 4P). Analysis at later stages should reveal whether tissue and root meristem identities are maintained.

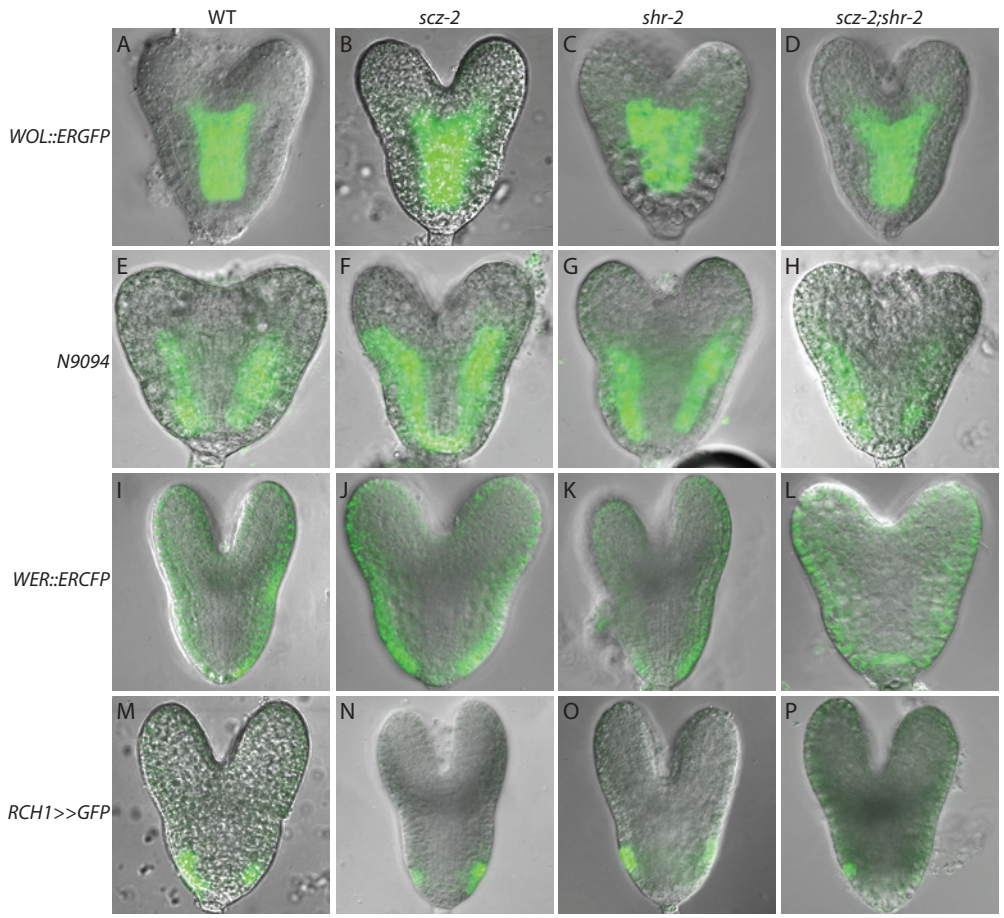

Figure 4. Cell fates are adequately set up in *scz-2;shr-2*.

(A-D) *WOL::$_{ER}$GFP* expression in wild-type (A), *scz-2* (B), *shr-2* (C) and *scz-2;shr-2* (D) heart stage embryos. (E-H) *N9094* expression in wild-type (E), *scz-2;QC46::$_{ER}$YFP* (F), *shr-2* (G) and *scz-2;shr-2* (H) heart stage embryos. (I-L) *WER::$_{ER}$CFP* expression in wild-type (I), *scz-2* (J), *shr-2* (K) and *scz-2;shr-2; QC46::$_{ER}$YFP* (L) heart stage embryos. (M-P) *RCH1>>$_{ER}$GFP* expression in wild-type (M), *scz-2* (N), *shr-2* (O) and *scz-2;shr-2* (P) heart stage embryos.

DISCUSSION

During embryogenesis the root stem cell niche is laid down, orchestrated by the combinatorial action of the auxin-responsive *PLT* genes and the independently initiated activity of the *SHR* and *SCR* genes. Here, we show that also *SCZ* is required for stem cell niche specification and root initiation. Genetic interactions and expression data indicate that *SCZ* operates partially downstream of *PLT* in niche maintenance and that normal *SCZ* expression requires *PLT* activity. However, to unequivocally position *SCZ* with respect to the *PLT* genes, it might be needed to test multiple mutant combinations

including redundant *plt3* and *plt4* alleles. Two other examples of well-studied rootless mutants are *mp* and *bodenlos* (*bdl*) (Berleth, Jurgens 1993, Hamann, Mayer & Jurgens 1999). *MP* and *BDL* act in the same pathway. Their action is vital for initiation of root formation at globular stage embryos. Here they mediate auxin response. This, together with activity of the protein phosphatases *POLTERGEIST* (*POL*) and *POL LIKE1,* directs an asymmetric division of the hypophysis, forming the lens shaped QC progenitor cell (Berleth, Jurgens 1993, Hamann, Mayer & Jurgens 1999, Gagne, Clark 2007, Gagne, Song & Clark 2008). Another strategy to genetically position *SCZ* in the process of QC initiation would be by using a weak *mp* allele, *mp-S319* and cross it to *scz-2*. *Mp-S319* results in 10-20% rootless seedlings (Cole et al. 2009, Donner, Sherr & Scarpella 2009, Schlereth et al. 2010). If *SCZ* acts fully downstream of *MP*, in *scz-2;mp-S319* double mutant this percentage will not change. If *SCZ* acts in parallel to *MP*, the incidence of rootless seedlings might increase.

Expression of *WOX5* and its upstream regulator *SCR* is initiated in the hypophysis and maintained in the lens shaped cell (Wysocka-Diller et al. 2000, Haecker et al. 2004) and joined by QC25, QC46 and QC184 expression at heart stage (Aida et al. 2004, Jenik, Barton 2005, Sarkar et al. 2007). Defects in *scz-2;shr-2* double mutants were traced back to late heart stage arguing that *SCZ* and *SHR* genes determine root formation from this stage onwards. Formative stem cell divisions are absent in *scz-2;shr-2* double mutants indicative of a failure to initiate these stereotypical divisions. These results correlate well with the overlapping expression of *SCZ* and *SHR* in the QC in these stages of embryogenesis. Further studies using QC specific markers that are still expressed in *shr-2* background (e.g. QC184) should clarify whether QC fate fails to be initiated is or not maintained in the *scz-2;shr-2* double mutant embryo. Pernas et al (2010) stated that *SCZ SCR* genetic interaction is indispensable for ground tissue identity (Pernas, Ryan & Dolan 2010). However, we detect the *N9094* ground tissue marker in *scz-2;shr-2* heart stage embryos indicating ground tissue fate is being set up in the double mutant. Analysis at later stages should confirm the role of *SCZ* and *SHR/SCR* in the maintenance of root ground tissue fate. We note that in *scz-2* embryos *N9094* expression initiates in ground tissue, including the future mutant subepidermal layer, whereas post embryonically expression is excluded from this layer (Mylona et al. 2002, Pernas, Ryan & Dolan 2010)). The fact that *scz-2* can be complemented by *N9094>>SCZ* (ten Hove et al. 2010), likely is a consequence of *N9094* being broader expressed in *scz-2* heart stage embryos.

Our genetic and expression data indicate that *SCZ* acts both together and in parallel to *SHR/SCR*. As in the interaction studies with *PLT* pathway genes, this indicates that it is not likely that *SCZ* can be positioned in linear pathways of *PLT* and *SHR/SCR* action. The non-linearity of the *PLT* and *SHR/SCR* pathways is by now well established (Cruz-Ramírez et al. 2012, Blilou et al. 2005). Our data suggest that genetic interaction data for such pathways do not yield the clear-cut outcomes which allow us to unambiguously position *SCZ* gene activity in either one of these pathways. Rather, our data indicate that *PLT* and *SHR/SCR* activity may both be connected to distinct *SCZ* functions, which

can explain that we find only partial interactions. Future studies should focus on understanding the mechanistic that underlies these genetic interactions. Yeast two hybrid experiments suggest that there is no interaction through direct protein interaction. Recently, however, it was found that PLT and SCR proteins can physically interact with TCP20 or TCP21 transcription factors. Genetic and biochemical data indicate that these proteins form complexes which together specify QC identity (A.Shimothono, BS, and RH, manuscr. in prep.). In this context it is highly relevant that we found protein interaction between SCZ and TCP20 (Chapter 4). It is tempting to speculate a role for TCP20 mediating SCZ-SCR and possibly even PLT protein interaction too. Future experiments should reveal if SCZ and SCR execute their function in multiprotein complexes, linked by TCP20. Studies to determine downstream effectors of such complexes should reveal to what extent *SCZ*, *PLT* and *SHR*/*SCR* act in parallel. The potential overlaps and differences between *SCZ, PLT*, *SHR* and *SCR* target genes will reveal their communality and authenticity. Common targets that are required for specifying QC may be found from transcriptome analysis of isolated QC cells or nuclei of *scz;shr* or *scz;scr* double mutant heart stage embryos.

EXPERIMENTAL PROCEDURES

Plant materials and growth conditions

Origins of mutant and transgenic lines: *shr-2, SHR::SHR:GFP* (Nakajima et al., 2001); *scr-4* (Fukaki et al., 1998); *SCR::$_{H2B}$YFP* (Heidstra et al., 2004); *plt1-4;plt2-2* (Aida et al., 2004); *PLT1::$_{ER}$CFP*, *PLT2::$_{ER}$CFP* (Galinha et al., 2007); *DR5::$_{ER}$GFP* (Benkova et al., 2003); *WOL/CRE1::$_{ER}$GFP* (Pischke et al. 2006); *WER::$_{ER}$CFP* (Galinha et al. 2007); *RCH1>>$_{ER}$GFP* (Casamitjana-Martinez 2003). Enhancer trap line *N9094* was obtained from the Nottingham *Arabidopsis* stock center (NASC, http://www.plantsci.cam.ac.uk/Haseloff). Seedlings and embryos were sterilized, plated and grown as described in (Scheres et al. 1995, Sabatini et al. 2003).

Microscopy

Light microscopy, starch granule staining, measurement of root length and aniline blue staining was performed as described in (Welch et al. 2007, Willemsen et al. 2008, Bougourd, Marrison & Haseloff 2000). For confocal microscopy, roots and dissected embryos were mounted in propidium iodide (PI; 20 µg/mL in distilled water) or 4% glucose (+/- PI), respectively.

Yeast two hybrid assay (Y2H)

Y2H interactions were studied according to (Welch et al. 2007) using the ProQuest Two Hybrid System (Invitrogen Life Technologies) and yeast strains Pj694α and Pj694a (James, Halladay & Craig 1996). The coding sequence of SCZ was amplified and fused into the *pDEST32* BD vector whereas the amplified coding sequences of PLT1, PLT2, SHR and SCR were recombined into the *pDEST22* AD vector. Although *pEXP-SCZ* did not show auto activation activity, 2mM 3-AT was used to eliminate

any background transcriptional activity. Primers used for SCZ are described in (ten Hove et al. 2010), for SHR and SCR in (Welch et al. 2007) and for PLT1, PLT2 primer sequences are:
PLT1-F: 5′-ggggacaagtttgtacaaaaaagcaggctgtatgaattctaacaactggcttggctttcctct-3′,
PLT1-R: 5′ggggaccactttgtacaagaaagctgggtcttactcattccacatagtgaaaacaccaccagg-3′,
PLT2-F: 5′-ggggacaagtttgtacaaaaaagcaggctgtatgaattctaacaactggctcgcgttccctc-3′,
PLT2-R: 5′-ggggaccactttgtacaagaaagctgggtcttattcattccacatcgtgaaaacacctcctgg-3′.

Chapter 4

Identification of proteins interacting with SCHIZORIZA

Jansweijer VMA[1,2], Gaillochet C[1], Krijnen M[1], Horváth B[1], Scheres B[1,2], Heidstra R[1,2]

[1] Department of Biology, section Molecular Genetics, Utrecht University, 3584 CH Utrecht, The Netherlands.
[2] Current affiliation: Plant Developmental Biology, Wageningen University Research, 6708 PB Wageningen, The Netherlands.

"L'Autunno"

ABSTRACT

Physical interaction between proteins, leading to formation of protein complexes is of key importance for many biological processes. In *Arabidopsis* roots, *SCHIZORIZA* (*SCZ*) promotes the specification and segregation of fates in tissues outside the stele. Genetic interaction between *SCZ* and *SCARECROW* (*SCR*) is required to specify the root stem cell niche during embryogenesis and in addition it has been reported that this interaction is responsible for setting up ground tissue identity. Here we test for proteins physically interacting with SCZ. We validate direct interaction of SCZ with transcription factors TEOSINTE BRANCHED1/ CYCLOIDEA/ PROLIFERATING CELL NUCLEAR ANTIGEN FACTOR19 (TCP19), TCP20, and FLC EXPRESSOR (FLX) in yeast and in plant nuclei. FLX as a binding partner may hint at a degradation mechanism through which SCZ segregates cell fate. We speculate that shared action of SCZ and SCR is facilitated by their common binding partner TCP20 as a chaperone.

INTRODUCTION

Protein complexes can be stable or transient and each complex conducts a discrete biological function (Hartwell et al. 1999). Protein complex formation is vital in directing developmental programs. Identification of proteins interacting with key regulators will therefore provide further insight in the molecular mechanisms underlying developmental events. The tetrameric MADS-box transcription factor complexes in *Arabidopsis* floral development are classic examples of regulatory complexes (Honma, Goto 2001, Pařenicová et al. 2003). MADS-box proteins PISTILLATA (PI) and APETALA3 (AP3), interact with AP1 through SEPALLATA3 (SEP3) to determine the identity of the floral organs. Overexpression of all four *PI*, *AP3*, *AP1*, and *SEP3* leads to a conversion of vegetative leaves to petal-like organs. SEP3 establishes the interaction between AP3-PI and AGAMOUS (AG). In absence of SEP3, AG fails to interact with the AP3-PI dimer. Overexpressing *AP3* and *PI* together with *SEP3* is sufficient to promote development of vegetative organs into petalloid organs whereas both *AP3* and *PI* together or *SEP3* alone is not (Honma, Goto 2001). Thus, SEP3 is needed as a scaffold between AP3-PI and AG and the protein complex as a whole determines its final action (Honma, Goto 2001). Also in *Arabidopsis* roots, protein complexes direct various developmental events. The two GRAS family transcription factors SHORTROOT (SHR) and its target SCARECROW (SCR) together direct the formation of ground tissue (endodermis and cortex) (Di Laurenzio et al. 1996, Helariutta et al. 2000). SHR protein moves from the stele to the endodermis and quiescent center (QC), where it activates transcription of- and physically interacts with SCR (Helariutta et al. 2000, Nakajima et al. 2001, Cui et al. 2007, Cui, Benfey 2009). SCR in turn restricts SHR movement with the aid of members of a class of zinc finger proteins that include JACKDAW (JKD) and MAGPIE (MGP) (Welch et al. 2007). JKD and MGP physically interact with both SHR and SCR but act in counteracting fashion. It is thought that the four proteins form nuclear complexes in cells where they are jointly expressed. Phenotype analyses show that formation of protein complexes is crucial for to regulation of cell type specification and stable boundary formation, by guiding the number of SHR–SCR-mediated asymmetric cell divisions (Welch et al. 2007). The SHR-SCR complex regulates a large set of targets, amongst which many cell-cycle related genes (Nakajima et al. 2001, Cui et al. 2007, Sozzani et al. 2010, Levesque et al. 2006). One of these, the D-type cyclin *CYCD6;1* is expressed in the cortex/ endodermis initial and its daughter. CYCD6;1 interacts with two redundant cyclin dependent kinases and regulators of cell cycle progression, CDKB2;1 and CDKB2;2, to facilitate asymmetric cell divisions in the cortex endodermis initial daughter (Sozzani et al. 2010). SCR also physically interacts with RETINOBLASTOMA RELATED (RBR) that has previously been shown to regulate the size of the stem cell population downstream of *SCR* through the CYCD/RB/E2F pathway (Wildwater et al. 2005). The CYCD6;1-CDK complex is a potential mediator of RBR phosphorylation, thereby counteracting SCR-RBR interaction and allowing the asymmetric division in a strictly regulated fashion (Cruz-Ramírez et al. 2012). This illustrates how different protein interactions and the interplay between the different complexes can precisely determine asymmetric cell divisions.

The B-type HEAT SHOCK FAMILY (HSF) transcription factor *SCHIZORIZA* (*SCZ*) has a role in the determination of cortex fate and is important for establishment of ground tissue stem cells in embryonic root. Furthermore, it segregates QC/columella and epidermis/lateral root cap fates non-cell autonomously (ten Hove et al. 2010, Pernas, Ryan & Dolan 2010). To date it remains unclear through what mechanism SCZ executes these intriguing functions in cell fate segregation. It has been suggested that SCZ conducts its non-cell autonomous action, through an unknown factor derived from ground tissue (ten Hove et al. 2010, Pernas, Ryan & Dolan 2010). SCZ together with SCR set up a functional root stem cell niche and ground tissue (Chapter 3 and (Pernas, Ryan & Dolan 2010)), possibly through protein complex formation. The SCZ protein contains multiple conserved domains through which a physical interaction with partner proteins could be accomplished. In the central part of SCZ protein, there is a heptad hydrophobic repeat (HR-A/B) oligomerization domain. Plant HSFs are divided into three classes, based on differences in flexible linkers in their oligomerization domain, the A class (HSFA, 16 members), the B class (HSFB, 4 members including SCZ) and the C class (HSFC, 1 member) (Scharf et al. 2011, Nover et al. 2001). HSFA5 is able to repress HSFA4A and HSFA4C through interaction with their oligomerization domain solely (Baniwal et al. 2007), illustrating the need of this domain for specific transcription factor interactions. Towards the C-terminus of the SCZ protein there are two distinct repressor domains, a nuclear localization signal and a nuclear export signal (ten Hove et al. 2010, Nover et al. 2001, Kagale, Links & Rozwadowski 2010, Ikeda, Ohme-Takagi 2009). HSF nuclear or cytoplasmic distribution changes dynamically depending on the balance between nuclear import and export (Scharf et al. 2011, Heerklotz et al. 2001). One of the repressor domains consists of a R/KLFGV conserved motif through which HSFB1 and HSFB2B were shown to exhibit repressive activity (Ikeda, Ohme-Takagi 2009). In contrast with other HSFBs, the C-terminal end of SCZ protein also contains an LxLxL type EAR motif with repressing potential (Kagale, Links & Rozwadowski 2010).

To identify interacting proteins, yeast two-hybrid (Y2H) screens and co-immunoprecipitation (co-IP) can be employed. Y2H experiments are simple, straightforward and can be high throughput by screening cDNA expression libraries. Additional advantage is that available cloned cDNAs simplify downstream studies (Causier, Davies 2002a). An advantage of the co-IP procedure is that entire protein complexes can be identified instead of single protein-protein interactions monitored with Y2H analysis. However, in contrast to Y2H, co-IP is not readily amenable for high throughput analysis. Here we use a Y2H approach to explore candidate proteins interacting with SCZ in *Arabidopsis* roots. To corroborate Y2H interactions we use Bimolecular Fluorescent Complementation (BiFC) assays in onion epidermal cells. We fail to show a direct physical interaction between SCZ and SHR/SCR proteins but do observe interaction with TCP19 and TCP20. We speculate how SCZ-TCP20 interaction could link SCZ to SHR/SCR and PLT pathways. Also, we find strong physical interaction of SCZ with a leucine zipper transcription factor FLX. This provides a first clue towards the SCZ mechanism of action in fate segregation through degradation of its protein interactors.

RESULTS

To investigate whether the interaction of SCZ with SHR and/or SCR occurs via protein interaction we performed Y2H assays. In addition, we decided to test interaction of SCZ with another 8 transcription factors involved in root (niche) development and that show an overlapping expression pattern with *SCZ*, i.e. JKD, RBR, PLETHORA1 (PLT1), PLT2, WUSCHEL RELATED HOMEOBOX 5 (WOX5), TCP19, TCP20 and TCP21. SHR, SCR, JKD and RBR are all expressed in both QC and ground tissue (Di Laurenzio et al. 1996, Helariutta et al. 2000, Wildwater et al. 2005, Sabatini et al. 2003). PLT1 and PLT2 show additional overlap with *SCZ* expression in vasculature (Aida et al. 2004) whereas *WOX5* is expressed solely in QC (Haecker et al. 2004, Sarkar et al. 2007). According to public expression data, *TCP19* is expressed in the QC, *TCP20* is found mostly in the differentiation region and *TCP21* is expressed in ground tissue (Brady et al. 2007).

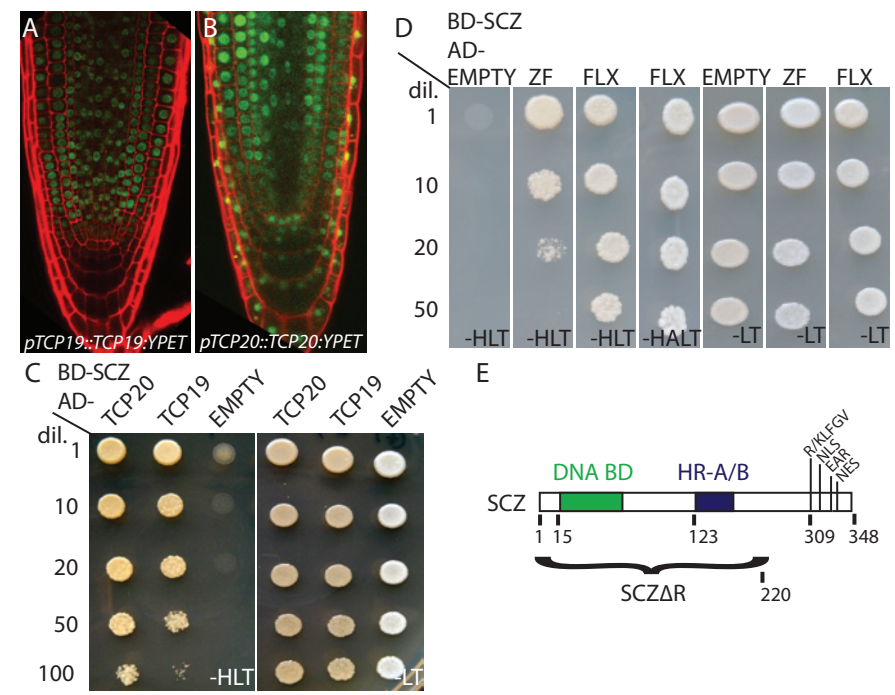

Figure 1. SCZ interacts with TCP19, TCP20 and FLX in yeast.

(A,B) 5-Day-old wild-type root expressing pTCP19::TCP19:YPET (A) and pTCP20::TCP20:YPET (B). (C,D) Y2H interaction of BD-SCZ with AD-TCP19, AD-TCP20 and AD-EMPTY (C) and BD-SCZ with AD-ZF, AD-FLX and AD-EMPTY (D). Colonies dropped in a series of dilutions. Growth on –HLT: interaction, growth on –HALT: strong interaction. Growth on –LT: transformation control. (E) Schematic representation of SCZ and SCZΔR baits. Conserved DNA binding domain (green), HR-A/B oligomerization domain (blue), NLS and NES according to (Nover et al. 2001). R/KLFGV motif (Ikeda, Ohme-Takagi 2009), EAR motif (Kagale, Links & Rozwadowski 2010). Location indicated in number of amino acids from start codon.

We focused on TCP19 and TCP20 expression in more detail and constructed protein:YPET fusions expressed from their endogenous promoter. We found *pTCP19::TCP19:YPET* expression in the root meristem largely excluding QC and root cap (Figure 1A). *pTCP20::TCP20:YPET* is expressed throughout the root meristem including QC and root cap (Figure 1B) and extending shootward towards and including the differentiation zone (not shown).

A stretch of 348 amino acids, comprising the entire SCZ coding sequence, was chosen as a bait and fused to the yeast GAL4 DNA binding domain (BD). Yeast expressing BD-SCZ did not grow on medium without Histidine and Tryptophan (–HT) nor when Adenine was omitted (-HAT) (not shown). This demonstrates that the bait fusion protein does not activate the *HIS* and *ADE* reporter genes by itself, indicating SCZ does not display transcription factor activity consistent with the lack of a typical transcription activation domain in the protein (Nover et al. 2001). Although BD-SCZ did not show any auto activation activity, we decided to use a low concentration of 2mM 3-AT to eliminate any background transcriptional activity. As a prey, SHR, SCR, JKD, RBR, PLT1, PLT2, WOX5, TCP19, TCP20 or TCP21 coding sequences were fused to the GAL4 transcription activation domain (AD), transformed to BD-SCZ yeast and tested for interaction by plating on -HLT and -HALT media. Only TCP19 and TCP20 appeared to interact (weakly) with SCZ in yeast as apparent from growth on –HLT media but not -HALT (Figure 1C, Table 1). Some HSFs are reported to become transcriptionally active when they form oligomers (Baniwal et al. 2007, Åkerfelt, Morimoto & Sistonen 2010). Therefore we tested the if SCZ can form homodimers using BD-SCZ with AD-SCZ in the Y2H assay. However we did not detect interaction (Table 1), suggesting that SCZ does not dimerize in yeast.

Table 1. SCZ physically interacts with FLX, TCP19 and TCP20.

AD-	BD-SCZ	BD-SCZΔR
SHR	0	NA
SCR	0	NA
JKD	0	NA
RBR	0	NA
PLT1	0	NA
PLT2	0	NA
WOX5	0	NA
TCP19	+	0
TCP20	+	0
TCP21	0	NA
SCZ	0	NA
EMPTY	0	0
FLX	+++	++
ZN	+	0

BD-SCZ and BD-SCZΔR were tested for interactions with root niche transcription factors fused to AD in Y2H assays. Empty vector was used as a negative control. (+) interaction, (0) no interaction, (NA) not analyzed.

In order to identify additional proteins that function in conjunction with SCZ, a large scale Y2H screen was employed. Again 2mM 3-AT was used to eliminate any background transcriptional activity. For prey proteins, we constructed a root cDNA library from roots of 5-day-old seedlings. BD-SCZ yeast was transformed with the AD-library. A total of ~$3x10^6$ transformants were assayed for interaction on –HLT medium in two independent screens. Growing colonies were subsequently re-plated onto –ALT (1^{rst} screen) or -HALT (2nd screen) media to reduce the number of false positives (James, Halladay & Craig 1996). Inserts in 403 clones were sequenced successfully. Herewith a list of 325 putative interaction partners of SCZ was generated of which 46 interacting proteins were obtained more than once (Table 2). A cDNA encoding the leucine zipper transcription factor FLC EXPRESSOR (FLX, At2G30120) was obtained from 8 independent yeast transformants (Table 2).

Table 2. List of putative SCZ interactors identified from Y2H screening.

Gene	Description	#
AT2G30120	**FLX (FLC expressor)**	**8**
AT2G36830	GAMMA-TIP(Tonoplast Intrinsic protein (TIP) gamma)	5
AT5G60390	elongation factor 1-alpha	5
AT2G41690	**AT-HSFB3 (A. thaliana heat shock TF B3)**	**4**
AT3G09260	PYK10 (phosphate starvation-response 3.1)	4
AT3G16240	PBP1 (PYK10-BINDING PROTEIN 1); copper ion binding	4
AT5G04460	protein binding / zinc ion binding	4
AT5G19510	elongation factor 1B alpha-subunit 2 (eEF1Balpha2)	4
AT1G04410	malate dehydrogenase, cytosolic, putative	3
AT1G65930	NADP+ isocitrate dehydrogenase, putative	3
AT1G74060	60S ribosomal protein L6 (RPL6B	3
AT3G04120	GAPC (Glyceraldehyde-3-Phosphate Dehydrogenase C subunit)	3
AT3G09200	60S acidic ribosomal protein P0 (RPP0B)	3
AT3G18780	ACT2 (ACTIN 2); structural constituent of cytoskeleton	3
AT4G08320	tetratricopeptide repeat (TPR)-containing protein	3
AT4G11210	disease resistance-responsive family/ dirigent family	3
AT4G17340	TONOPLAST INTRINSIC PROTEIN 2;2 (TIP2;2)	3
AT5G11770	NADH-ubiquinone oxidoreductase 20 kDa subunit	3
AT1G06760	histone H1, putative	2
AT1G07930	elongation factor 1-alpha / EF-1-alpha	2
AT1G13440	GlycerAldehyde-3-Phosphate dehydrogenase C2 (GAPC2)	2
AT1G30230	elongation factor 1-beta / EF-1-beta	2
AT1G62660	beta-fructosidase (BFRUCT3)	2

Genes involved in transcriptional regulation (bold).

Table 2. List of putative SCZ interactors identified from Y2H screening.

Gene	Description	#
AT2G37130	peroxidase 21 (PER21) (P21) (PRXR5)	2
AT2G47730	ATGSTF8 | glutathione S-transferase 6 (GST6)	2
AT3G03960	chaperonin, putative	2
AT3G08530	clathrin heavy chain, putative	2
AT3G09470	UNC93-like protein	2
AT3G17390	MTO3 (METHIONINE OVER-ACCUMULATOR 3)	2
AT3G20390	endoribonuclease L-PSP family protein	2
AT3G25520	ATL5 (A. THALIANA RIBOSOMAL PROTEIN L5)	2
AT3G63080	ATGPX5 (glutathione peroxidase 5); glutathione peroxidase	2
AT4G13940	MEE58(Maternal Effect Embryo Arrest 58); copper ion binding	2
AT4G15160	lipid transfer protein (LTP) family protein	2
AT4G18100	60S ribosomal protein L32 (RPL32A)	2
AT4G26660	unknown protein located in the chloroplast	2
AT4G28830	methyltransferase/ nucleic acid binding	2
AT5G03240	UBQ3 (POLYUBIQUITIN 3)	2
AT5G08680	Encodes the mitochondrial ATP synthase beta-subunit	2
AT5G23250	succinyl-CoA ligase (GDP-forming)	2
AT5G26260	meprin and TRAF homology domain-containing protein	2
AT5G27930	protein phosphatase 2C, putative	2
AT5G44020	acid phosphatase class B family protein	2
AT5G48230	ACAT2/EMB1276(Acetoacetyl-CoaThiol2/EmbryoDefective)	2
AT5G56030	HSP81-2 (HEAT SHOCK PROTEIN 81-2); ATP binding	2
AT5G58860	CYP86A1; fatty acid (omega-1)-hydroxylase/ oxygen binding	2
AT1G17880	**NAC domain-containing prot/BTF3b-like TF**	**1**
AT1G34370	**STOP1 (sensitive to proton rhizotoxicity 1)**	**1**
AT1G53190	**zinc finger (C3HC4-type RING finger) family protein**	**1**
AT1G55520	**TBP2 (TATA BINDING PROTEIN 2)**	**1**
AT1G75340	**zinc finger (CCCH-type) family protein**	**1**
AT3G20770	**EIN3 (ETHYLENE-INSENSITIVE3)**	**1**
AT3G58030	**zinc finger (C3HC4-type RING finger) family protein**	**1**
AT4G28440	**DNA-binding protein-related**	**1**
AT5G29000	**PHL1, Myb-type TF**	**1**
AT1G01100	60S acidic ribosomal protein P1 (RPP1A);	1
AT1G02100	leucine carboxyl methyltransferase family protein	1

Genes involved in transcriptional regulation (bold).

Table 2. List of putative SCZ interactors identified from Y2H screening.

Gene	Description	#
AT1G03220	extracellular dermal glycoprotein, putative	1
AT1G03870	FLA9 (FASCICLIN-LIKE ARABINOOGALACTAN 9)	1
AT1G04480	60S ribosomal protein L23 (RPL23A)	1
AT1G04510	MAC3A, a U-box protein	1
AT1G05860	unknown protein	1
AT1G06290	ACX3 (ACYL-COA OXIDASE 3	1
AT1G06980	unknown protein	1
AT1G07670	calcium-transporting ATPase	1
AT1G07820	histone H4	1
AT1G07890	APX1 (Ascorbate Peroxidase1, Maternal Effect Embryo Arrest6)	1
AT1G09690	60S ribosomal protein L21 (RPL21C);	1
AT1G09940	glutamyl-tRNA reductase, HEMA2	1
AT1G10700	ribose-phosphate pyrophosphokinase 3 (PRS3)	1
AT1G11580	PMEPCRA (Methylesterase PCRA); enzyme inhibitor	1
AT1G11910	aspartyl protease family protein	1
AT1G12000	beta subunit, putative	1
AT1G13830	beta-1,3-glucanase-related	1
AT1G14320	SAC52 (Suppressor Of Acaulis52); struct constituent ribosome	1
AT1G14620	DECOY	1
AT1G14690	MAP65-7 (MICROTUBULE-ASSOCIATED PROTEIN 65-7)	1
AT1G15500	chloroplast ADP, ATP carrier, putative /ADP,ATP translocase	1
AT1G16810	similar to CGI-144-like protein [Solanum lycopersicum]	1
AT1G16920	small GTP-binding protein (Rab11)	1
AT1G18080	ATARCA; nucleotide binding	1
AT1G18090	exonuclease, putative	1
AT1G19600	pfkB-type carbohydrate kinase family protein	1
AT1G19910	vacuolar H+-pumping ATPase 16 kDa proteolipid (ava-p2)	1
AT1G20200	EMB2719 (EMBRYO DEFECTIVE 2719)	1
AT1G20480	4-coumarate--CoA ligase family protein	1
AT1G20696	Encodes subgroup of HMGB prot (high mobility group B)	1
AT1G20950	unknown protein	1
AT1G21310	ATEXT3 (EXTENSIN 3); structural constituent of cell wall	1
AT1G22300	GRF10 (GENERAL REGULATORY FACTOR 10)	1
AT1G22410	2-dehydro-3-deoxyphosphoheptonate aldolase, putative	1

Genes involved in transcriptional regulation (bold).

Table 2. List of putative SCZ interactors identified from Y2H screening.

Gene	Description	#
AT1G22530	PATELLIN 2 (PATL2), transporter activity	1
AT1G23100	10 kDa chaperonin, putative	1
AT1G23950	unknown protein;	1
AT1G25490	RCN1 (Roots Curl In NPA); prot phosphatase type 2A regulator	1
AT1G28330	DYL1 (Dormancy-associated protein-like 1)	1
AT1G29320	transducin family protein / WD-40 repeat family protein	1
AT1G29900	CARB; ATP binding / carbamoyl-phosphate synthase	1
AT1G30110	A. Thaliana Nudix Hydrolase Homolog25 (ATNUDX25)	1
AT1G31060	unknown protein	1
AT1G32580	plastid developmental protein DAG	1
AT1G34040	alliinase family protein	1
AT1G36310	methyltransferase	1
AT1G43170	Encodes a cytoplasmic ribosomal protein	1
AT1G47250	Encodes 20S proteasome subunit PAF2	1
AT1G48850	embryo defective 1144 (EMB1144)	1
AT1G50200	ALANYL-TRNA SYNTHETASE (ALATS);	1
AT1G51060	HTA10; DNA binding	1
AT1G51580	KH domain-containing protein	1
AT1G53240	malate dehydrogenase (NAD)	1
AT1G53500	MUM4, encodes a putative NDP-L-rhamnose synthase	1
AT1G53580	GLY3 (Glyoxalase II 3); hydroxyacylglutathione hydrolase	1
AT1G53590	NTMC2T6.1	1
AT1G56070	LOS1; copper ion-/nucleic acid binding/translation elongation	1
AT1G57720	elongation factor 1B-gamma, putative	1
AT1G58270	ZW9 mRNA	1
AT1G59359	40S ribosomal protein S2 (RPS2B)	1
AT1G60710	encodes ATB2, oxidoreductase	1
AT1G60790	unknown protein	1
AT1G62380	1-aminocyclopropane-1-carboxylic oxidase (ACC oxidase)	1
AT1G62390	PB1 domain-containing protein/ TPR-containing protein	1
AT1G63000	NRS/ER (Nucleotide-Rhamnose Synthase/Epimerase-Reductase)	1
AT1G66670	caseinolytic protease	1
AT1G66680	unknown function	1
AT1G70850	MLP-LIKE PROTEIN 34 (MLP34)	1

Genes involved in transcriptional regulation (bold).

Table 2. List of putative SCZ interactors identified from Y2H screening.

Gene	Description	#
AT1G71780	unknown protein	1
AT1G72370	P40; structural constituent of ribosome	1
AT1G73250	ATFX/GER1	1
AT1G74050	60S ribosomal protein L6 (RPL6C)	1
AT1G74960	Encodes a plastidic beta-ketoacyl-ACP synthase II	1
AT1G75400	protein binding / zinc ion binding	1
AT1G75990	(RPN3) 26S proteasome regulatory subunit S3, putative	1
AT1G76180	ERD14 (EARLY RESPONSE TO DEHYDRATION 14)	1
AT1G78900	Encodes catalytic subunit A of the vacuolar ATP synthase.	1
AT1G79550	Encodes cytosolic phosphoglycerate kinase (PGK).	1
AT1G79750	Malic enzyme encoded by AtNADP-ME4	1
AT1G80510	Encodes a close relative of the amino acid transporter ANT1	1
AT2G01720	ribophorin I family protein	1
AT2G02040	PTR2 (PEPTIDE TRANSPORTER 2)	1
AT2G02390	ATGSTZ1 (A. Thaliana Glutathione S-Transferase Zeta1)	1
AT2G05220	40S ribosomal protein S17 (RPS17B)	1
AT2G10410	transposable element gene	1
AT2G17280	phosphoglycerate/bisphosphoglycerate mutase family protein	1
AT2G17350	unknown protein	1
AT2G17440	PIRL5, member of the Plant Intracellular Ras-group-related LRRs	1
AT2G20630	protein phosphatase 2C	1
AT2G21250	mannose 6-phosphate reductase (NADPH-dependent)	1
AT2G21870	ATP synthase 24 kDa subunit, mitochondrial precursor	1
AT2G23350	PAB4 (POLY(A)Binding Prot4); RNA binding /translation initiation	1
AT2G23810	TET8 | TET8 (TETRASPANIN8)	1
AT2G26730	leucine-rich repeat transmembrane protein kinase	1
AT2G27530	Encodes ribosomal protein L10aP	1
AT2G29620	unknown protein	1
AT2G30100	ubiquitin family protein	1
AT2G31810	acetolactate synthase small subunit, putative	1
AT2G33210	HSP60-2 (HEAT SHOCK PROTEIN 60-2)	1
AT2G33590	cinnamoyl-CoA reductase family	1
AT2G33830	dormancy/auxin associated family protein	1
AT2G34560	katanin, putative	1

Genes involved in transcriptional regulation (bold).

Table 2. List of putative SCZ interactors identified from Y2H screening.

Gene	Description	#
AT2G34680	AIR9 (Auxin-Induced in Root cultures 9); protein binding	1
AT2G35200	unknown protein	1
AT2G37180	RD28 (plasma membrane intrinsic protein 2;3); water channel	1
AT2G37310	pentatricopeptide (PPR) repeat-containing protein	1
AT2G38700	Encodes mevalonate diphosphate decarboxylase	1
AT2G39900	LIM domain-containing protein	1
AT2G41770	unknown protein	1
AT2G42400	VOZ2(Vascular plant One Zinc finger prot2)	1
AT2G43150	proline-rich extensin-like family protein	1
AT2G44160	methylenetetrahydrofolate reductase MTHFR2 mRNA, complete	1
AT2G44790	UCC2 (UCLACYANIN 2); copper ion binding / electron carrier	1
AT2G45960	PIP1B, TMP-A, ATHH2, PIP1;2; water channel	1
AT2G47320	peptidyl-prolyl cis-trans isomerase cyclophilin-type family protein	1
AT2G47620	ATSWI3A (Switch/Sucrose NonFermenting3A); DNA binding	1
AT3G01420	DOX1 alpha-dioxygenase	1
AT3G02900	unknown protein	1
AT3G03010	aminoacyl-tRNA hydrolase	1
AT3G03780	AtMS2; methionine synthase	1
AT3G04840	40S ribosomal protein S3A (RPS3aA)	1
AT3G08580	mitochondrial ADP/ATP carrier	1
AT3G09440	heat shock cognate 70 kDa protein 3 (HSC70-3) (HSP70-3)	1
AT3G09820	salvage synthesis of adenylates and methyl recycling	1
AT3G10710	pectinesterase family protein	1
AT3G10720	pectinesterase, putative	1
AT3G11710	ATKRS-1 (A. Thaliana Lysys-TRNA Synthetase 1)	1
AT3G11820	SYP121 (SYNTAXIN OF PLANTS 121); receptor/ protein anchor	1
AT3G12260	complex 1 family protein / LVR family protein	1
AT3G13310	DNAJ heat shock N-terminal domain-containing protein	1
AT3G14010	CID4 (CTC-Interacting Domain 4)	1
AT3G16780	60S ribosomal protein L19	1
AT3G16810	APUM24 (Arabidopsis Pumilio 24); RNA binding / binding	1
AT3G17440	NOVEL PLANT SNARE 13	1
AT3G20720	unknown protein	1
AT3G21550	unknown protein	1

Genes involved in transcriptional regulation (bold).

Table 2. List of putative SCZ interactors identified from Y2H screening.

Gene	Description	#
AT3G22270	unknown protein	1
AT3G24170	ATGR1 Glutathione disulfide reductase	1
AT3G24570	peroxisomal membrane 22 kDa family protein	1
AT3G25585	AAPT2 (AminiAlcoholPhosphoTransferase)	1
AT3G25830	ATTPS-CIN (terpene synthase-like sequence-1,8-cineole)	1
AT3G26520	TIP2 (TONOPLAST INTRINSIC PROTEIN 2); water channel	1
AT3G28710	H+-transporting two-sector ATPase	1
AT3G42790	AL3 nuclear-localized PhD domain containing homeodomain	1
AT3G44330	unknown protein	1
AT3G44750	Encodes a histone deacetylase	1
AT3G45430	lectin protein kinase family protein	1
AT3G46010	Actin-depolymerizing factor (ADF)	1
AT3G48680	GCAL2 (Gamma Carbonic Anhydrase-Like2); acyltransferase	1
AT3G48990	AMP-dependent synthetase and ligase family protein	1
AT3G49910	60S ribosomal protein L26 (RPL26A)	1
AT3G50880	HhH-GPD base excision DNA repair family protein	1
AT3G52930	fructose-bisphosphate aldolase, putative	1
AT3G53110	LOS4 (Low expression of osmotically responsive genes 1)	1
AT3G53730	histone H4	1
AT3G54400	aspartyl protease family protein	1
AT3G54580	proline-rich extensin-like family protein	1
AT3G54760	dentin sialophosphoprotein-related	1
AT3G55010	PUR5 (phosphoribosylformylglycinamidine cyclo-ligase 5)	1
AT3G55230	disease resistance-responsive family protein	1
AT3G55460	SCL30 (SC35-like splicing factor 30); RNA binding	1
AT3G56340	40S ribosomal protein S26 (RPS26C	1
AT3G57000	nucleolar essential protein-related	1
AT3G58610	ketol-acid reductoisomerase	1
AT3G59480	pfkB-type carbohydrate kinase family protein	1
AT3G59920	ATGDI2 (RAB GDP DISSOCIATION INHIBITOR 2)	1
AT3G59970	MTHFR1 (METHYLENETETRAHYDROFOLATE REDUCTASE 1	1
AT3G60340	palmitoyl protein thioesterase family protein	1
AT3G60440	Unknown protein	1
AT3G60600	VAP27-1 (Vamp/synaptobrevin-Associated-Prot27-1)	1

Genes involved in transcriptional regulation (bold).

Table 2. List of putative SCZ interactors identified from Y2H screening.

Gene	Description	#
AT3G60750	transketolase, putative	1
AT3G60820	PBF1; peptidase/ threonine-type endopeptidase	1
AT3G61800	unknown protein	1
AT3G61930	unknown protein	1
AT3G62290	ATARFA1E (ADP-ribosylation factor A1E)	1
AT3G62650	unknown protein	1
AT3G62870	60S ribosomal protein L7A (RPL7aB)	1
AT4G01870	tolB protein-related	1
AT4G02890	Polyubiquitin gene containing 4 ubiquitin repeats	1
AT4G04830	SelR domain-containing protein;	1
AT4G05050	UBQ11 (UBIQUITIN 11); protein binding	1
AT4G08980	F-box family protein (FBW2)	1
AT4G09040	RNA recognition motif (RRM)-containing protein;	1
AT4G09160	SEC14 cytosolic factor protein	1
AT4G09800	RPS18C (S18 RIBOSOMAL PROTEIN)	1
AT4G11320	cysteine proteinase, putative	1
AT4G13330	unknown protein	1
AT4G13890	Embryo Sac Development Arrest37 (EDA36), enzymatic activity	1
AT4G14150	PAKRP1 (PHRAGMOPLAST-ASSOCIATED KINESIN-RELATED1)	1
AT4G14880	cytosolic isoform of cytosolic O-acetylserine(thiol)lyase	1
AT4G15070	DC1 domain-containing protein	1
AT4G16520	ATG8F (AUTOPHAGY 8F); microtubule binding	1
AT4G17350	phosphoinositide binding protein	1
AT4G17390	60S ribosomal protein L15 (RPL15B)	1
AT4G20960	diaminohydroxyphosphoribosylaminopyrimidine deaminase	1
AT4G22150	A. thaliana CDC48-interacting UBX-domain protein (PUX3)	1
AT4G22270	involved in regulation organ size development	1
AT4G23470	hydroxyproline-rich glycoprotein family protein	1
AT4G24190	SHD (SHEPHERD); ATP binding	1
AT4G24520	Encodes a cyp450 reductase	1
AT4G24830	arginosuccinate synthase family	1
AT4G25630	FIB2 (FIBRILLARIN 2)	1
AT4G25672	Upstream open reading frames (uORFs)	1
AT4G26940	galactosyltransferase family protein	1

Genes involved in transcriptional regulation (bold).

Table 2. List of putative SCZ interactors identified from Y2H screening.

Gene	Description	#
AT4G27130	eukaryotic translation initiation factor SUI1	1
AT4G30190	cation-transporting ATPases AHA2	1
AT4G31170	protein kinase family protein	1
AT4G34140	D111/G-patch domain-containing protein	1
AT4G37070	PLA IVA/PLP1; nutrient reservoir	1
AT4G37180	myb family transcription factor	1
AT4G38350	hedgehog receptor	1
AT4G38510	vacuolar ATP synthase subunit B, putative	1
AT4G38740	Encodes cytosolic cyclophilin ROC1.	1
AT5G02260	ATEXPA9 (ARABIDOPSIS THALIANA EXPANSIN A9)	1
AT5G02500	encodes a member of heat shock protein 70 family	1
AT5G04430	BTR1L (BINDING TO TOMV RNA 1L (LONG FORM))	1
AT5G05010	clathrin adaptor complexes medium subunit-related	1
AT5G05600	oxidoreductase, 2OG-Fe(II) oxygenase family protein	1
AT5G07090	40S ribosomal protein S4 (RPS4B)	1
AT5G08590	SNRK2.1 (SNF1-Related protein kinase 2.1)	1
AT5G11000	unknown protein;	1
AT5G11520	ASP3 (ASPARTATE AMINOTRANSFERASE 3)	1
AT5G11700	glycine-rich protein	1
AT5G11730	expressed protein	1
AT5G12860	dicarboxylate transporter 1 (DiT1)	1
AT5G13070	MSF1-like family protein	1
AT5G13420	transaldolase, putative	1
AT5G13520	peptidase M1 family protein	1
AT5G15650	Reversibly Glycosylated Polypeptide-2	1
AT5G16720	unknown protein	1
AT5G17620	unknown protein	1
AT5G17920	cytosolic cobalamin-independent methionine synthase	1
AT5G18380	40S ribosomal protein S16 (RPS16C)	1
AT5G18910	protein kinase family protein	1
AT5G19770	TUA3 (tubulin alpha-3)	1
AT5G20290	40S ribosomal protein S8 (RPS8A);	1
AT5G27540	similarity to GTPases, localized to mitochondrion	1
AT5G27850	60S ribosomal protein L18 (RPL18C)	1

Genes involved in transcriptional regulation (bold).

Table 2. List of putative SCZ interactors identified from Y2H screening.

Gene	Description	#
AT5G33320	CUE1 (CAB Underexpressed 1); transmembrane transporter	1
AT5G38640	eukaryotic translation initiation factor 2B family protein	1
AT5G38690	similar to unknown protein	1
AT5G39320	UDP-glucose 6-dehydrogenase, putative	1
AT5G39790	chloroplast localized protein with starch binding activity	1
AT5G40890	voltage-gated chloride channel	1
AT5G42590	CYP71A16; electron carrier/ heme-/ iron-/ oxygen binding	1
AT5G43350	Encodes an inorganic phosphate transporter Pht1;1	1
AT5G44340	TUB4 (tubulin beta-4 chain)	1
AT5G45428	CPuORF24 (Conserved peptide upstream ORF 24)	1
AT5G46430	60S ribosomal protein L32 (RPL32B)	1
AT5G47200	ATRAB1A small GTPase mediated signal transduction	1
AT5G47700	60S acidic ribosomal protein P1 (RPP1C)	1
AT5G47770	FPS1 (FARNESYL DIPHOSPHATE SYNTHASE 1)	1
AT5G52470	FIB1 (FIBRILLARIN 1); snoRNA binding	1
AT5G52840	NADH-ubiquinone oxidoreductase-related	1
AT5G53000	PP2A-associated protein	1
AT5G53250	ArabinoGalactan Protein 22 (AGP22), GPI anchored protein	1
AT5G53850	haloacid dehalogenase-like hydrolase family protein	1
AT5G54160	caffeic acid/5-hydroxyferulic acid O-methyltransferase	1
AT5G56760	ATSERAT1;1 (A. Thaliana Serine Acetyltransferase 1;1)	1
AT5G60790	ATGCN1 (A. thaliana general control non-repressible 1)	1
AT5G62580	binding	1
AT5G63980	Encodes a bifunctional protein bisphosphate	1
AT5G64100	peroxidase, putative	1
AT5G64120	Cell wall bound peroxidase induced by hypo-osmolarity	1
AT5G64260	EXORDIUM LIKE 2 (EXL2)	1
AT5G64940	ATATH13 (ABC2 homolog 13)	1
AT5G66950	unknown protein	1
ATMG00020	RRN26, MITOCHONDRIAL RIBOSOMAL RNA 26S	1

Genes involved in transcriptional regulation (bold).

SCZ possibly exerts its function through interacting with a transcription factor obtained from the Y2H screening. Therefore we decided to validate the interaction with all 11 genes involved in transcriptional regulation (Table 2, bold) in a directed Y2H assay. Clones isolated from the library for

each of these were independently retransformed into BD-SCZ yeast and tested for interaction by plating on -HLT and -HALT media. Interaction with a C3HC4-type RING-type Zinc finger protein with unknown function (ZF) encoded by At1g53190 (Kosarev, Mayer & Hardtke 2002) conferred growth on –HLT media. Transformation with clone encoding FLX (At2G30120) conferred growth on both –HLT and –HALT (Figure 1D). *ZF* expression is not reported in literature and the gene is not present in public expression databases (e.g. eFP browser) since it is not represented on the Affymetrix ATH1 GeneChip. Consequently we lack information on its precise expression in the root meristem. FLX is important for flowering as a component of the FRIGIDA (FRI)-complex, which activates the transcription of FLOWERING LOCUS C (FLC) (Choi et al. 2011, Andersson et al. 2008). Public gene expression databases indicate that FLX is not expressed in any root tissue ((Brady et al. 2007) & eFP browser). However, a study focused on FLX puts forward that FLX is expressed in the root meristem (Andersson et al. 2008) and high *FLX* gene expression was found in shoot and root apical meristems and vascular tissues (Choi et al. 2011). We investigated FLX expression in root in more detail using the gFLX::FLX:GFP;*flx* line and detected weak expression in the meristematic epidermis which fades when the cells mature (Figure 2A).

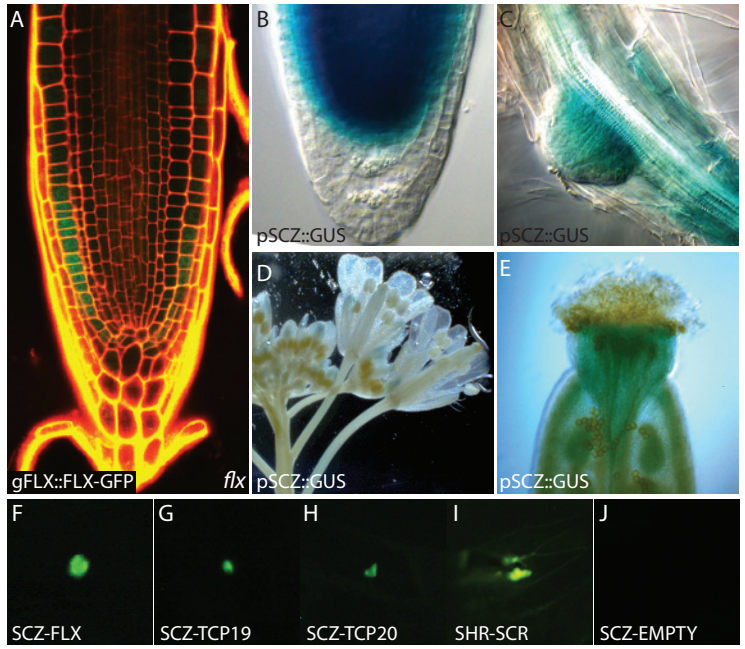

Figure 2. In planta analysis of SCZ interactors.

(A) *pFLX::FLX:GFP* expression in 5-day-old *flx* root. (B-E) *pSCZ::GUS* expression in root meristem (B), lateral root emergence (C) and flower/carpel (D,E) ((E) is a blow up of (D)). (F-J) BiFC in onion epidermal cell nuclei of SCZ-FLX (F), SCZ-TCP19 (G) and SCZ-TCP20 (H). SHR-SCR is taken as a positive control (I). SCZ-EMPTY is taken as a negative control (J).

This expression pattern does not overlap with that of *SCZ* gene root expression. We checked *SCZ* expression in other parts of the plant, using *pSCZ::GUS* (ten Hove et al. 2010). *SCZ* was expressed not only in meristematic vasculature, ground tissue and QC (Figure 2B), but also in emerging lateral roots (Figure 2C) and in carpels, just below the stigma (Figure 3 D,E). Also, *SCZ* was expressed in the meristemoid cells ((Pillitteri et al. 2011) & eFP browser)), whereas *FLX* was highly expressed in guard cells ((Andersson et al. 2008) & eFP browser)). This led us to hypothesize that SCZ is needed for the exclusion of FLX, in a spatial sense in the root meristem and in temporal fashion during stomatal development.

The *scz-2* mutation confers a frame shift at amino acid 307, leading to a stop codon 32 amino acids into the altered reading frame and an apparently dysfunctional protein (ten Hove et al. 2010). This indicates that the C-terminal end, amino acid 307-349, of the SCZ protein is essential for its function. This region harbors both repressor domains, the NLS and NES sites; four putative domains through which SCZ may bind other proteins. We investigated the importance of this region for the interaction with the identified partners, using a truncated version of SCZ lacking of the C-terminal 128 amino acids, designated SCZΔR (Figure 1E). Interestingly, ZF, TCP19 and TCP20 no longer interact with SCZΔR, suggesting that these proteins interact with SCZ through the C-terminal region of the SCZ protein. On the contrary, FLX is still able to strongly interact with SCZΔR (Table 1) indicating FLX binds SCZ within the first 220 amino acids. To substantiate the interactions found in Y2H assays we used the Bimolecular Fluorescent Complementation (BiFC) assay targeted in onion epidermal cells. In BiFC, enhanced Yellow Fluorescence Protein (eYFP, Clontech Laboratories) is split into an N-terminal domain of 154 amino acids (YFP/N) and a C-terminal domain of 84 amino acids (YFP/C). Each protein to be tested, is fused in frame to one of these BiFC probes. If two proteins are able to physically interact, their interaction brings together the two fragments of YFP resulting in fluorescence. SCZ CDS was fused to YFP/N and FLX, TCP19 and TCP20 were fused to YFP/C. After introducing the constructs pairwise into onion epidermal cells, we observed YFP fluorescence complementation for SCZ with FLX (Figure 2F), SCZ with TCP19 (Figure 2G) and SCZ with TCP20 (Figure 2H). SHR-SCR was taken as a positive control (Figure 2I), SCZ-empty vector serves as a negative control (Figure 2J). Using BiFC we were able to confirm the interactions found by Y2H indicating that SCZ efficiently binds its partners in the nucleus of plant cells.

The effect of the identified interaction partners can be studied by looking at their corresponding mutant phenotypes. To see the effect of absence of FLX on roots, we used a *flx* null allele (Andersson et al. 2008) to analyze single mutant and *scz;flx* double mutant roots. However, *flx* single mutant roots appear wild-type (Figure 3A) and double mutant *scz;flx* roots resemble *scz-2* roots both in organization (Figure 3B) and root length (not shown). Apparently the *flx* mutation does not have an additive effect to the *scz-2* mutant phenotype. Finally, we analyzed roots of transheterozygote seedlings. Heterozygous *scz* mutants appear wild type, therefore lowered levels of FLX and SCZ and thus of protein complex might have a subtle effect on the phenotype.

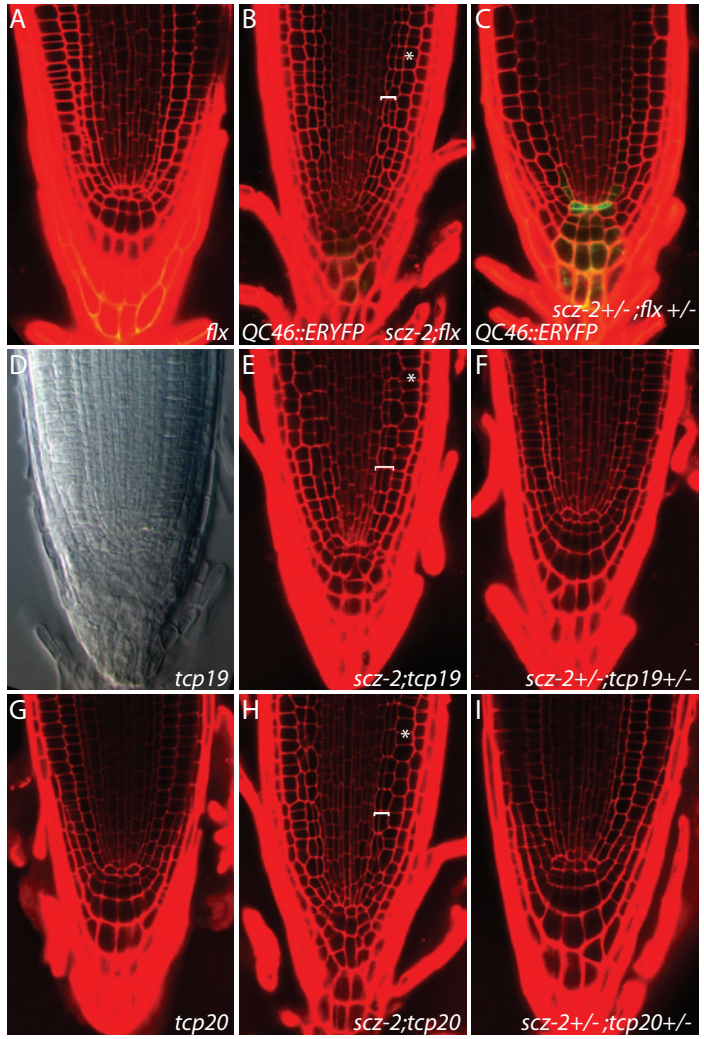

Figure 3. *flx*, *tcp20* and *tcp19* mutant analyses.

(A-C) 5-Day-old *flx* single mutant (A), *scz-2;flx* double mutant (B) and *scz-2;flx* transheterozygote (C) roots. (D-F) 5 Day old *tcp19* single mutant (D), *scz-2;tcp19* double mutant (E) and *scz-2;tcp19* transheterozygote (F) roots. (G-I) 5-Day-old *tcp20* single mutant (G), *scz-2;tcp20* double mutant (H) and *scz-2;tcp20* transheterozygote (I) roots. Square bracket indicates *scz* typical ectopic endodermal layers, asterisk indicates *scz* mutant subepidermal layer.

However, roots of F1 seedlings of a *scz-2 x flx* cross resemble wild-type (Figure 3C), showing that even low levels of SCZ-FLX are sufficient to sustain wild-type phenotype. A similar analysis was done with *tcp19* and *tcp20* single mutants, *scz-2;tcp19* and *scz-2;tcp20* double mutants and the corresponding transheterozygote seedlings. For *tcp19* an inducible knockdown line was generated, in which transcript is down regulated to 44% compared to wild-type levels (not shown). *tcp19* and *tcp20* single

mutant roots appear wild-type (Figure 3D,G). Both double mutant *scz-2;tcp19* and *scz-2;tcp20* roots resemble *scz-2* roots in organization (Figure 3 E,H, hash/asterisk) and growth (not shown). Roots of *scz-2;tcp19* and *scz-2;tcp20* transheterozygote resemble wild-type (Figure 3 F,I). Thus, neither TCP19 nor TCP20 alone affect root meristem organization and knock down of the genes does not cause an additional effect on *scz-2* phenotype. Recently, TCP20 was found to interact with both SCR and PLT proteins in planta (A.Shimotohno, RH and BS manuscript in prep). As described above, SCR and SCZ do not physically interact, but they do genetically interact ((Pernas, Ryan & Dolan 2010) & Chapter 3)). Hypothetically, TCP20 can form the physical link between SCZ and SCR, facilitating their protein interaction by acting as a chaperone protein.

DISCUSSION

We chose to conduct a Y2H screen using a root cDNA library in order to find proteins interacting with SCZ. If the percentage of interactors we have confirmed in directed Y2H is characteristic for the success rate in our library screen, it means that 80 percent of the identified candidate genes are false positives. This seems to be a general issue in Y2H large scale screens (Causier, Davies 2002b). Some studies show that the reliability of high-throughput Y2H assays is about 50% or lower (Sprinzak, Sattath & Margalit 2003). One explanation for finding false positives is that frequently yeast clones harbor multiple target plasmids. The plasmid isolated is not involved in the actual protein-protein interaction responsible for growth on selective media (Sprinzak, Sattath & Margalit 2003). Also, it is known that using a higher number of selection markers will give a lower percentage false positives. The minimum number selection markers to be used is two, HIS and ADE (ProQuest System). Once confirmed in yeast, interactions might not occur in situ because in context of the organism proteins are not expressed in the same cells. Nevertheless, Y2H can aid to assign a putative function to uncharacterized proteins. For improved reliability the data obtained by different screening procedures (e.g. Y2H, BiFC and co-IP) should be integrated in the biological network around the protein of interest. Here, we conducted a BiFC assay targeted in onion epidermal cells and show that SCZ-FLX, SCZ-TCP19 and SCZ-TCP20 interactions also occur in the nucleus of plant cells. We confirmed SCZ-ZF interaction in Y2H, however at the time of this study a knockout line for ZF was not available from public seed collection databases. Therefore, ZF was not considered for further study.

From our Y2H screen a total of 325 putative interaction partners of SCZ was found. Some proteins were identified multiple times as interactors from the screen. Generally, the incidence per interactor is seen as an indication for strength of interaction. In our screen, FLX was obtained from 8 independent yeast transformants. In directed Y2H SCZ-FLX interaction was shown to be very strong, supporting this hypothesis. FLX still interacts with SCZΔR, meaning that FLX must bind within the first 220 amino acids of SCZ sequence. Potentially, SCZ-FLX interact through the HR-A/B oligomerization

domain, that has been shown to mediate protein complex formation between HSFs. For example, HSFA1A dissociates from Hsp90 and Hsp70 through its oligomerization domain and activates transcription of stress related genes (Nover et al. 2001, Baniwal et al. 2007, Åkerfelt, Morimoto & Sistonen 2010, Miller, Mittler 2006). Further investigation to the exact binding location of FLX to SCZ, should reveal if the two interact via SCZs oligomerization domain. SCZ-FLX complex could be formed in the root meristem and execute its function there. However, in the double mutant combination no additional effect was seen. There are multiple ways to explain this. First, the severity of *scz-2* mutant phenotype might mask any additional effect of *flx* mutation. It is imaginable that SCZ is the core of a protein complex responsible for the strong phenotypes observed in the mutant and that removing just one of the secondary proteins from the remaining crippled complex, has no additional effect. Also, it is possible that, in absence of FLX other structurally related proteins take over their function. This option can be checked by testing multiple knock-outs with for example members of the same transcription factor family. Second, it is possible that the functional SCZ-FLX interaction takes place in a domain other than that of SCZ expression in the root. We have not yet analyzed phenotypes in those domains, but we know from expression studies that both SCZ and FLX are present outside the root. Expression of SCZ and FLX does overlap in carpels, leaving open a possibility for SCZ and FLX to interact there. The *flx* mutant flowers earlier than wild-type, indicating the gene plays a role in flowering time (Andersson et al. 2008). This is shown to correlate with a loss of FLC expression and a lowered expression of two FLC paralogs, MADS AFFECTING FLOWERING 1 (MAF1) and MAF2, genes shown to be repressors of flowering (Ratcliffe et al. 2001, Scortecci, Michaels & Amasino 2001). It was found that FLX is a component the FRI-complex. Through this complex the transcription of FLC is activated and thereby flowering is repressed (Choi et al. 2011). Furthermore, (Choi et al. 2011) describe high FLX protein expression in shoot and root apical meristems and vascular tissues. Here, we show that FLX resides in the root epidermis, where *SCZ* is not expressed. Although *SCZ* expression outside of the ground tissue cannot complement its mutant phenotype (ten Hove et al. 2010), it cannot be ruled out that SCZ protein moves outside its expression domain and interacts with FLX in the epidermis. SCZ and FLX proteins could interact in a complex which targets protein degradation. Also, *SCZ* was found to be expressed in the meristemoid cells (Pillitteri et al. 2011) and *FLX* is expressed highly in guard cells (eFP browser). Potentially, the SCZ-FLX complex has a role in stomatal development. Judging from the mutually exclusive *SCZ* and *FLX* expression patterns it is tempting to speculate that SCZ is essential for degradation of FLX protein or vice versa. Such a complex which targets protein degradation, is also seen in Skp, Cullin, F-box containing (SFC) complexes. These multiprotein ubiquitin ligase complexes catalyze the ubiquitination of proteins and mark them for destruction (Morgan 2007). The target specific component of such complexes is an F-box family protein. For example, SCF- Transport Inhibitor Response 1 (TIR1) is such an F-box family protein. TIR1 is found to interact with AUX/IAA proteins and is herewith responsible for the turnover of AUX/IAA proteins. The regulated degradation of AUX/IAA proteins is essential for expression or repression of downstream auxin inducible genes (Gray et al. 2001). Notably, amongst the putative SCZ

interactors there is one F-box family protein, FBW2 (Table 2, AT4G08980), leaving open a possibility for SCZ to interact with FLX in an SFC complex and be marked for destruction both in space in the root epidermis and in temporal fashion during stomatal development. Of course, first interaction of SCZ and FBW2 needs to be confirmed. If the two interact, analysis of corresponding mutant plants could shed more light on SCZ-FLX interaction possibly in a SCF complex. Also, if reasoning is true, in *scz-2* mutant background, FLX protein should be detected ectopically in SCZ expression domain, for example in the cortex. From microarray analysis we do not see a change in FLX expression (Chapter 2), although transcript levels may be too low, which is supported by the absence of root FLX expression as judged by online expression databases (eFP browser). Furthermore, in vivo BiFC, with SCZ and FLX expressed from their own promoter, may reveal SCZ-FLX complex formation in root tissues. However, it should be considered that possibly the SCZ-FLX complex is quickly degraded *in situ* and therefore not traceable in such a system.

From a directed Y2H we found physical interaction of SCZ with TCP19 and TCP20 in yeast cells. We were able to confirm these interactions in onion epidermal cells. We showed that interaction of SCZ with TCP19 and TCP20 must occur through the C-terminal part of the protein in yeast, potentially through the R/KLFGV type or LxLxL type EAR repressor domain. Other plant proteins are also shown to physically interact through their repressor domain. For example, IAA12/BDL belongs to the AUX/IAA family of transcriptional repressors and its N-terminal region contains a LxLxL type EAR motif. The transcription factor TOPLESS (TPL) physically interacts with IAA12/BDL through this EAR motif (Szemenyei, Hannon & Long 2008). Furthermore, Novel Interactor of JAZ (NINJA), which contains a LxLxL type EAR motif, acts as a transcriptional repressor in jasmonic acid responses. Also, NINJA physically interacts with TPL through this EAR repression motif (Pauwels et al. 2010). To investigate the exact binding location of TCP19 and TCP20 to SCZ, further studies, focussing on binding potential of more detailed regions in the C-terminal end of SCZ protein should be performed. Possibly, SCZ-TCP19 and SCZ-TCP20 complexes are formed in the root meristem. TCP20 was previously shown to bind type II DNA motifs present in the promoter of genes involved in cell cycle regulation (Miller, Mittler 2006). *TCP20* transcript is expressed ubiquitously in the embryo, whereas in the root, it is localized to the vascular tissues, the root cap, and the endodermis of the elongation zone (Åkerfelt, Morimoto & Sistonen 2010). *TCP19* is expressed specifically in the QC and in mature xylem (Brady et al. 2007). Here, we find that TCP19 and TCP20 localize throughout the root meristem. These differences might be because we looked at a protein fusion line, instead of transcriptional expression patterns. However, neither of the single mutants display a phenotype, nor does knocking out *TCP19* or *TCP20* in the *scz-2* mutant background enhance *scz-2* phenotype. Like we have indicated for FLX, also here this might be due to the severity of *scz-2* mutant phenotype masking any additional effect. Alternatively, it might be because SCZ-TCP19 and SCZ-TCP20 interaction takes place in a different domain. Regarding TCP20 we come up with a different explanation. In QC, TCP20 physically interacts in (*in vivo*) BiFC assays with SCR and PLT proteins (Shimothono & Heidstra unpublished).

Thus, TCP20 connects the PLT and the SCR/SHR pathway through protein complex formation. *SCZ* and *SCR* genetically interact, as double mutant *scz;scr* roots display a very severe phenotype that can be traced back to heart stage embryos where QC and root fail to be properly initiated (Chapter 3 & (Pernas, Ryan & Dolan 2010)). However, we could not show direct protein interaction (Table 1). Instead, SCZ can also physically interact with TCP20. Therefore, we speculate that TCP20 acts as a chaperone, linking PLT, SHR/SCR and SCZ in their job to initiate and maintain QC. An additional BiFC of SCZ with SCR and/or PLT in presence of TCP20 might reveal whether SCZ can form complexes with SCR and PLT via TCP20. And if one QC protein complex is formed or several. Finally, an *in vivo* BiFC assay, in which SCZ, SCR, PLTs and TCP are expressed from their own promoter, may validate protein complex formation in the root, possibly in the QC. If SCZ forms a complex with SCR facilitated by TCP20, a role for FLX could be to degrade the entire complex and keep SCZ-TCP20-SCR complex out of the epidermis. With this, FLX would limit SCZ and SCR action to the ground tissue, patterning cortex and endodermis.

In conclusion, we find interaction of SCZ with transcription factors FLX, TCP19 and TCP20 in Y2H and in plant nuclei. We hypothesize that SCZ acts to mark FLX protein for degradation at specific places in the root or at specific moments in development in complex with F-Box protein FBW2. This scenario would fit with SCZs role as a cell fate segregator. We provide evidence supporting a potential link of SCZ with both SCR and PLT proteins through TCP20, possibly in QC. Our ideas serve a starting point for further study and provide clues that aid to unravel the biological working mechanism of SCZ and its relation to other patterning factors.

EXPERIMENTAL PROCEDURES

Yeast two hybrid assay (Y2H)

Y2H interactions were studied according to (Welch et al. 2007) using the ProQuest Two Hybrid System (Invitrogen Life Technologies) and yeast strain Pj694A (James, Halladay & Craig 1996). Pj694A yeast carries mutations in *TRP*, *LEU*, *HIS* and *URA* or *ADE* genes required for amino acid biosynthesis. Pj694A cannot grow if these amino acids are dropped from the medium. Plasmids that are used in Y2H assay carry genes complementing these mutations, therefore allowing for selection of transformant yeast with interacting proteins. The GAL4 transcriptional activator is split into two fragments: the binding domain (BD), bound to an upstream activating sequence (UAS) and the activation domain (AD), needed for activation of transcription. In Y2H, proteins to be tested are fused to either BD or AD domain. If the two proteins interact, AD and BD come together, resulting into activation of the reporter gene (Causier, Davies 2002a). The prey encoding cDNAs were fused to the yeast GAL4 DNA-binding domain (BD) of pDEST32 that carries a TRP gene for selection in yeast. The BD-SCZ plasmid was transformed into yeast strain PJ694A where it binds to the upstream activating sequences (UAS) of the HIS and ADE loci (James, Halladay & Craig 1996). Since, *HIS* reporter gene can be leaky

in some yeast strains, a low concentration (2mM) of 3-Amino-1,2,4-Triazole (3-AT) was be added to the media. 3-AT reduces background growth because it is a competitive inhibitor of the *HIS3* gene product (Causier, Davies 2002a, Gietz et al. 1997). To test for transcriptional auto-activation by the BD-SCZ fusion protein, we plated yeast containing only the bait construct on medium lacking auxotrophic markers.

Library screen

The Y2H root library was cloned using the pDEST22 destination vector that harbors the GAL4 transcriptional activation domain (AD) and carries a LEU gene for selection in yeast. Plasmid DNA from positive colonies was extracted as described in (Li, Li & Sheen 2010) and transformed in *E.coli* DH5α. Plasmid DNA was then isolated from *E.coli* according to (Li et al, 2010) and sequenced on the Macrogen Platform using M13F, M13R, PD22F and PD22R primers (Supplementary Table 1). Alternatively, PCR was performed on positive yeast colonies and PCR products were sequenced. Sequencing reactions were analyzed with BlastN software.

Directed Y2H

The coding sequences of *SCZ* and *SCZΔR* were amplified and cloned into both the pDEST32-BD and pDEST22-AD vectors. *PLT1, PLT2, SCR, SHR, WOX5, RBR, JKD, TCP19, TCP20 en TCP21* coding sequences were amplified from whole seedling cDNA and cloned into pDEST22-AD vectors. An empty prey vector was used as a negative control (EMPTY). Primers used to amplify full-length CDS and deletion fragments are described in Supplementary Table 1.

Bimolecular fluorescence complementation assay (BiFC)

Gateway compatible pARC233 (N-terminal domain of 154 amino acids (YFP/N)) and pARC234 (C-terminal domain of 84 amino acids (YFP/C)) vectors (Tonaco et al. January 2006) were used for a BiFC assay as described in (Walter et al. 2004). *SCZ, FLX, TCP19, TCP20*, *SCR*, and *SHR* CDS were inserted to generate N-terminal split-YFP fusions. Primers used to amplify cDNA and deletion fragments are described in Supplementary Table 1. Gold particles (1,0 µm diameter, BioRad) were coated with 5µL (1µg/mL) plasmid DNA and bombarded into onion epidermal cells, using a helium-driven particle accelerator (PDS-100/He; BioRad) at 1100 psi rupture disc bursting pressure. The bombarded tissue was incubated in darkness for 10-12h. YFP fluorescence was recorded using a Leica binocular microscope with YFP filter.

Plant growth conditions and materials

Seedling were sterilized, plated and grown as described (Sabatini et al. 2003, Scheres et al. 1995). Mutant alleles used were *flx* and *flx*;pFLX::FLX:GFP (Andersson et al. 2008), *scz-2* (ten Hove et al. 2010), *tcp20* (SALK016203). Primers used for genotyping *tcp20* were SALK016203_LP, SALK016203_RP and LBb1.3. For *flx* T-DNA insertion was sequenced according to (Andersson et al. 2008). *scz-2* mutation was identified according to (ten Hove et al. 2010). TCP translational fusions (pTCP::gTCP:YFP) were constructed with Multisite Gateway (Invitrogen), by PCR amplifying the promoters (~3 kb upstream translation start site) and genes from Col-0 genomic DNA using primers pTCP19F/R, gTCP19F/R, pTCP20F/R and gTCP20F/R. Fragments were cloned into pGII0125 (Heidstra, Welch & Scheres 2004), with the promoter in the first box, the gene in the second box and a YFP coding sequence in the third box (fused to the 3'-end of the genomic fragment). Transformation was performed on Columbia ecotype plants according to the floral dip method (Clough, Bent 1998). Knockdown of TCP19 was achieved by creating a tcp19 artificial microRNA (ami) line (http://wmd3.weigelworld.org/cgi-bin/webapp.cgi). pG1090XVE::amiTCP19::nosT was made by pG1090XVE promoter in the first box, Ami19 in the second box and the nosT terminator in the third box of pGII0125 (Multisite Gateway). Independent transformants T2 lines were grown on media with and without 25uM b-estradiol and RNA was isolated. Lines were and tested for TCP19 transcript levels by qRT-PCR, using primers QPCRTCP19F/R. All primers sequences are found in Supplementary Table 1. Line 19.1 (used in this study) has 44% of transcript levels compared to non-induced/ wild-type. Transformation was performed on Col-0 ecotype plants according to the floral dip method (Clough, Bent 1998).

Microscopy

Light microscopy and β-glucoronidase activity was done as described (Welch et al. 2007, Willemsen et al. 1998, Bougourd, Marrison & Haseloff 2000). For confocal microscopy roots were mounted in propidium iodide (PI; 20 µg/mL in distilled water) (Berg et al. 1995) and examined with a Zeiss LSM710.

ACKNOWLEDGEMENTS

We would like to thank Carol R. Andersson and Elisabeth S. Dennis from CSIRO Plant Industry, Canberra, Australia, for kindly providing us with *flx* and *flx;FLXFLXGFP* seeds.

CHAPTER 4 SUPPLEMENTARY INFORMATION

Supplemental Table 1. Primer sequences

Primer	Sequence
cloning	
SCZFattb1	GGGGACAAGTTTGTACAAAAAAGCAGGCTTGatggcgatgatggtcgag
SCZF2attB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTtttgctcgtgatcttcttcct
SCZR2attB2noR	GGGGACCACTTTGTACAAGAAAGCTGGGTtgtctttctcatattactctgatcatg
FLXattB1	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCGGAGAAGATTATCGGAGA
FLXattB2	GGGGACCACTTTGTACAAGAAAGCTGGGTAAAGTAAAACCGCAATCTTATCA
35s mini	ttctactgcagcaagacccttcctctatat
pTCP19F	GGGGACAACTTTGTATAGAAAAGTTGggtcttagatccgaccactactttcac
pTCP19R	GGGGACTGCTTTTTTGTACAAACTTGttaccacaaaaccctagaatatctgaa
gTCP19F	GGGGACAAGTTTGTACAAAAAAGCAGGCTGGatggaatcgaatcacgaagg
gTCP19R	GGGGACCACTTTGTACAAGAAAGCTGGGTGagtctcatgacatgaagacggag
pTCP20F	GGGGACAACTTTGTATAGAAAAGTTGtttgcaggtgcagtagcatc
pTCP20R	GGGGACTGCTTTTTTGTACAAACTTGaagctggttctgattctgactcttc
gTCP20F	GGGGACAAGTTTGTACAAAAAAGCAGGCTGGatggatcccaagaacctaaatcg
gTCP20R	GGGGACCACTTTGTACAAGAAAGCTGGGTGacgacctgagccttgagaatc
sequencing	
M13F(-20)	GTAAAACGACGGCCAGT
M13R(-20)	GCGGATAACAATTTCACACAGG
PD22F	CGGTCCGAACCTCATAACAACTC
PD22R	AGCCGACAACCTTGATTGGAGAC
genotyping	
SALK016203_LP	AAATTTCTTGAACCCACCACC
SALK016203_RP	CCACCTGCAAGTTCAAACAAC
LBb1.3	ATTTTGCCGATTTCGGAAC
qRT-PCR	
QPCR TCP19F	AAAGAAACGGAGGAAGAGAAAC
QPCR TCP19R	TGAGCCACCCAAAAACTGAA

Chapter 5

Strategies for genetic dissection of *SCHIZORIZA* cell fate separation roles

Jansweijer VMA[1,2], van Liere S[1,2], Scheres B[1,2], Heidstra R[1,2]

[1] Department of Biology, section Molecular Genetics, Utrecht University, 3584 CH Utrecht, The Netherlands.
[2] Current affiliation: Plant Developmental Biology, Wageningen University Research, 6708 PB Wageningen, The Netherlands.

"L'Inverno"

ABSTRACT

The *SCHIZORIZA* (*SCZ*) transcription factor is crucial for cellular patterning of the *Arabidopsis* root. *SCZ* action determines fate cell autonomously in ground tissue and acts non-cell autonomously to separate QC/columella and epidermis/lateral root cap fates. Here we test a forward genetics approach to uncover functional targets of *SCZ* in different regions of the root meristem. We describe two suppression phenotypes of *scz-2* that uncouple two different fate separation events. One line restores predominantly columella tissue defects, whereas the other partially restores radial tissue organization. These findings support the idea that *SCZ* acts through different targets in order to establish its diverse roles in fate separation in the root meristem. However, both suppression events are rare and do not segregate in Mendelian fashion. We discuss how *scz* suppressor screening should be adapted for higher efficiency.

INTRODUCTION

In plants, orientation and asymmetry of divisions is of major importance in generating developmental pattern, since cells cannot migrate because of their shared cell walls (ten Hove, Heidstra 2008, Abrash, Bergmann 2009, Paciorek, Bergmann 2010). The *Arabidopsis* root acquires its organization through a fixed pattern of asymmetric cell divisions of particular stem cells (or initials) resulting in distinct organs and tissues. The formation of lateral roots starts with asymmetric cell divisions of two pericycle cells. These founder cells form the core of a lateral root primordium. Ultimately, the lateral root primordium starts to resemble the primary root tip (Petricka, Winter & Benfey 2012, Malamy, Benfey 1997)(Malamy, Benfey 1997). In the tip of the mature primary root, the quiescent center (QC) consists of a small group of rarely dividing cells, which is surrounded by the stem cells that make up the tissues of the root. The ground tissue (cortex and endodermis) share a cortex/endodermis initial. Asymmetric division of the cortex endodermis initial daughter is directed by *SHORTROOT* (*SHR)* and *SCARECROW (SCR)* transcription factors (Benfey et al. 1993, Di Laurenzio et al. 1996, Helariutta et al. 2000). Furthermore, epidermis and lateral root cap share their initial and two plant specific class NAM, ATAF1/2, and CUC2 (NAC) domain transcription factors *FEZ* and *SOMBRERO* (*SMB*) are instructive for its asymmetric cell division (Willemsen et al. 2008). Laser ablation studies have shown that the QC is crucial for maintenance of the surrounding stem cells (Berg et al. 1995, Scheres et al. 1997). Distal to the QC, the columella stem cells reside. Their daughter cells differentiate into columella cells, which contain starch and are able to sense gravity. *PLETHORA* (*PLT*), *SHR* and *SCR* transcription factors together are required for QC fate specification and maintenance (Aida et al. 2004, Galinha et al. 2007, Sabatini et al. 2003). The (*SCZ*) transcription factor is identified as a novel actor in cell fate separation in plants, specifically relevant for asymmetric cell divisions in stem cell areas (ten Hove et al. 2010, Pernas, Ryan & Dolan 2010). Although *SCZ* is not needed to set up QC fate during embryogenesis, it was found that *SCZ* is crucial for the maintenance of QC identity post-germination (ten Hove et al. 2010, Pernas, Ryan & Dolan 2010). Defects in *scz* lead to roots which grow indeterminate, but always lag behind in length compared to wild-type (ten Hove et al. 2010, Pernas, Ryan & Dolan 2010). In *scz* mutant roots, cells at the position of QC and columella are morphologically abnormal and accumulate starch granules which normally mark only differentiated columella cells. QC markers are displaced and expressed diffusely in the starch containing cells indicating *SCZ* acts to separate QC and columella fate. *SCZ* is also required for cortex fate specification. The first defect described in *scz* mutants is a premature periclinal division of the ground tissue initial in heart stage embryos. Subsequently, ectopic ground tissue layers are formed of which the outermost layer fails to adopt proper cortex fate judged by absence of cortex markers and ectopic root hair initiation (ten Hove et al. 2010, Pernas, Ryan & Dolan 2010, Mylona et al. 2002). Furthermore, *SCZ* functions to correctly separate epidermis and lateral root cap fate (ten Hove et al. 2010, Pernas, Ryan & Dolan 2010).

Fate separation defects in *scz-2* are associated with compromised stem cell activity. Like *SCZ*, also *WUSCHEL RELATED HOMEOBOX5 (WOX5)* is expressed in the QC as early as the first QC founder cells are established during embryogenesis (Sarkar et al. 2007). *WOX5* is required for the maintenance of QC function specifically with respect to columella stem cells. *WOX5* overexpression results in increased columella stem cell activity and increased columella stem cell numbers (Sarkar et al. 2007). A mildly enhanced columella stem cell activity also occurs in the knockout of *SMB* (Willemsen et al. 2008). Both *scz-2;35S::WOX5* and *scz-2;smb-3* double mutant roots show restored QC morphology/function visualized by QC marker expression and reoccurrence of columella stem cells, although radial patterning and root growth defects remain (ten Hove et al. 2010). These observations suggest the possibility to dissect the *scz* mutant phenotype through second site mutagenesis. The notion that *SCZ* acts both cell autonomously to specify cortical cell identity and non-cell autonomously to separate cell fates in surrounding layers (ten Hove et al. 2010), also suggests *SCZ* may also act through separate molecular mechanisms. In order to unravel cell type specific effects and mechanisms behind *SCZ* action we set up an EMS mutagenesis screen in the *scz-2* mutant background to identify modifiers of the mutant phenotype. We find two different genetic constitutions that each suppress distinct aspects of the *scz* phenotype, enforcing the idea that *SCZ* is implicated in different asymmetric cell divisions the *Arabidopsis* root.

RESULTS

Mutagenesis screen for suppression of *scz-2*

As one component of the *scz* phenotype comprises the fate separation defects in QC/columella region, we chose to use a QC marker (*QC46::$_{ER}$YFP*) in *scz* mutant background to find additional specific modifiers for the control of QC maintenance and QC/columella fate segregation. *QC46::$_{ER}$YFP* marker in *scz-2* mutant background no longer shows its wild-type peak expression. Instead it is broadly expressed in the starch granule containing columella cells (ten Hove et al. 2010) (Figure 1, 2B). We applied two screening criteria, restoration of root length and reversion of the *QC46::$_{ER}$YFP* marker expression to wild-type-like expression (Figure 1).

Theoretically, by screening less than 5000 plants from an EMS mutagenized M1 generation it is possible to find a mutation in any given gene (Greene et al. 2003). Here, roughly 50.000 EMS mutagenized *scz-2;QC46::$_{ER}$YFP* seeds were divided into 274 pools and sown on soil. Each pool thus ideally consist of seeds from ~182 M1 plants not considering seedling lethality, e.g. due to mutation or poor germination. From the progeny of each pool, approximately 150 M2 seeds were screened on plate for suppression of *scz-2* root length defect and restored expression of the *QC46::$_{ER}$YFP* marker. For each recessive single-gene modifier, more than 8 seedlings would be found in the total M2 screening (150/182*50.000/5000= 8,3). We did find *monopteros* (*mp*) mutants which are recognized by their rootless phenotype (Berleth, Jurgens 1993) in 6 distinct pools. Together with an albino

frequency of 3,5% amongst all screened seedlings, this suggest that mutagenesis has occurred with albeit low, but sufficient penetrance (Alonso, Ecker 2006, Page, Grossniklaus 2002).

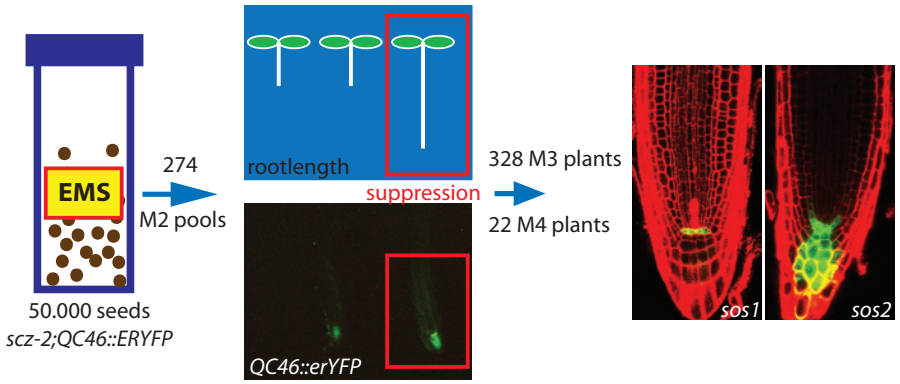

Figure 1. EMS mutagenesis screen.
Schematic representation of the EMS mutagenesis screen done on *scz-2;QC46::$_{ER}$YFP* seeds.

In the M2 generation we selected 328 putative *scz-2* suppression phenotypes. For only two of these, the phenotype was confirmed in homozygous M3 and M4 lines. We will refer to these lines as 'suppression of *scz 1*' (*sos1*) and *sos2* (Figure 1, 2C,D). For detailed phenotypic analysis of the two suppression phenotypes *sos1* and *sos2* seeds from the M4 generation with homozygous *scz-2* background were used. We confirmed that both *sos1* and *sos2* still harbor the *scz-2* mutation and found no additional intragenic mutations in the *SCZ* genomic sequence from -223 bp to +1800 bp relative to start codon (data not shown). The F1 progeny of *sos1* crossed to *sos2* (both still in *scz-2* background) does not show suppression of *scz-2* phenotype (not shown) demonstrating that these suppressors are not allelic. The *smb-3* mutation is able to suppress *scz-2* QC defects (ten Hove et al. 2010). Therefore we tested if *sos1* or *sos2* were allelic to *smb*. In the F1 of *sos1* and *sos2* crossed to *scz-2;smb-3* we found no suppression of *scz-2* phenotype (not shown), demonstrating that *sos1* and *sos2* are not allelic to *smb*. The fact that we do not find a suppression phenotype resembling the one caused by the *smb* mutant illustrates that our screening for suppression of *scz-2* needs to be optimized.

sos1 and *sos2* do not correspond to single genetic loci

To identify mutations corresponding to the suppression phenotype, we initiated a backcross population of *sos1* and *sos2* (both still in *scz-2* genetic background) crossed to *scz-2* to clean up the genetic load of mutations in each suppressor line. If suppression was caused by mutations in a single locus, we expect to find suppression of *scz-2* phenotype in 25% of the F2 progeny. If mutations in two or three separate loci together are responsible, there should be *scz-2* phenotype suppression

in 6,25% and 1,5% of the F2 progeny, respectively. In our hands neither of the two lines yielded F2 progeny in which the suppression phenotype reappeared (not shown). Therefore, either multiple loci are mutated in each suppressor of *scz-2*, or the suppression is caused by epigenetic changes of a complex nature.

sos1 and *sos2* suppress different aspects of *scz* phenotype

We imaged *scz-2*, *sos1* and *sos2* mutant roots at different days post germination (dpg) using confocal microscopy to analyze the progression of phenotypes in time. At 3dpg we observe differences in *QC46::$_{ER}$YFP* marker expression and cell morphology. In both *sos1* and *sos2* lines, marker expression is more focused to the QC and not spread through the whole QC/columella area as in *scz-2,* at 5dpg, this distinction remains (Figure 2 A-G). Cells in QC/columella region of *sos1* roots display a more regular morphology, and QC cells can be distinguished (Figure 2C,F white arrowhead). Strikingly, in *sos2* roots the radial organization is partially restored (Figure 2D,G), ectopic divisions giving rise to a double endodermis layer (Figure 2D) occur less frequent than in *scz-2*. However, the QC/columella region remains irregular. When roots grow older (11dpg) the differences between *scz-2*, *sos1* and *sos2* remain (Figure 2H-J).

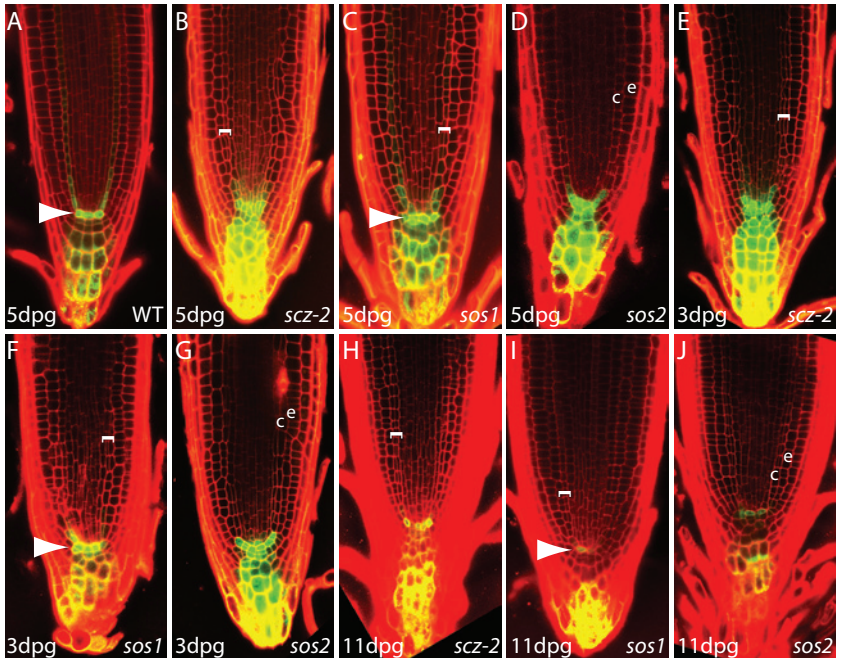

Figure 2. *sos1* and *sos2* suppress distinct aspects of *scz-2* phenotype.

(A-D) *QC46::$_{ER}$YFP* marker expression in wild-type (A), *scz-2* (B), *sos1* (C) and *sos2* (D) roots (5dpg). (E-G) *QC46::$_{ER}$YFP* in 3-Day old *scz-2* (E), *sos1* (F) and *sos2* (G) roots. (H-J) *QC46::$_{ER}$YFP* in 11-Day old *scz-2* (H), *sos1* (I) and *sos2* (J) roots. White arrowhead marks QC. Square bracket indicates *scz* ectopic endodermal layers, cortex (c), epidermis (e), days post germination (dpg).

To investigate if *sos1* and *sos2* suppressed the root growth defect of *scz-2*, we compared the root lengths of each suppressor to *scz-2* and wild-type. Similar to the *scz-2* single mutant, both *sos1* and *sos2* roots continued to grow indeterminate but lagged behind approximately half in growth compared to wild-type (Figure 3A,B). Furthermore, the subepidermal root hair initiation phenotype remains in *sos1* and *sos2* (not shown). *scz-2* shows no specific defects in shoot development. Similarly, *sos1* plants grow to a normal height and flowering is not affected (not shown). On the contrary, *sos2* plants lag behind in growth and remain smaller, reaching 10 cm in height approximately. In addition, *sos2* flowers generate fewer seeds and pollen formation is impaired (not shown).

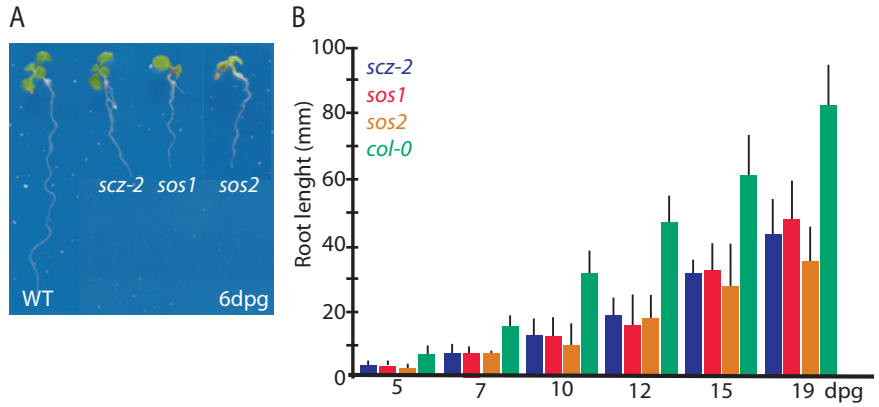

Figure 3. *sos1* and *sos2* do not restore decreased root growth.

(A) 6-Day-old wild-type, *scz-2*, *sos1* and *sos2* seedlings. (B) Root length measurements of *scz-2*, *sos1*, *sos2* and Col-0 roots at 5, 7, 10, 12, 15 and 19 days post germination (dpg).

Columella stem cell loss is restored in *sos1*

The morphology of cells in the stem cell niche and the restored focused expression of *QC46::$_{ER}$YFP* in the QC (Figure 2) suggested that the *scz-2* QC fate defect is restored by *sos1*. To determine whether the QC in *sos1* roots is functional and able to sustain surrounding stem cells we examined the presence of starch granules in columella tissue as a marker for differentiation in 5-day-old seedling roots. In wild-type there is a single layer of stem cells devoid of starch between QC and differentiated columella cells (Figure 4A) whereas in *scz-2* all cells in the QC/columella area contain starch (Figure 4B). In *sos1*, a QC can be appointed and a layer of columella stem cells is restored based on the absence of starch granules in cells just below the QC (Figure 4C). Similar analysis in *sos2*, reveals that all cells in the QC/columella in *sos2* roots contain starch granules (Figure 4D), indicating QC function and columella stem cells are not restored by *sos1*. Thus, the genetic constitution of *sos1* is able to specifically suppress the loss of columella stem cells of the *scz-2* mutant, whereas *sos2* cannot.

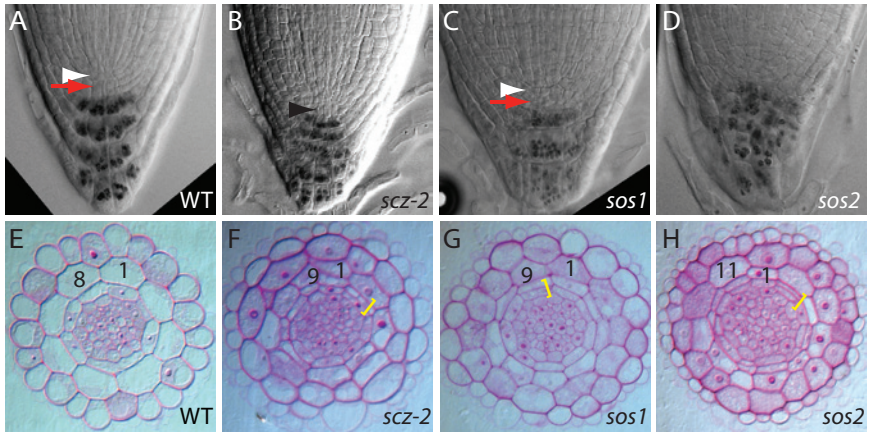

Figure 4. *sos1* rescues QC and *sos2* rescues radial patterning defects.

(A-D) Lugol stained wild-type (A), *scz-2* (B), *sos1* (C) and *sos2* (D) roots (5 dpg). White arrowhead marks QC, red arrow indicates columella stem cells devoid of starch granules. Black arrowhead points to accumulation of starch granules in mutant QC region. (E-H) Transverse histological sections of 5-day-old wild-type (E), *scz-2* (F), *sos1* (G) and *sos2* (H) roots. Number of subepidermal cells are indicated. Square yellow bracket indicates ectopic endodermal divisions.

Radial patterning defects are partially restored in *sos2*

From the longitudinal confocal images of *sos2* the *scz-2* radial patterning defects appear to be restored in around 50% of the roots analyzed. This indicates suppression is not fully penetrant. Alternatively, suppression may occur in particular cell files that can be out of the imaging focus plane at the time of analysis. To examine the suppression phenotype in more detail, we made transverse sections along the root longitudinal axis of Col-0, *scz-2, sos1* and *sos2*. Cross-sections of *scz-2* root meristems confirm that vascular tissues are normal, and a double layer of endodermis-like cells is present. Epidermal cell number is reduced from the normal ~22 cells to ~12 cells and the cells resemble the underlying cortex-like cells. An additional layer of lateral root cap-like cells surrounds the epidermis, see also (ten Hove et al. 2010) (Figure 4F). In cross-sections of *sos2* root meristems, on average only 2-3 out of 9 endodermis files of *sos2* roots have divided ectopically (n=4, Figure 4H), whereas in *scz-2* nearly all endodermis files are doubled. The duplicated endodermal cell files appear not to be correlated with a specific position in the root with respect to the vascular organization. The number of epidermis cells in *sos2* is increased to 18 cells (stdev= 1) compared to *scz-2* (12 cells, stdev= 1), also the cells are rounder, more resembling wild-type epidermis cells. However, a wild-type number of around 22 epidermis cells is never found. Wild-type roots typically show 8 cortex cells (Figure 4E). *scz-2* mutant roots lack a normal cortex layer, instead there is a mutant subepidermal layer below epidermis. The *scz-2* subepidermal layer consist of 10 cells on average (stdev= 1) (Figure 4F). The number of cells in the subepidermal layer in *sos2* also varies from 9 to 11 (Figure 4H). In agreement with the longitudinal images of *sos1* roots, the radial sections through the root meristem did not reveal any additional suppression phenotypes (Figure 4G). Summarizing, in *sos2* we see a partial rescue of the number of epidermis cells and reduced ectopic divisions in the ground tissue, whereas *sos1* does not rescue *scz-2* radial defects. Therefore, it seems that *sos2* represents an intermediate phenotype between *scz-2* and wild-type regarding *scz-2* radial patterning phenotype.

DISCUSSION

We describe a forward genetics pilot screen in attempt to isolate functional *SCZ* targets, that are responsible for different aspects of *scz-2* phenotype. Using EMS mutagenesis we found two distinct suppression phenotypes: *sos1* and *sos2*. We found that mutations underlying each suppression phenotype reside outside the *SCZ* gene. However, we failed to identify the loci related to suppression phenotypes, because suppression phenotypes that were stable upon inbreeding were not maintained in next generations upon outcrossing. Potentially the mutations causing underlying suppression phenotypes reside at multiple loci or are epigenetic in origin, or both. Even with two loci involved, the relative mild suppression phenotype may become undetectable as a quantitative trait upon segregation. During the course of screening M2 and M3 generations a suppressor resembling the *smb* mutant was not found. In a saturated screen it is expected to find multiple mutant alleles for each gene that gives a phenotype of interest when mutated. The fact that *smb* was not detected amongst our mutants, together with the fact that we found only one allele for each suppressor, indicates that the mutagenesis was not done to saturation. However, we did find a chlorophyll mutant occurrence of 3,5% and we were able to find multiple *mp* mutant seedlings indicating that the mutagenesis was conducted with albeit low, but sufficient penetrance (Alonso, Ecker 2006, Page, Grossniklaus 2002). In addition, it turned out to be challenging to see differences in expression of the *QC46::$_{ER}$YFP* marker in the M2 generation. The marker is not spread out evenly through the columella in all cases, but sometimes seems to focus slightly to QC in *scz-2* mutants. Even though in *scz-2* background *QC46::$_{ER}$YFP* expression never resembles wild-type completely, this may explain the low number of suppressors identified. A large number of M2 candidates selected with the aid of a fluorescence binocular in the initial screening, turned out to be false positives upon validation using confocal microscopy. A way to overcome this, is to start the initial screening step using confocal microscopy. However, screening will then become more labor intensive. Ideally, in a full screen focused on QC suppressors, a QC marker should be used in which a more obvious distinction can be made between *scz-2* and wild-type phenotype using a fluorescence binocular. For example, QC marker *WOX5* could be used. However, in *scz-2 WOX5* is still expressed in one or two cells at the QC region, although expression is not maintained when *scz-2* roots grow older (ten Hove et al. 2010). In addition, an EMS mutagenesis screen focused on restoration of the radial organization with markers for cortex and lateral root cap might reveal more suppressors of cortex specification and/or epidermis/lateral root cap segregation defects in *scz-2*. Furthermore, to unravel *SCZ* action in specific region in the root meristem, targets of *SCZ* identified in transcriptome analyses

may be combined with the *scz* mutation and analyzed for suppression. That this approach is feasible comes from the identification of *SMB* and *ZN7571* (*smb-3* and *zn7571* can suppress *scz* QC defects) as differentially regulated genes in wild-type and *scz-2* mutant roots (Chapter 2).

Interestingly, here we find two suppression phenotypes, each related to different aspects of *scz-2* phenotype. On the one hand, *sos1* restores *scz* QC/columella phenotypes specifically including the reappearance of columella stem cells that lack starch granules. On the other hand *sos2* partly restores *scz-2* radial patterning defects. Due to focal plane limitations with confocal imaging a suppression by *sos2* was not detected in all seedling roots. However, by looking at transverse sections we did find suppression of ectopic endodermal divisions in all *sos2* roots analyzed. In addition, in all roots suppression was observed of epidermal cell numbers to intermediate levels compared to wild-type.

Table 1. components *scz-2* phenotype are suppressed by different genetic constitutions.

	defects in columella	QC	cortex	endodermis	lateral root cap	epidermis
scz-2	+	+	+	+	+	+
sos1	-	-	+	+	+	+
sos2	+	+	-	-	-	-
scz-2;smb-3	-	-	+	+	+	+
scz-2;zn7571	-	-	+	+	+	+

(+) defects present, (-) defects suppressed.

The fact that different genetic constitutions can suppress different aspects of the *scz-2* phenotype like *smb* (ten Hove et al. 2010), *zn7571* (Chapter 2), *sos1*, and *sos2* (Table 1) indicates that the defects seen in the QC/columella area and the radial pattern disorganization can be uncoupled. This reinforces the notion that *SCZ* executes separable activities. Together, these results suggest that a specific combination of multiple gene products is needed to restore all aspects of the *scz-2* phenotype to wild-type. In future, it might be interesting to combine *sos1* with *sos2* to see if together they can rescue the *scz-2* phenotype to a greater extent. Both suppression phenotypes, seem to be related to a specialized *SCZ* defect in the root meristem, since neither *sos* rescued the *scz-2* root growth defect nor the root hair initiation pattern. Like *scz-2*, *sos1* does not affect shoot development. However, *sos2* plants do stay very small and give few seeds, possibly because pollen formation is affected. *sos2* could have an additional effect on shoot growth and flower development. However, at this point it cannot be ruled out that these phenotypes are not linked to the suppression. Although the exact genetic constitution of each suppression phenotype described here remains elusive, they do enforce our idea that *SCZ* influences the many different asymmetric cell division events in the root meristem via distinct targets.

EXPERIMENTAL PROCEDURES

Plant growth conditions and materials

Seedling and embryos were sterilized, plated and grown as described (Sabatini et al. 2003, Scheres et al. 1995). For each EMS M2 pool around 150 seeds were plated for analysis.

Mutagenesis

EMS mutagenesis was done as described in (Willemsen et al. 1998) on approximately 50.000 dry seeds of double homozygous double insertion *scz-2;QC46::ERYFP* (ten Hove et al. 2010) *Arabidopsis thaliana* ecotype Columbia-0 plants.

Sequencing

For sequencing *SCZ* genomic region the following primers were used: SCZF (gtagttacacatgagga-cagatacaca), SCZseq1 (gtgattacccatctcttaaagttcc), SCZseq2 (gaggttgttgttgttgttgcttctg), SCZseq3 (cagaccaaacaagcaaaacg) and SCZR (acgtgaatgcattaaataactgg).

Microscopy

Screening was done using a fluorescence binocular (Leica MZ16F) and confocal laser scanning microscope (Zeiss LSM701). Light microscopy, starch granule staining and measurement of root length was performed as described (Willemsen et al. 1998). For confocal microscopy, roots were prepared in propidium iodide (PI; 20 µg/mL) in distilled water according to (Berg et al. 1995). Histological sections of roots were prepared as described (Scheres et al. 1995). For each histological sample a number of 4 roots was sectioned.

Chapter 6

Summary and concluding remarks

SUMMARY AND CONCLUDING REMARKS

SCHIZORIZA (*SCZ*) defines an intriguing pathway for asymmetric cell division in plants. We have explored transcriptomics, genetic interaction and protein interaction studies and attempted second site mutagenesis to get a deeper understanding of the role *SCZ* plays in root patterning. In this chapter I will discuss the results obtained and highlight opportunities for future research.

Chapter 2 focusses on downstream effectors of *SCZ* obtained by transcriptional profiling of *scz* mutant versus wild-type roots. Interestingly, the distribution of obtained differentially expressed genes matches the proposed actions of *SCZ*. Comparison with the high-resolution spatiotemporal gene expression map (Brady et al. 2011), shows that down regulated genes are mainly expressed in cortex. Genes affected in *scz* can be classified into gene ontology categories such as 'response to abiotic stimulus', 'phototropism' and 'oxidation-reduction process'. In angiosperm plant roots, the cortex functions as a 'canal' for transport through the root. It can contain numerous intercellular spaces, essential for aeration of the root and cortex cells are in close contact with one another via plasmodesmata. Substances that move across the cortex may move via the plasmodesmata, via the cortex cell walls or both (Raven, Evert & Eichorn 1986, Leyser, Day 2009). Inside the cortex the endodermis resides, which lacks air spaces and contains Casparian strips (perpendicular to the root surface) that block passage of water through the endodermal cell walls. As a result, water and minerals that are taken up from the soil by root hairs, have to travel from the cortex, through the endodermal cytoplasm or through numerous plasmodesmata before reaching the vascular tissue. This way, the passages of water and minerals is regulated in the ground tissue and excessive water loss is prevented (Raven, Evert & Eichorn 1986, Leyser, Day 2009). The *SCZ* effectors we identified hint not only to a role in cortex specification but also a role for *SCZ* in cortex differentiation. On the other hand, genes up regulated in the mutant root are enriched for peripheral tissue expression, i.e. columella, lateral root cap and epidermis. The NAC genes *BEARSKIN1* (*BRN1)*, *BRN2*, *FEZ* and *SOMBRERO* (*SMB*), involved in root cap development (Willemsen et al. 2008, Bennett et al. 2010), are found in this gene set. Interestingly, whereas *BRN* gene expression is maintained longer in maturing lateral root cap cells, FEZ and SMB reporters cross tissue boundaries and both invade epidermis in *scz* mutant roots. The latter agrees with a function for *SCZ* in fate segregation. Repression of FEZ and SMB ectopic expression is not observed upon short inducible GR:SCZ rescue conditions in the *scz* mutant indicating *SCZ* may not act directly on their promoters.

In addition to the NAC genes, we identified three transcription factor genes, *ANTHOCYANINLESS2* (*ANL2*), *TUBBY LIKE PROTEIN8* (*TLP8*) and *ZN7571* with functions in root cap patterning, whose transcripts were up regulated in *scz* roots. Like FEZ and SMB, also *TLP8* and *ZN7571* expression is ectopic, invading more inner tissues of *scz* roots, enforcing the hypothesis that *SCZ* acts to segregate cell fates by confining gene expression patterns (Figure 1). In spite of regulation of *ANL2*, *TLP8* and *ZN7571* genes by *SCZ*, no statistically significant differential expression is accomplished upon short

inducible GR:SCZ rescue conditions, failing to link the regulation of those directly to *SCZ*. Possibly, the effect of SCZ on these target promoters requires more time e.g. by recruiting additional binding partners to effectively shut down expression in ectopically invaded tissue types. Alternatively, SCZ may not bind to their promoters and repression of these genes is accomplished via a mediating target or mechanism. *zn7571* mutant roots show a specific defect in columella stem cell maintenance. Such a defect is also seen in *wuschel related homeobox5* (*wox5*) mutants, and both *WOX5* and *ZN7571* were placed genetically downstream of *SHORTROOT/SCARECROW* (*SHR/SCR*) genes ((Sarkar et al. 2007) Chapter 3). However, whereas *WOX5* is expressed in the QC, *SCZ* acts to segregate *ZN7571* to the root cap (Figure 1). In such a scenario, *ZN75771* may act in a cell autonomous fashion to promote columella differentiation possibly downstream of and/or interfering with *WOX5* signaling from the QC. In agreement with this scenario, elimination of ectopic *ZN7571* expression in *scz;zn7571* double mutants restores QC function.

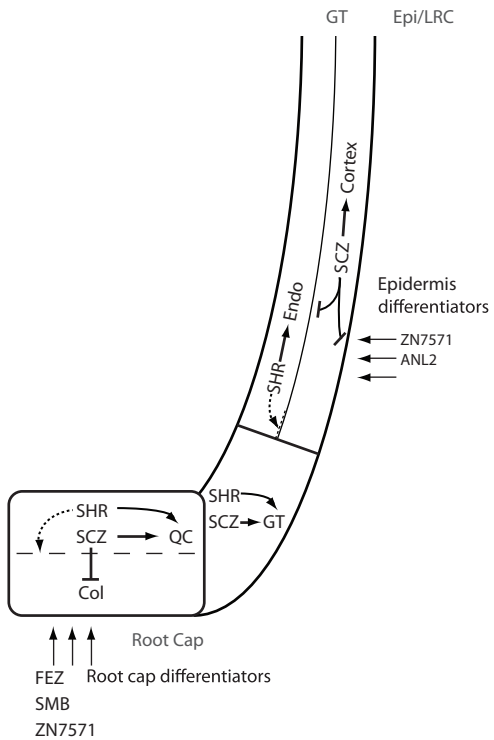

Figure 1. Schematic view on *SCZ* action in root meristem.

SCZ acts to determine cortex fate in the cortex ground tissue layer. Additionally, *SCZ* inhibits columella fate in QC. Herewith *SCZ* specifies both cortex and QC fate respectively. Ectopic (root cap/ epidermis) fate determinants are excluded from QC and ground tissue by *SCZ* action to confine their gene expression patterns. Ground tissue (GT), Lateral root cap (LRC), Epidermis (Epi), Endodermis (Endo). Arrow indicates induction of cell fate, dashed arrow indicates induction of cell division.

SCZ acts to specify cortex fate and to segregate QC and columella fate and epidermis and lateral root cap fate (Pernas, Ryan & Dolan 2010, ten Hove et al. 2010). Recently it was found that root cap layers are slowly replenished by the QC itself (Cruz et al. submitted). This places *SCZs* effect in columella in a somewhat different light, since it may comprise both a fate segregating (keeping out root cap fate from QC) and fate determining role from within the QC itself, acting together with SHR to specify QC (Figure 1). Nevertheless, the question remains how *SCZ* acts to segregate outer tissue fates in a non-cell autonomous fashion e.g. in epidermis/lateral root cap. Three scenarios come to mind. First, *SCZ* may specify cortex, and as a consequence spreading of fates across boundaries is prevented via ligand–receptor signalling. Such a scenario is reminiscent of the function of RECEPTOR-LIKE PROTEIN KINASE1 (RPK1) and TOADSTOOL2 (TOAD2) receptors in embryogenesis. Both RPK1 and TOAD2 receive intercellular signals and they mediate intracellular responses that are necessary for embryonic pattern formation (Nodine, Yadegari & Tax 2007). Tissue boundaries in the *Arabidopsis* root niche can indeed shift by excess CLAVATA3/ESR-RELATED (CLE) peptide application (Fiers et al. September 2005, Stahl et al. 2009). The CLE40 - CRINKLY4 (ACR4) - CLAVATA1 (CLV1) module acts from the columella and promotes columella differentiation (Stahl et al. 2009, De Smet et al. 2008, Stahl et al. 2013). *ZN7571* may be a member of such signalling pathway and it will be interesting to determine the relation of *ZN7571* with respect to the putative CLE40-ACR4-CLV1 signaling loop. Second, *SCZ* activity may regulate small RNAs (sRNA) emanating from the cortex to pattern and maintain surrounding tissue fates or invading neighbouring tissues. In analogy, endodermis-expressed microRNAs activated by *SHR/SCR* were shown to act non-cell-autonomously to repress vascular *PHABULOSA* mRNA levels that directs xylem development in a dosage dependent fashion (Carlsbecker et al. 2010). Third, *SCZ* mediated cortex specification may result in the induction of a transcription factor moving outward and controlling gene expression in surrounding tissues. Among the transcription factors identified here, candidates not examined in detail should be tested for protein movement. In this respect, SCZ may itself move to surrounding tissues. However, complementation tests in tissues other than cortex cannot rescue any aspect of the *scz* mutant phenotype. Thus, a more complicated scenario would be required possibly involving signals from the specified cortex that together with translocated SCZ maintain fate segregation in the surrounding tissues. Interesting in this respect is the observed direct activation of the cap gene *BRN1* upon *SCZ* induction (Chapter 2) whereas *SCZ* is not expressed in the root cap. The direct activation of *BRN1* by *SCZ* at least supports *SCZ* identity as a transcription factor, being able to transcriptionally regulate target genes in direct fashion. Comparing the localization of GFP-SCZ with the larger 3xGFP-SCZ protein fusion is a straightforward method to test SCZ movement.

Although the profiling described here delivered new factors involved in root development, to get closer to the function of *SCZ* with respect to downstream effectors in scenarios described above, short inducible rescue experiments are required. We have shown that GR:SCZ is functional and suitable for such experiments. Cell or tissue specific GR:SCZ induction experiments may even be

attempted to separate spatial responses in e.g. initial cortex specification and subsequent outer tissue fate segregation. Second, we need an unbiased platform to identify any size/type of transcript unlike the ATH1 microarrays (Affymetrics) we employed here. Rather, the AGRONOMICS1 microarray with the advantage of analysis using standard statistics (Rehrauer et al. February 2010) or RNAseq (Zheng et al. 2010) should be used. In depth investigation of relevant and direct *SCZ* target genes will aid to decipher its mechanism of action.

Chapter 3 addresses the relation of *SCZ* to the notorious root meristem initiation and maintenance transcription factor genes *SHR*, *SCR*, *PLETHORA1* (*PLT1*) and *PLT2*. Triple mutant *scz-2;plt1-4;plt2-2* roots resemble *plt1-4;plt2-2* double mutant roots, suggesting *SCZ* acts downstream. However, its expression pattern is only mildly affected with reduced peak expression of *SCZ* in *plt1-4;plt2-2* double mutant roots. Contrarily, *scz-2;shr-2* and *scz-2;scr-4* double mutants display a severely enhanced rootless phenotype compared to each single mutant that finds its origin in the aberrant development of the QC and surrounding stem cells from heart stage embryogenesis onward. Nevertheless, mutual expression patterns only become affected post-embryonically in mutant backgrounds. Therefore, *SCZ* cannot unambiguously be positioned in linear pathways of *PLT* and *SHR*/*SCR* action. Non-linearity of the *PLT* and *SHR*/*SCR* pathways is by now well established (Blilou et al. 2005, Cruz-Ramírez et al. 2012). Non-trivial genetic interactions, as seen between *SCZ*, *PLT* and *SHR*/*SCR*, often occur when proteins act together in complex (Collins et al. 2007). Although a direct physical link between SCZ and PLT or SHR/SCR proteins was not found, in **Chapter 4** we describe protein interaction between SCZ and TEOSINTE BRANCHED1/ CYCLOIDEA/ PROLIFERATING CELL NUCLEAR ANTIGEN FACTOR20 (TCP20). Recently, we obtained evidence that TCP20 connects SCR with PLT proteins via protein interaction in the QC (Shimotohno, Heidstra, Scheres manuscr in prep). This leads us to hypothesize that multiprotein complexes are formed in the QC. SCZ would be part of such complex, linked to SCR and PLT via TCP20 to execute their shared function in patterning and maintenance of the stem cell niche. Taking away single components of such a complex may have different effects depending on the genetic redundancy of the particular partner and the effect it may have on the integrity and stability of the complex (Collins et al. 2007). Together, this shared function suggests a set of target genes regulated by related protein complexes that specify the few cells that make up the niche during heart stage embryogenesis. The identification of specific target genes is challenging given the inability to culture specific plant (stem) cells in vitro, in contrast to animal systems. Thus, we require sophisticated techniques, such as sorting of GFP marked cells, focused on the isolation of individual cell types for analysis. The fact that for most profiling studies and Chromatin Immune Precipitation (ChIP) one does not need whole cells but just nuclei, allows a combination of nuclear localized fluorescent transcription factor with sorting of nuclei from embryos (Borges et al. 2012) to be applied. Alternatively, in the INTACT (isolation of nuclei tagged in specific cell types) method the nucleus of the cell type of interest is labeled by expression of a biotinylated nuclear envelope protein. Consequently, the nuclei of interest can get affinity purified (Deal, Henikoff 2010). In both methods, a high yield and purity of nuclei from the desired cell types

can be obtained. Comparing expression profiles at stages just prior and after phenotypic demise should identify common downstream target genes important during embryogenic patterning of the niche. Alternatively, promoters for commonly regulated genes may be identified upon ChIP of SCZ, SHR/SCR and PLT protein-DNA complexes in root niche progenitors in heart stage embryos.

A mechanistic foundation to asymmetric cell division of the cortex/endodermis initial was recently described by (Cruz-Ramírez et al. 2012). RETINOBLASTOMA-RELATED (RBR) directly regulates asymmetric cell divisions in the cortex-endodermis initial daughter by interacting with the SHR/SCR pathway. Physical interaction between RBR and SCR suppresses asymmetric cell division, this is counteracted by CYCLIN D6;1 (CYCD6;1) which phosphorylates RBR and thus prevents SCR-RBR interaction. The balance of SCR-RBR interaction on the one hand and CYCD6;1 on the other defines the exact position of asymmetric cell divisions, generating a very robust bistable system. Additionally, SHR and auxin distribution restrict asymmetric division to the stem cell region (Cruz-Ramírez et al. 2012). Complex formation involving SCZ, SHR and SCR proteins mediated by TCP chaperones together with the specificity of the CYCD6;1 expression in the ground tissue initial may confine the role of separate complexes to the correct tissue. If and how SCZ influences this SCR-RBR-CYCD6 bistable ground tissue patterning system is not clear. Ground tissue divisions still occur in *scz* and are even ectopic creating double endodermis. Rather, proper ground tissue fate specification is hampered in *scz*, with inner layers accumulating SHR and SCR proteins suggestive of endodermis fate whereas the outer cortical layer lacks cortex fate markers.

SCZ lacks the canonical transcription activation domain of related heat shock transcription factors, consistent with absence of any transcriptional auto-activation in yeast (Chapter 4). Instead, the protein harbors two putative transcriptional repression domain at the C-terminus. However, until date, SCZs repressive activity is not confirmed (Guan et al. 2012). Therefore it is conceivable that *SCZ* acts as both repressor and activator depending on the type of cofactor that is recruited. Screening for the putative interaction partners by yeast two hybrid (Chapter 4) delivered one such putative cofactor: FLC EXPRESSOR (FLX). FLX has been shown to contain transcription activation activity (Choi et al. 2011), but the FLX protein localization is exclusive to that of the *SCZ* gene expression pattern. Complex formation thus would require translocation of SCZ to the epidermis to activate downstream events ensuring stable maintenance of tissue fates. Alternatively, a role for FLX could be to timely degrade SCZ protein complexes and with this limit SCZ (and SCR) action to the ground tissue, patterning cortex and endodermis. To find additional SCZ binding co-factors regulating transcription, directed yeast two hybrid using only transcription factors as bait should be performed, e.g. using the REGIA transcription factor ORF Library (Paz-Ares 2002). Phenotypic and transcriptomic analysis of mutant roots or tissues of such candidate cofactors may reveal their involvement in a complex with SCZ. It is imaginable that composition of such complexes highly depends on its localization, therefore dynamic in vivo localization studies should be used to determine complex formation in the root meristem.

The presumption that *SCZ* exerts its role via distinct mechanisms is strengthened in **Chapter 5** where we have tried to dissect different aspects of the *scz-2* phenotype by a classical forward genetics approach. We find two genetic constitutions that each rescue part of the *scz-2* phenotype. The genetic foundation to *suppression of scz1* (*sos1*) rescues *scz* QC/columella fate separation defects. Whereas *sos2* (partially) restores the *scz* radial fate separation defect in ground tissue and epidermis. However, neither suppression phenotype rescued *scz-2* impaired root growth, suggesting that a combined restoration of patterning defects is required or that *SCZ* influences growth via a different, as yet uncharacterized, mechanism. In Chapter 2 we identified *SMB* as a downstream effector of *SCZ* and we confirmed the previously observed suppression of the QC/columella aspect of *scz-2* phenotype in double mutant *scz;smb* roots (ten Hove et al. 2010). Also *zn7571* suppresses the *scz-2* QC/columella phenotype.

Factors and mechanisms underlying asymmetric cell division are likely to overlap between different regions of the plant. Although cell polarity events preceding an asymmetric cell division are still poorly understood, the cell needs to polarize in order to get an asymmetric outcome of the cell division. Recently, it was found that *PLT* transcription factors can direct reorientation of the cell division plane. They do so, by activating auxin signaling locally, resulting in increased *MICROTUBULE ASSOCIATED PROTEIN65* (*MAP65*) expression, which, in turn, guides localization of CLIP-ASSOCIATED PROTEINs (CLASPs) (Dhonukshe et al. 2012). CLASP is a microtubule cell cortex interaction mediator, that is able to precisely orient the cytoskeleton and, with that, cell division plane (Dhonukshe et al. 2012, Ambrose et al. 2011). This finding illustrates how a plant transcription factor can cause rearrangement of the cytoskeleton for division plane reorientation. It will be interesting to see if the described network can be connected to *SCZ* and to what extent it is disturbed in *scz* defective cell fate segregation events in the epidermis/lateral root cap. Although this connection might be modest, since in *scz* mutant periclinal divisions still occur (Pernas, Ryan & Dolan 2010, ten Hove et al. 2010). Interestingly, in a transcriptome analysis of stomatal development, *SCZ* was found to be transcriptionally enriched in mutant epidermis consisting of solely meristemoids (*scrmD;mute)*. Indeed, *SCZ::CFP* is specifically expressed in meristemoids during stomatal development (Pillitteri et al. 2011), notwithstanding no role has been ascribed to *SCZ* in stomatal development yet. It was shown that restriction of symplastic movement might be an essential step for the proper segregation of cell-fate determinants during stomatal development (Guseman et al. 2010). Support for such a scenario in roots comes from the observation that ACR4 and CLV1 receptors can interact at plasmodesmata, possibly directly regulating or restricting the movement of columella stemness determinants thereby confining stem cell identity to a single layer contacting the QC (Stahl et al. 2013). Investigation of plasmodesmata aperture in *scz* mutant roots could reveal if *SCZ* ensures the closure of lateral exits in peripheral tissues to prevent movement of fate determinants.

Since *SCZ* was identified as determinant of cortex fate and fate segregator for epidermis/lateral root cap and QC/columella (Pernas, Ryan & Dolan 2010, ten Hove et al. 2010), we were able to provide a more nuanced view on *SCZ* action. We identified effectors on which *SCZ* exerts its cell-autonomous and non-cell autonomous actions. The connection of *SCZ* with the *PLT* and *SHR*/*SCR* pathways, together with the identification of common co-factors, unveils protein complex formation as a potential mechanism of *SCZ* action. Finally, genetic analyses support the view that different *SCZ* functions during development can be dissected. Unravelling the different mechanistic aspects of *SCZ* function in autonomous control of cell specification and non-autonomous regulation of fate segregation should gain a deeper understanding of asymmetric division and plant development in general.

Chapter 7

Nederlandse samenvatting

NEDERLANDSE SAMENVATTING

Uit slechts één enkele cel (de zygote), ontwikkelen meercellige organismen een enorme variatie aan celtypen. Om deze verscheidenheid te kunnen bereiken maken planten en dieren beide gebruik van hetzelfde mechanisme: asymmetrische celdeling. In tegenstelling tot symmetrische celdelingen, waarbij twee equivalente dochtercellen ontstaan, resulteert een asymmetrische celdeling in twee verschillende dochtercellen (Horvitz, Herskowitz 1992).

De ontwikkeling van planten onderscheidt zich met name post-embryonaal van de animale ontwikkeling. Terwijl bij de animale ontwikkeling, alle organen en weefsels al gevormd worden tijdens embryogenese, worden tijdens de ontwikkeling van planten nieuwe weefsels en organen ook nog na de embryogenese nog gevormd. Een volwassen plantenembryo vertoont hierdoor nog weinig overeenkomsten met een post-embryonale plant (Steeves, Sussex 1989). In tegenstelling tot dieren, zijn planten sessiele organismen, die voorbestemd zijn tot een leven op een vaste plaats. Daarbij vormen planten voortdurend nieuwe organen en weefsels, hun hele leven lang. Ook animale cellen verschillen ten opzichte van plantencellen. Animale cellen kunnen bewegen naar de plaats van bestemming, maar plantencellen zijn immobiel, omdat de celwand die de celmembraan omringt hen fixeert tot een vaste plaats in de cellulaire matrix. Om toch de nodige flexibiliteit te bewerkstelligen hebben planten het vermogen ontwikkeld om te schakelen tussen cel identiteiten wanneer dat nodig is. Een mix van cel identiteiten binnen één cel, door een mutatie in één van de genetische factoren die deze identiteit bepaalt, komt daarom uitsluitend voor bij plantencellen (Costa, Shaw 2007). De verfijnde manier waarop planten asymmetrische celdelingen toepassen en het vermogen om deze asymmetrische celdelingen opnieuw te programmeren, hun hele leven lang, maakt het mogelijk voor planten om hun indrukwekkende bouwplan te verkrijgen.

Planten groeien vanuit verschillende groepen van delende cellen die zich bevinden in de meristemen. Binnen zo'n meristeem, zijn de stamcel niches van cruciaal belang voor de balans tussen zelf-vernieuwing van de stamcellen en differentiatie. Stamceldelingen in de wortel van de modelplant *Arabidopsis thaliana* zijn asymmetrisch. Elke stamceldeling genereert een nieuwe stamcel en een dochtercel, welke laatste vervolgens het traject van differentiatie ingaat. Het *Arabidopsis* wortelmeristeem is een mooi voorbeeld van het gebruik van asymmetrische celdeling om patronen en diversiteit te creëren. De fundamentele vragen welke ontwikkelingsbiologen bezighouden zijn; hoe de verschillende cellulaire uitkomsten van asymmetrische celdelingen worden bepaald; hoe een lichaamsplan tot stand komt en behouden blijft en welke factoren hierbij een cruciale rol spelen.

De transcriptiefactor *SCHIZORIZA* (*SCZ*) vertegenwoordigt een nieuw mechanisme om cel identiteiten te specificeren en van elkaar te scheiden. Het moleculaire mechanisme dat ten grondslag ligt aan de werking van *SCZ* is nog onbekend. Om de functie van *SCZ* verder te doorgronden is het van

belang dat er een antwoord wordt gevonden op vragen als; Via welke effectoren voert *SCZ* haar cel-autonome en niet-cel-autonome acties uit; Hoe verhoudt *SCZ* zicht tot de bekende wortel factoren *PLETHORA* (*PLT*) en *SHORTROOT/SCARECROW* (*SHR/SCR)*; Wat zijn mogelijke cofactoren die SCZ gebruikt; en kan het ingewikkelde fenotype van de *scz* mutant worden opgedeeld in verschillende functies van *SCZ*. Het onderzoek dat hier gepresenteerd wordt draagt bij aan een beter begrip van asymmetrische celdeling en segregatie van cel identiteiten door de verschillende aspecten van de functie van *SCZ* tijdens de ontwikkeling van de wortel te bestuderen.

Om effectoren van *SCZ* te vinden, is er een microarray analyse gedaan (Chapter 2). De targetgenen die gevonden zijn, versterken onze visie op de functie van *SCZ* in de specificatie van de cortex en de segregatie van cel identiteiten in de buitenste weefsels. De relatie tussen *SCZ* en de genen *FEZ*, *SOMBRERO* (*SMB)*, *BEARSKIN1* (*BRN1) en BRN2,* allen uit de genfamilie van de NAC-domein transcriptiefactoren, wordt onderzocht. Daarnaast wordt de functie beschreven van drie transcriptiefactoren, waarvan de rol in patroonvorming van het wortelmeristeem nog niet bekend was: *ANTHOCYANINLESS2* (*ANL2*), *TUBBY LIKE PROTEIN 8* (*TLP8*) en een C2H2-type Zinc-Finger transcriptiefactor; *ZN7571*. Een onderzoek naar de relatie tussen *SCZ* en de goed gekarakteriseerde transcriptiefactorgenen *SHR*, *SCR*, *PLT1* en *PLT2*, geeft nieuwe informatie over de taak van *SCZ* in het vormgeven van de stamcelniche (Chapter 3). De genetische interactie tussen *SCZ* en *SHR/ SCR* wordt bevestigd en analyses van de dubbelmutanten laten zien dat *SCZ* en *SHR/SCR* samen nodig zijn om de stamcelniche vorm te geven tijdens de embryogenese.

Drie eiwitten die een fysieke interactie met SCZ aan kunnen gaan worden beschreven (Chapter 4). Ten eerste laten eiwitinteractiestudies zien dat de TEOSINTE BRANCHED1/ CYCLOIDEA/ PROLIFERATING CELL NUCLEAR ANTIGEN FACTOR (TCP) transcriptiefactoren, TCP19 en TCP20 kunnen interacteren met SCZ. Daarnaast wordt een directe fysieke interactie tussen SCZ en de Leucine-Zipper transcriptiefactor FLC-expressor (FLX) vastgesteld. Naar aanleiding van de gevonden eiwitinteracties en nog niet gepubliceerde data welke interactie tussen TCP20 en SCR aantonen, stellen we de hypothese op dat TCP20 een fysieke link vormt in de interactie tussen SCZ en SCR. Mogelijke gebeurt dit in het 'quiescent center' (QC), waar al een sterke genetische interactie tussen de laatste twee genen gevonden is (Chapter 3). De eiwitinteractie tussen FLX en SCZ, leidt ons naar een speculatie over een mogelijk mechanisme van degradatie via welke SCZ cel identiteiten segregeert. De verschillende aspecten van het *scz* mutante fenotype worden van elkaar losgekoppeld via een klassieke 'forward genetics' benadering (Chapter 5). Twee verschillende genetische constituties worden beschreven welke twee verschillende aspecten van het *scz* fenotype onderdrukken. Deze constatering onderschrijft onze hypothese dat *SCZ* de verschillende asymmetrische celdelingen in het wortelmeristeem beïnvloedt via verschillende targets. Tenslotte worden de hoofdstukken samengevat en bediscussieerd en een aantal mogelijke vervolgstudies wordt aangestipt (Chapter 6).

Chapter 8

References

Abe, M., Katsumata, H., Komeda, Y. & Takahashi, T. 2003, "Regulation of shoot epidermal cell differentiation by a pair of homeodomain proteins in Arabidopsis", *Development,* vol. 130, no. 4, pp. 635-643.

Abe, M., Takahashi, T. & Komeda, Y. 1999, "Cloning and characterization of an L1 layer-specific gene in Arabidopsis thaliana", *Plant and cell physiology,* vol. 40, no. 6, pp. 571-580.

Abrash, E.B. & Bergmann, D.C. 2009, "Asymmetric cell divisions: a view from plant development", *Developmental cell,* vol. 16, no. 6, pp. 783-796.

Agarwal, P., Kapoor, S. & Tyagi, A.K. 2011, "Transcription factors regulating the progression of monocot and dicot seed development", *BioEssays,* vol. 33, no. 3, pp. 189-202.

Aichinger, E., Kornet, N., Friedrich, T. & Laux, T. 2012, "Plant stem cell niches", *Annual Review of Plant Biology,* vol. 63, pp. 615-636.

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

Åkerfelt, M., Morimoto, R.I. & Sistonen, L. 2010, "Heat shock factors: integrators of cell stress, development and lifespan", *Nature reviews Molecular cell biology,* vol. 11, no. 8, pp. 545-555.

Alonso, J.M. & Ecker, J.R. 2006, "Moving forward in reverse: genetic technologies to enable genome-wide phenomic screens in Arabidopsis", *Nature Reviews Genetics,* vol. 7, no. 7, pp. 524-536.

Ambrose, C., Allard, J.F., Cytrynbaum, E.N. & Wasteneys, G.O. 2011, "A CLASP-modulated cell edge barrier mechanism drives cell-wide cortical microtubule organization in Arabidopsis", *Nature communications,* vol. 2, pp. 430.

Andersson, C.R., Helliwell, C.A., Bagnall, D.J., Hughes, T.P., Finnegan, E.J., Peacock, W.J. & Dennis, E.S. 2008, "The FLX gene of Arabidopsis is required for FRI-dependent activation of FLC expression", *Plant and cell physiology,* vol. 49, no. 2, pp. 191-200.

Arabidopsis Interactome Mapping Consortium 2011, "Evidence for Network Evolution in an Arabidopsis Interactome Map", *Science,* vol. 333, no. 6042, pp. 601-607.

Austin, R.S., Vidaurre, D., Stamatiou, G., Breit, R., Provart, N.J., Bonetta, D., Zhang, J., Fung, P., Gong, Y., Wang, P.W., McCourt, P. & Guttman, D.S. 2011, "Next-generation mapping of Arabidopsis genes", *The Plant Journal,* vol. 67, no. 4, pp. 715-725.

Balazadeh, S., Riaño-Pachón, D. & Mueller-Roeber, B. 2008, "Transcription factors regulating leaf senescence in Arabidopsis thaliana", *Plant Biology,* vol. 10, pp. 63-75.

Baniwal, S.K., Chan, K.Y., Scharf, K. & Nover, L. 2007, "Role of Heat Stress Transcription Factor HsfA5 as Specific Repressor of HsfA4", *Journal of Biological Chemistry,* vol. 282, no. 6, pp. 3605-3613.

Begum, T., Reuter, R. & Schöffl, F. 2013, "Overexpression of AtHsfB4 induces specific effects on root development of Arabidopsis", *Mechanisms of development,* vol. 130, no. 1, pp. 54-60.

Benfey, P.N., Linstead, P.J., Roberts, K., Schiefelbein, J.W., Hauser, M.T. & Aeschbacher, R.A. 1993, "Root development in Arabidopsis: four mutants with dramatically altered root morphogenesis", *Development,* vol. 119, no. 1, pp. 57-70.

Benfey, P.N. & Scheres, B. 2000, "Root development", *Current Biology,* vol. 10, pp. 813-815.

Benjamini, Y. & Hochberg, Y. 1995, "Controlling the false discovery rate: a practical and powerful approach to multiple testing", *Journal of the Royal Statistical Society.Series B (Methodological),* pp. 289-300.

Bennett, T., van den Toorn, A., Sanchez-Perez, G.F., Campilho, A., Willemsen, V., Snel, B. & Scheres, B. 2010, "SOMBRERO, BEARSKIN1, and BEARSKIN2 regulate root cap maturation in Arabidopsis", *The Plant Cell Online,* vol. 22, no. 3, pp. 640-654.

Berg, C.v.d., Willemsen, V., Hage, W., Weisbeek, P. & Scheres, B.J.G. 1995, "Cell fate in the Arabidopsis root meristem determined by directional signalling", *Nature,* vol. 378, no. 6552, pp. 62.

Berleth, T. & Jurgens, G. 1993, "The role of the monopteros gene in organising the basal body region of the Arabidopsis embryo", *Development,* vol. 118, no. 2, pp. 575-587.

Birnbaum, K., Shasha, D.E., Wang, J.Y., Jung, J.W., Lambert, G.M., Galbraith, D.W. & Benfey, P.N. 2003, "A Gene Expression Map of the Arabidopsis Root", *Science,* vol. 302, no. 5652, pp. 1956-1960.

Blilou, I., Xu, J., Wildwater, M., Willemsen, V.A., Paponov, I., Friml, J., Heidstra, R., Aida, M., Palme, K. & Scheres, B.J.G. 2005, "The PIN auxin efflux facilitator network controls growth and patterning in Arabidopsis roots", pp. 39-44.

Boggon, T.J., Shan, W.S., Santagata, S., Myers, S.C. & Shapiro, L. 1999, "Implication of tubby proteins as transcription factors by structure-based functional analysis", *Science,* vol. 286, no. 5447, pp. 2119-2125.

Borges, F., Gardner, R., Lopes, T., Calarco, J.P., Boavida, L.C., Slotkin, R.K., Martienssen, R.A. & Becker, J.D. 2012, "FACS-based purification of Arabidopsis microspores, sperm cells and vegetative nuclei", *Plant Methods,* vol. 8, no. 1, pp. 44.

Bougourd, S., Marrison, J. & Haseloff, J. 2000, "An aniline blue staining procedure for confocal microscopy and 3D imaging of normal and perturbed cellular phenotypes in mature Arabidopsis embryos", *The Plant Journal,* vol. 24, no. 4, pp. 543-550.

Bowman, J.L., Smyth, D.R. & Meyerowitz, E.M. 1989, "Genes directing flower development in Arabidopsis.", *The Plant Cell Online,* vol. 1, no. 1, pp. 37-52.

Brady, S.M., Zhang, L., Megraw, M., Martinez, N.J., Jiang, E., Yi, C.S., Liu, W., Zeng, A., Taylor-Teeples, M. & Kim, D. 2011, "A stele-enriched gene regulatory network in the Arabidopsis root", *Molecular systems biology,* vol. 7, no. 1.

Brady, S.M., Orlando, D.A., Lee, J., Wang, J.Y., Koch, J., Dinneny, J.R., Mace, D., Ohler, U. & Benfey, P.N. 2007, "A High-Resolution Root Spatiotemporal Map Reveals Dominant Expression Patterns", *Science,* vol. 318, no. 5851, pp. 801-806.

Brand, U., Fletcher, J.C., Hobe, M., Meyerowitz, E.M. & Simon, R. 2000, "Dependence of stem cell fate in Arabidopsis on a feedback loop regulated by CLV3 activity", *Science Signalling,* vol. 289, no. 5479, pp. 617.

Breuninger, H., Rikirsch, E., Hermann, M., Ueda, M. & Laux, T. 2008, "Differential Expression of WOX Genes Mediates Apical-Basal Axis Formation in the Arabidopsis Embryo", *Developmental cell,* vol. 14, no. 6, pp. 867-876.

Bryksin, A.V. & Matsumura, I. 2010, "Overlap extension PCR cloning: a simple and reliable way to create recombinant plasmids", *BioTechniques,* vol. 48, no. 6, pp. 463.

Busch, W., Miotk, A., Ariel, F.D., Zhao, Z., Forner, J., Daum, G., Suzaki, T., Schuster, C., Schultheiss, S.J. & Leibfried, A. 2010, "Transcriptional control of a plant stem cell niche", *Developmental cell,* vol. 18, no. 5, pp. 841-853.

Cai, M., Qiu, D., Yuan, T., Ding, X., Li, H., Duan, L., Xu, C., Li, X. & Wang, S. 2007, "Identification of novel pathogen-responsive cis-elements and their binding proteins in the promoter of OsWRKY13, a gene regulating rice disease resistance", *Plant, Cell & Environment,* vol. 31, no. 1, pp. 86-96.

Carlsbecker, A., Lee, J.Y., Roberts, C.J., Dettmer, J., Lehesranta, S., Zhou, J., Lindgren, O., Moreno-Risueno, M.A., Vatén, A. & Thitamadee, S. 2010, "Cell signalling by microRNA165/6 directs gene dose-dependent root cell fate", *Nature,* vol. 465, no. 7296, pp. 316-321.

Casamitjana-Martinez, E. 2003, "Receptor kinase signaling in Arabidopsis root meristem maintenance", *Department of Developmental Genetics.Volume PhD dissertation*.

Causier, B. & Davies, B. 2002, "Analysing protein-protein interactions with the yeast two-hybrid system", *Plant Molecular Biology,* vol. 50, no. 6, pp. 855-870.

Causier, B., Ashworth, M., Guo, W. & Davies, B. 2012, "The TOPLESS interactome: a framework for gene repression in Arabidopsis", *Plant Physiology,* vol. 158, no. 1, pp. 423-438.

Choi, K., Kim, J., Hwang, H., Kim, S., Park, C., Kim, S.Y. & Lee, I. 2011, "The FRIGIDA Complex Activates Transcription of FLC, a Strong Flowering Repressor in Arabidopsis, by Recruiting Chromatin Modification Factors", *The Plant Cell Online,* vol. 23, no. 1, pp. 289-303.

Clark, S.E., Jacobsen, S.E., Levin, J.Z. & Meyerowitz, E.M. 1996, "The CLAVATA and SHOOT MERISTEMLESS loci competitively regulate meristem activity in Arabidopsis", *Development,* vol. 122, no. 5, pp. 1567-1575.

Clark, S.E., Running, M.P. & Meyerowitz, E.M. 1993, "CLAVATA1, a regulator of meristem and flower development in Arabidopsis", *Development,* vol. 119, no. 2, pp. 397-418.

Clough, S.J. & Bent, A.F. 1998, "Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana", *The Plant Journal,* vol. 16, no. 6, pp. 735-743.

Cole, M., Chandler, J., Weijers, D., Jacobs, B., Comelli, P. & Werr, W. 2009, "DORNRÖSCHEN is a direct target of the auxin response factor MONOPTEROS in the Arabidopsis embryo", *Development,* vol. 136, no. 10, pp. 1643-1651.

Collins, S.R., Miller, K.M., Maas, N.L., Roguev, A., Fillingham, J., Chu, C.S., Schuldiner, M., Gebbia, M., Recht, J. & Shales, M. 2007, "Functional dissection of protein complexes involved in yeast chromosome biology using a genetic interaction map", *Nature,* vol. 446, no. 7137, pp. 806-810.

Costa, S. & Shaw, P. 2007, "'Open minded cells: how cells can change fate", *Trends in cell biology,* vol. 17, no. 3, pp. 101-106.

Cruz-Ramírez, A., Díaz-Triviño, S., Blilou, I., Grieneisen, V.A., Sozzani, R., Zamioudis, C., Miskolczi, P., Nieuwland, J., Benjamins, R. & Dhonukshe, P. 2012, "A Bistable Circuit Involving SCARECROW-RETINOBLASTOMA Integrates Cues to Inform Asymmetric Stem Cell Division", *Cell,* vol. 150, no. 5, pp. 1002-1015.

Cui, H. & Benfey, P.N. 2009, "Interplay between SCARECROW, GA and LIKE HETEROCHROMATIN PROTEIN 1 in ground tissue patterning in the Arabidopsis root", *The Plant Journal,* vol. 58, no. 6, pp. 1016-1027.

Cui, H., Levesque, M.P., Vernoux, T., Jung, J.W., Paquette, A.J., Gallagher, K.L., Wang, J.Y., Blilou, I., Scheres, B. & Benfey, P.N. 2007, "An evolutionarily conserved mechanism delimiting SHR movement defines a single layer of endodermis in plants", *Science Signalling,* vol. 316, no. 5823, pp. 421.

De Smet, I., Vassileva, V., De Rybel, B., Levesque, M.P., Grunewald, W., Van Damme, D., Van Noorden, G., Naudts, M., Van Isterdael, G. & De Clercq, R. 2008, "Receptor-like kinase ACR4 restricts formative cell divisions in the Arabidopsis root", *Science Signalling,* vol. 322, no. 5901, pp. 594.

Deal, R.B. & Henikoff, S. 2010, "A simple method for gene expression and chromatin profiling of individual cell types within a tissue", *Developmental cell,* vol. 18, no. 6, pp. 1030-1040.

Dharmasiri, N., Dharmasiri, S., Weijers, D., Lechner, E., Yamada, M., Hobbie, L., Ehrismann, J.S., Jürgens, G. & Estelle, M. 2005, "Plant development is regulated by a family of auxin receptor F box proteins", *Developmental cell,* vol. 9, no. 1, pp. 109-119.

Dhonukshe, P., Weits, D., Cruz-Ramirez, A., Deinum, E., Tindemans, S., Kakar, K., Prasad, K., Mähönen, A., Ambrose, C., Sasabe, M., Wachsmann, G., Luijten, M., Bennett, T., Machida, Y., Heidstra, R., Wasteneys, G., Mulder, B. & Scheres, B. 2012, "A PLETHORA-Auxin Transcription Module Controls Cell Division Plane Rotation through MAP65 and CLASP", *Cell,* vol. 149, no. 2, pp. 383-396.

Di Cristina, M., Sessa, G., Dolan, L., Linstead, P., Baima, S., Ruberti, I. & Morelli, G. 1996, "The Arabidopsis Athb-10 (GLABRA2) is an HD-Zip protein required for regulation of root hair development", *The Plant Journal,* vol. 10, no. 3, pp. 393-402.

Di Laurenzio, L., Wysocka-Diller, J., Malamy, J.E., Pysh, L., Helariutta, Y., Freshour, G., Hahn, M.G., Feldmann, K.A. & 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,* vol. 86, no. 3, pp. 423-433.

Diévart, A. & Clark, S.E. 2003, "Using mutant alleles to determine the structure and function of leucine-rich repeat receptor-like kinases", *Current opinion in plant biology,* vol. 6, no. 5, pp. 507-516.

Dolan, L., Janmaat, K., Willemsen, V., Linstead, P., Poethig, S., Roberts, K. & Scheres, B. 1993, "Cellular organisation of the Arabidopsis thaliana root", *Development,* vol. 119, no. 1, pp. 71-84.

Donner, T.J., Sherr, I. & Scarpella, E. 2009, "Regulation of preprocambial cell state acquisition by auxin signaling in Arabidopsis leaves", *Development,* vol. 136, no. 19, pp. 3235-3246.

Englbrecht, C., Schoof, H. & Böhm, S. 2004, "Conservation, diversification and expansion of C2H2 zinc finger proteins in the Arabidopsis thaliana genome", *Bmc Genomics,* vol. 5, no. 1, pp. 39.

Eshed, Y., Baum, S.F., Perea, J.V. & Bowman, J.L. 2001, "Establishment of polarity in lateral organs of plants", *Current Biology,* vol. 11, no. 16, pp. 1251-1260.

Favaro, R., Pinyopich, A., Battaglia, R., Kooiker, M., Borghi, L., Ditta, G., Yanofsky, M.F., Kater, M.M. & Colombo, L. 2003, "MADS-Box Protein Complexes Control Carpel and Ovule Development in Arabidopsis", *The Plant Cell Online,* vol. 15, no. 11, pp. 2603-2611.

Ferreira, F.J. & Kieber, J.J. 2005, "Cytokinin signaling", *Current opinion in plant biology,* vol. 8, no. 5, pp. 518-525.

Fiers, M., Golemiec, E., Xu, J., van der Geest, L., Heidstra, R., Stiekema, W. & Liu, C. September 2005, "The 14–Amino Acid CLV3, CLE19, and CLE40 Peptides Trigger Consumption of the Root Meristem in Arabidopsis through a CLAVATA2-Dependent Pathway", *The Plant Cell Online,* vol. 17, no. 9, pp. 2542-2553.

Friend, S.H., Bernards, R., Rogelj, S., Weinberg, R.A., Rapaport, J.M., Albert, D.M. & Dryja, T.P. 1986, "Identification of a human DNA segment having properties of the gene that predisposes to retinoblastoma and osteosarcoma", *Nature,* vol. 323, no. 6089, pp. 643-646.

Gagne, J.M. & Clark, S.E. 2007, "The protein phosphatases POL and PLL1 are signaling intermediates for multiple pathways in Arabidopsis", *Plant signaling & behavior,* vol. 2, no. 4, pp. 245-246.

Gagne, J.M., Song, S.K. & Clark, S.E. 2008, "POLTERGEIST and PLL1 are required for stem cell function with potential roles in cell asymmetry and auxin signaling", *Communicative & integrative biology,* vol. 1, no. 1, pp. 53-55.

Galinha, C., Hofhuis, H., Luijten, M., Willemsen, V., Blilou, I., Heidstra, R. & Scheres, B. 2007, "PLETHORA proteins as dose-dependent master regulators of Arabidopsis root development", *Nature,* vol. 449, no. 7165, pp. 1053-1057.

Gallagher, K.L. & Benfey, P.N. 2008, "Both the conserved GRAS domain and nuclear localization are required for SHORT-ROOT movement", *The Plant Journal,* vol. 57, no. 5, pp. 785-797.

Gallagher, K.L., Paquette, A.J., Nakajima, K. & Benfey, P.N. 2004, "Mechanisms regulating SHORT-ROOT intercellular movement", *Current biology,* vol. 14, no. 20, pp. 1847-1851.

Gietz, R.D., Triggs-Raine, B., Robbins, A., Graham, K.C. & Woods, R.A. 1997, "Identification of proteins that interact with a protein of interest: applications of the yeast two-hybrid system", *Molecular and cellular biochemistry,* vol. 172, no. 1, pp. 67-79.

Gorte, M., Horstman, A., Page, R.B., Heidstra, R., Stromberg, A. & Boutilier, K. 2011, "Microarray-based identification of transcription factor target genes", *Methods Mol Biol,* vol. 754, pp. 119-141.

Grafi, G., Burnett, R.J., Helentjaris, T., Larkins, B.A., DeCaprio, J.A., Sellers, W.R. & Kaelin, W.G. 1996, "A maize cDNA encoding a member of the retinoblastoma protein family: involvement in endoreduplication", *Proceedings of the National Academy of Sciences,* vol. 93, no. 17, pp. 8962-8967.

Gray, W.M., Kepinski, S., Rouse, D., Leyser, O. & Estelle, M. 2001, "Auxin regulates SCFTIR1-dependent degradation of AUX/IAA proteins", *Nature,* vol. 414, no. 6861, pp. 271-276.

Greene, E.A., Codomo, C.A., Taylor, N.E., Henikoff, J.G., Till, B.J., Reynolds, S.H., Enns, L.C., Burtner, C., Johnson, J.E., Odden, A.R., Comai, L. & Henikoff, S. 2003, "Spectrum of Chemically Induced Mutations From a Large-Scale Reverse-Genetic Screen in Arabidopsis", *Genetics,* vol. 164, no. 2, pp. 731-740.

Grieneisen, V.A., Xu, J., Marée, A.F.M., Hogeweg, P. & Scheres, B. 2007, "Auxin transport is sufficient to generate a maximum and gradient guiding root growth", *Nature,* vol. 449, no. 7165, pp. 1008-1013.

Griffiths, A., Miller, J., Suzuki, D., Lewontin, R. & Gelbart, W. 2000, *An introduction to genetic analysis,* 7th edn, W.H. Freeman and Company, USA.

Grigg, S.P., Galinha, C., Kornet, N., Canales, C., Scheres, B. & Tsiantis, M. 2009, "Repression of Apical Homeobox Genes Is Required for Embryonic Root Development in Arabidopsis", *Current Biology,* vol. 19, no. 17, pp. 1485-1490.

Guan, Q., Wen, C., Zeng, H. & Zhu, J. 2012, "A KH Domain-Containing Putative RNA-Binding Protein Is Critical for Heat Stress-Responsive Gene Regulation and Thermotolerance in Arabidopsis", *Molecular Plant,* .

Guseman, J.M., Lee, J.S., Bogenschutz, N.L., Peterson, K.M., Virata, R.E., Xie, B., Kanaoka, M.M., Hong, Z. & Torii, K.U. 2010, "Dysregulation of cell-to-cell connectivity and stomatal patterning by loss-of-function mutation in Arabidopsis CHORUS (GLUCAN SYNTHASE-LIKE 8)", *Development,* vol. 137, no. 10, pp. 1731-1741.

Haecker, A., Groß-Hardt, R., Geiges, B., Sarkar, A., Breuninger, H., Herrmann, M. & Laux, T. 2004, "Expression dynamics of WOX genes mark cell fate decisions during early embryonic patterning in Arabidopsis thaliana", *Development,* vol. 131, no. 3, pp. 657-668.

Hamann, T., Benkova, E., Bäurle, I., Kientz, M. & Jürgens, G. 2002, "The Arabidopsis BODENLOS gene encodes an auxin response protein inhibiting MONOPTEROS-mediated embryo patterning", *Genes & development,* vol. 16, no. 13, pp. 1610-1615.

Hamann, T., Mayer, U. & Jurgens, G. 1999, "The auxin-insensitive bodenlos mutation affects primary root formation and apical-basal patterning in the Arabidopsis embryo", *Development,* vol. 126, no. 7, pp. 1387-1395.

Hartwell, L.H., Hopfield, J.J., Leibler, S. & Murray, A.W. 1999, "From molecular to modular cell biology", *Nature,* vol. 402, pp. C47-C52.

Hassan, H., Scheres, B. & Blilou, I. 2010, "JACKDAW controls epidermal patterning in the Arabidopsis root meristem through a non-cell-autonomous mechanism", *Development,* vol. 137, no. 9, pp. 1523-1529.

Heerklotz, D., Döring, P., Bonzelius, F., Winkelhaus, S. & Nover, L. 2001, "The balance of nuclear import and export determines the intracellular distribution and function of tomato heat stress transcription factor HsfA2", *Molecular and cellular biology,* vol. 21, no. 5, pp. 1759-1768.

Heidstra, R., Welch, D. & Scheres, B. 2004, "Mosaic analyses using marked activation and deletion clones dissect Arabidopsis SCARECROW action in asymmetric cell division", *Genes & development,* vol. 18, no. 16, pp. 1964-1969.

Helariutta, Y., Fukaki, H., Wysocka-Diller, J., Nakajima, K., Jung, J., Sena, G., Hauser, M. & Benfey, P.N. 2000, "The SHORT-ROOT Gene Controls Radial Patterning of the Arabidopsis Root through Radial Signaling", *Cell,* vol. 101, no. 5, pp. 555-567.

Hellens, R.P., Edwards, E.A., Leyland, N.R., Bean, S. & Mullineaux, P.M. 2000, "pGreen: a versatile and flexible binary Ti vector for Agrobacterium-mediated plant transformation", *Plant Molecular Biology,* vol. 42, no. 6, pp. 819-832.

Hervé, C., Dabos, P., Bardet, C., Jauneau, A., Auriac, M.C., Ramboer, A., Lacout, F. & Tremousaygue, D. 2009, "In vivo interference with AtTCP20 function induces severe plant growth alterations and deregulates the expression of many genes important for development", *Plant Physiology,* vol. 149, no. 3, pp. 1462-1477.

Hervé, C., Dabos, P., Bardet, C., Jauneau, A., Auriac, M.C., Ramboer, A., Lacout, F. & Tremousaygue, D. 2009, "In vivo interference with AtTCP20 function induces severe plant growth alterations and deregulates the expression of many genes important for development", *Plant Physiology,* vol. 149, no. 3, pp. 1462-1477.

Heyl, A. & Schmülling, T. 2003, "Cytokinin signal perception and transduction", *Current opinion in plant biology,* vol. 6, no. 5, pp. 480-488.

Honma, T. & Goto, K. 2001, "Complexes of MADS-box proteins are sufficient to convert leaves into floral organs", *Nature,* vol. 409, no. 6819, pp. 525-529.

Horvitz, H.R. & Herskowitz, I. 1992, "Mechanisms of asymmetric cell division: two Bs or not two Bs, that is the question.", *Cell,* vol. 68, pp. 237-255.

Ikeda, M., Mitsuda, N. & Ohme-Takagi, M. November 2009, "Arabidopsis WUSCHEL Is a Bifunctional Transcription Factor That Acts as a Repressor in Stem Cell Regulation and as an Activator in Floral Patterning", *The Plant Cell Online,* vol. 21, no. 11, pp. 3493-3505.

Ikeda, M. & Ohme-Takagi, M. 2009, "A Novel Group of Transcriptional Repressors in Arabidopsis", *Plant and Cell Physiology,* vol. 50, no. 5, pp. 970-975.

Irizarry, R.A., Hobbs, B., Collin, F., Beazer-Barclay, Y.D., Antonellis, K.J., Scherf, U. & Speed, T.P. 2003, "Exploration, normalization, and summaries of high density oligonucleotide array probe level data", *Biostatistics,* vol. 4, no. 2, pp. 249-264.

Ito, Y., Nakanomyo, I., Motose, H., Iwamoto, K., Sawa, S., Dohmae, N. & Fukuda, H. 2006, "Dodeca-CLE peptides as suppressors of plant stem cell differentiation", *Science Signalling,* vol. 313, no. 5788, pp. 842.

James, P., Halladay, J. & Craig, E.A. 1996, "Genomic Libraries and a Host Strain Designed for Highly Efficient Two-Hybrid Selection in Yeast", *Genetics,* vol. 144, no. 4, pp. 1425-1436.

Jan, Y. & Jan, L.Y. 2001, "Asymmetric cell division in the Drosophila nervous system", *Nature Reviews Neuroscience,* vol. 2, no. 11, pp. 772-779.

Jenik, P.D. & Barton, M.K. 2005, "Surge and destroy: the role of auxin in plant embryogenesis", *Development,* vol. 132, no. 16, pp. 3577-3585.

Jeong, S., Volny, M. & Lukowitz, W. 2012, "Axis formation in Arabidopsis transcription factors tell their side of the story", *Current opinion in plant biology,* vol. 15, no. 1, pp. 4-9.

Jürgens, G. & Mayer, U. 1994, "Arabidopsis, A colour atlas of developing embryos" , pp. 7-21.

Jürgens, G. 1992, "Pattern formation in the flowering plant embryo", *Current opinion in genetics & development,* vol. 2, no. 4, pp. 567-570.

Kagale, S., Links, M.G. & Rozwadowski, K. 2010, "Genome-Wide Analysis of Ethylene-Responsive Element Binding Factor-Associated Amphiphilic Repression Motif-Containing Transcriptional Regulators in Arabidopsis", *Plant Physiology,* vol. 152, no. 3, pp. 1109-1134.

Kakimoto, T. 2003, "Perception and signal transduction of cytokinins", *Annual Review of Plant Biology,* vol. 54, no. 1, pp. 605-627.

Kamiya, N., Itoh, J.I., Morikami, A., Nagato, Y. & Matsuoka, M. 2003, "The SCARECROW gene's role in asymmetric cell divisions in rice plants", *The Plant Journal,* vol. 36, no. 1, pp. 45-54.

Kiba, T., Yamada, H., Sato, S., Kato, T., Tabata, S., Yamashino, T. & Mizuno, T. 2003, "The type-A response regulator, ARR15, acts as a negative regulator in the cytokinin-mediated signal transduction in Arabidopsis thaliana", *Plant and cell physiology,* vol. 44, no. 8, pp. 868-874.

Kinoshita, A., Nakamura, Y., Sasaki, E., Kyozuka, J., Fukuda, H. & Sawa, S. 2007, "Gain-of-function phenotypes of chemically synthetic CLAVATA3/ESR-related (CLE) peptides in Arabidopsis thaliana and Oryza sativa", *Plant and cell physiology,* vol. 48, no. 12, pp. 1821-1825.

Knoblich, J.A. 2008, "Mechanisms of Asymmetric Stem Cell Division", *Cell,* vol. 132, no. 4, pp. 583-597.

Koizumi, K., Hayashi, T. & Gallagher, K. 2012, "SCARECROW reinforces SHORT-ROOT signaling and inhibits periclinal cell divisions in the ground tissue by maintaining SHR at high levels in the endodermis", *Plant Signaling & Behavior,* vol. 7, no. 12, pp. 1573-1577.

Koizumi, K., Wu, S., MacRae-Crerar, A. & Gallagher, K.L. 2011, "An essential protein that interacts with endosomes and promotes movement of the SHORT-ROOT transcription factor", *Current Biology,* vol. 21, no. 18, pp. 1559-1564.

Kornet, N.G. 2008, *Role of chromatin factors in Arabidopsis root stem cell maintenance*. PhD dissertation.

Kosarev, P., Mayer, K. & Hardtke, C.S. 2002, "Evaluation and classification of RING-finger domains encoded by the Arabidopsis genome", *Genome Biol,* vol. 3, no. 4, pp. 0016.1-0016.12.

Krizek, B.A., Prost, V. & Macias, A. 2000, "AINTEGUMENTA Promotes Petal Identity and Acts as a Negative Regulator of AGAMOUS", *The Plant Cell Online,* vol. 12, no. 8, pp. 1357-1366.

Kubo, H. & Hayashi, K. 2011, "Characterization of root cells of anl2 mutant in Arabidopsis thaliana", *Plant Science,* vol. 180, no. 5, pp. 679-685.

Kubo, H., Kishi, M. & Goto, K. 2008, "Expression analysis of ANTHOCYANINLESS2 gene in Arabidopsis", *Plant Science,* vol. 175, no. 6, pp. 853-857.

Kubo, H., Peeters, A.J.M., Aarts, M.G.M., Pereira, A. & Koornneef, M. 1999, "ANTHOCYANINLESS2, a Homeobox Gene Affecting Anthocyanin Distribution and Root Development in Arabidopsis", *The Plant Cell Online,* vol. 11, no. 7, pp. 1217-1226.

Kuhlemeier, C. 2007, "Phyllotaxis", *TRENDS in plant science,* vol. 12, no. 4, pp. 143-150.

Kwak, S.H. & Schiefelbein, J. 2008, "A Feedback Mechanism Controlling SCRAMBLED Receptor Accumulation and Cell-Type Pattern in Arabidopsis", *Current Biology,* vol. 18, no. 24, pp. 1949-1954.

Lai, C.P., Lee, C.L., Chen, P.H., Wu, S.H., Yang, C.C. & Shaw, J.F. 2004, "Molecular analyses of the Arabidopsis TUBBY-like protein gene family", *Plant Physiology,* vol. 134, no. 4, pp. 1586-1597.

Lawrence, E. (ed) 2005, *Henderson's dictionary of biology*, 13th edn, Pearson Education Limited, Essex, England.

Leibfried, A., To, J.P.C., Busch, W., Stehling, S., Kehle, A., Demar, M., Kieber, J.J. & Lohmann, J.U. 2005, "WUSCHEL controls meristem function by direct regulation of cytokinin-inducible response regulators", *Nature,* vol. 438, no. 7071, pp. 1172-1175.

Levesque, M.P., Vernoux, T., Busch, W., Cui, H., Wang, J.Y., Blilou, I., Hassan, H., Nakajima, K., Matsumoto, N. & Lohmann, J.U. 2006, "Whole-genome analysis of the SHORT-ROOT developmental pathway in Arabidopsis", *PLoS biology,* vol. 4, no. 5, pp. e143.

Leyser, O. & Day, S. 2009, *Mechanisms in plant development,* Wiley-Blackwell.

Li, C., Potuschak, T., Colón-Carmona, A., Gutiérrez, R.A. & Doerner, P. 2005, "Arabidopsis TCP20 links regulation of growth and cell division control pathways", *Proceedings of the National Academy of Sciences of the United States of America,* vol. 102, no. 36, pp. 12978-12983.

Li, J.F., Li, L. & Sheen, J. 2010, "Protocol: a rapid and economical procedure for purification of plasmid or plant DNA with diverse applications in plant biology", *Plant methods,* vol. 6, no. 1, pp. 1.

Lohmann, J.U., Hong, R.L., Hobe, M., Busch, M.A., Parcy, F., Simon, R. & Weigel, D. 2001, "A molecular link between stem cell regulation and floral patterning in Arabidopsis", *Cell,* vol. 105, no. 6, pp. 793-804.

Lu, P., Porat, R., Nadeau, J.A. & O'Neill, S.D. 1996, "Identification of a meristem L1 layer-specific gene in Arabidopsis that is expressed during embryonic pattern formation and defines a new class of homeobox genes.", *The Plant Cell Online,* vol. 8, no. 12, pp. 2155-2168.

Luijten, M. 2009, *Regulation of stem cell maintenance and cell differentiation states in Arabidopsis root development*, PhD dissertation.

MacAlister, C.A., Ohashi-Ito, K. & Bergmann, D.C. 2006, "Transcription factor control of asymmetric cell divisions that establish the stomatal lineage", *Nature,* vol. 445, no. 7127, pp. 537-540.

Madlung, A. & Comai, L. 2004, "The Effect of Stress on Genome Regulation and Structure", *Annals of Botany,* vol. 94, no. 4, pp. 481-495.

Malamy, J.E. & Benfey, P.N. 1997, "Organization and cell differentiation in lateral roots of Arabidopsis thaliana", *Development,* vol. 124, no. 1, pp. 33-44.

Mansfield, S. & Briarty, L. 1991, "Early embryogenesis in Arabidopsis thaliana. II. The developing embryo", *Canadian journal of botany,* vol. 69, no. 3, pp. 461-476.

Martín-Trillo, M. & Cubas, P. 2010, "TCP genes: a family snapshot ten years later", *Trends in plant science,* vol. 15, no. 1, pp. 31-39.

Masucci, J.D., Rerie, W.G., Foreman, D.R., Zhang, M., Galway, M.E., Marks, M.D. & Schiefelbein, J.W. 1996, "The homeobox gene GLABRA2 is required for position-dependent cell differentiation in the root epidermis of Arabidopsis thaliana", *Development,* vol. 122, no. 4, pp. 1253-1260.

Mendenhall, E.M. & Bernstein, B.E. 2008, "Chromatin state maps: new technologies, new insights", *Genetics & Development,* vol. 18, pp. 109.

Meyer, S., Scholz-Starke, J., De Angeli, A., Kovermann, P., Burla, B., Gambale, F. & Martinoia, E. 2011, "Malate transport by the vacuolar AtALMT6 channel in guard cells is subject to multiple regulation", *The Plant Journal,* vol. 67, no. 2, pp. 247-257.

Miller, G. & Mittler, R. 2006, "Could heat shock transcription factors function as hydrogen peroxide sensors in plants?", *Annals of Botany,* vol. 98, no. 2, pp. 279-288.

Mokry, M., Nijman, I.J., van Dijken, A., Benjamins, R., Heidstra, R., Scheres, B. & Cuppen, E. 2011, "Identification of factors required for meristem function in Arabidopsis using a novel next generation sequencing fast forward genetics approach", *BMC genomics,* vol. 12, no. 1, pp. 256.

Möller, B. & Weijers, D. 2009, "Auxin control of embryo patterning", *Cold Spring Harbor Perspectives in Biology,* vol. 1, no. 5.

Moreno-Risueno, M.A., Van Norman, J.M., Moreno, A., Zhang, J., Ahnert, S.E. & Benfey, P.N. 2010, "Oscillating gene expression determines competence for periodic Arabidopsis root branching", *Science,* vol. 329, no. 5997, pp. 1306.

Morgan, D.O. 2007, *The cell cycle: principles of control,* Sinauer Associates Incorporated.

Morillo, S.A. & Tax, F.E. 2006, "Functional analysis of receptor-like kinases in monocots and dicots", *Current opinion in plant biology,* vol. 9, no. 5, pp. 460-469.

Muller, P.Y., Janovjak, H., Miserez, A.R. & Dobbie, Z. 2002, "Short technical report processing of gene expression data generated by quantitative Real-Time RT-PCR", *BioTechniques,* vol. 32, no. 6, pp. 1372-1379.

Mylona, P., Linstead, P., Martienssen, R. & Dolan, L. 2002, "SCHIZORIZA controls an asymmetric cell division and restricts epidermal identity in the Arabidopsis root", *Development,* vol. 129, no. 18, pp. 4327-4334.

Nakajima, K., Sena, G., Nawy, T. & Benfey, P.N. 2001, "Intercellular movement of the putative transcription factor SHR in root patterning", *Nature,* vol. 413, no. 6853, pp. 307-311.

Nakamura, M., Katsumata, H., Abe, M., Yabe, N., Komeda, Y., Yamamoto, K.T. & Takahashi, T. August 2006, "Characterization of the Class IV Homeodomain-Leucine Zipper Gene Family in Arabidopsis", *Plant Physiology,* vol. 141, no. 4, pp. 1363-1375.

Nawy, T., Lee, J., Colinas, J., Wang, J.Y., Thongrod, S.C., Malamy, J.E., Birnbaum, K. & Benfey, P.N. 2005, "Transcriptional Profile of the Arabidopsis Root Quiescent Center", *The Plant Cell Online,* vol. 17, no. 7, pp. 1908-1925.

Neumüller, R.A., Knoblich, J.A. 2009, "Dividing cellular asymmetry: asymmetric cell division and its implications for stem cells and cancer", *Genes and Development,* vol. 23, pp. 2675-2699.

Nodine, M.D., Yadegari, R. & Tax, F.E. 2007, "RPK1 and TOAD2 Are Two Receptor-like Kinases Redundantly Required for Arabidopsis Embryonic Pattern Formation", *Developmental cell,* vol. 12, no. 6, pp. 943-956.

Nover, L., Bharti, K., Döring, P., Mishra, S.K., Ganguli, A. & Scharf, K.D. 2001, "Arabidopsis and the heat stress transcription factor world: how many heat stress transcription factors do we need?", *Cell stress & chaperones,* vol. 6, no. 3, pp. 177.

Nowack, M.K., Harashima, H., Dissmeyer, N., Zhao, X.A., Bouyer, D., Weimer, A.K., De Winter, F., Yang, F. & Schnittger, A. 2012, "Genetic Framework of Cyclin-Dependent Kinase Function in Arabidopsis", *Developmental cell,* vol. 22, no. 5, pp. 1030-1040.

Ogawa, M., Shinohara, H., Sakagami, Y. & Matsubayashi, Y. 2008, "Arabidopsis CLV3 peptide directly binds CLV1 ectodomain", *Science,* vol. 319, no. 5861, pp. 294-294.

Paciorek, T. & Bergmann, D.C. 2010, "The secret to life is being different: asymmetric divisions in plant development", *Current opinion in plant biology,* vol. 13, no. 6, pp. 661-669.

Page, D.R. & Grossniklaus, U. 2002, "The art and design of genetic screens: Arabidopsis thaliana", *Nature Reviews Genetics,* vol. 3, no. 2, pp. 124-136.

Pařenicová, L., De Folter, S., Kieffer, M., Horner, D.S., Favalli, C., Busscher, J., Cook, H.E., Ingram, R.M., Kater, M.M. & Davies, B. 2003, «Molecular and phylogenetic analyses of the complete MADS-box transcription factor family in Arabidopsis new openings to the MADS world», *The Plant Cell Online,* vol. 15, no. 7, pp. 1538-1551.

Park, J., Ahn, J., Kim, Y., Kim, S.J., Kim, J., Kim, W.T. & Pai, H. 2005, "Retinoblastoma protein regulates cell proliferation, differentiation, and endoreduplication in plants", *The Plant Journal,* vol. 42, no. 2, pp. 153-163.

Pauwels, L., Barbero, G.F., Geerinck, J., Tilleman, S., Grunewald, W., Pérez, A.C., Chico, J.M., Bossche, R.V., Sewell, J. & Gil, E. 2010, "NINJA connects the co-repressor TOPLESS to jasmonate signalling", *Nature,* vol. 464, no. 7289, pp. 788-791.

Paz-Ares, J. 2002, "REGIA, an EU project on functional genomics of transcription factors from Arabidopsis thaliana", *Comparative and Functional Genomics,* vol. 3, no. 2, pp. 102-108.

Peris, C.I.L., Rademacher, E.H. & Weijers, D. 2010, "Chapter One-Green Beginnings–Pattern Formation in the Early Plant Embryo", *Current topics in developmental biology,* vol. 91, pp. 1-27.

Pernas, M., Ryan, E. & Dolan, L. 2010, "SCHIZORIZA controls tissue system complexity in plants", *Current Biology,* vol. 20, no. 9, pp. 818-823.

Peteranderl, R., Rabenstein, M., Shin, Y.K., Liu, C.W., Wemmer, D.E., King, D.S. & Nelson, H.C.M. 1999, "Biochemical and biophysical characterization of the trimerization domain from the heat shock transcription factor", *Biochemistry,* vol. 38, no. 12, pp. 3559-3569.

Petricka, J.J., Winter, C.M. & Benfey, P.N. 2012, "Control of Arabidopsis Root Development", *Annual Review of Plant Biology,* vol. 63, pp. 563-590.

Pillitteri, L.J., Peterson, K.M., Horst, R.J. & Torii, K.U. 2011, "Molecular profiling of stomatal meristemoids reveals new component of asymmetric cell division and commonalities among stem cell populations in Arabidopsis", *The Plant Cell Online,* vol. 23, no. 9, pp. 3260-3275.

Pischke, M.S., Sussman, M.R., Helariutta, Y. & Kakimoto, T. 2006, "Cytokinins regulate a bidirectional phosphorelay network in Arabidopsis", *Current Biology,* vol. 16, pp. 1116-1122.

Pruneda-Paz, J.L., Breton, G., Para, A. & Kay, S.A. 2009, "A functional genomics approach reveals CHE as a component of the Arabidopsis circadian clock", *Science Signalling,* vol. 323, no. 5920, pp. 1481.

Pruneda-Paz, J.L., Breton, G., Para, A. & Kay, S.A. 2009, "A Functional Genomics Approach Reveals CHE as a Component of the Arabidopsis Circadian Clock", *Science,* vol. 323, no. 5920, pp. 1481-1485.

Przemeck, G.K., Mattsson, J., Hardtke, C.S., Sung, Z.R. & Berleth, T. 1996, "Studies on the role of the Arabidopsis gene MONOPTEROS in vascular development and plant cell axialization", *Planta,* vol. 200, no. 2, pp. 229-237.

Rademacher, E.H., Lokerse, A.S., Schlereth, A., Llavata-Peris, C.I., Bayer, M., Kientz, M., Freire Rios, A., Borst, J.W., Lukowitz, W. & Jürgens, G. 2012, "Different auxin response machineries control distinct cell fates in the early plant embryo", *Developmental cell,* vol. 22, no. 1, pp. 211-222.

Ratcliffe, O.J., Nadzan, G.C., Reuber, T.L. & Riechmann, J.L. 2001, "Regulation of Flowering in Arabidopsis by an FLCHomologue", *Plant Physiology,* vol. 126, no. 1, pp. 122-132.

Raven, P., Evert, R. & Eichorn, S. 1986, "Biology of plants. Worth Publ", *Inc, New York*.

Rehrauer, H., Aquino, C., Gruissem, W., Henz, S.R., Hilson, P., Laubinger, S., Naouar, N., Patrignani, A., Rombauts, S., Shu, H., Van de Peer, Y., Vuylsteke, M., Weigel, D., Zeller, G. & Hennig, L. February 2010, "AGRONOMICS1: A New Resource for Arabidopsis Transcriptome Profiling", *Plant Physiology,* vol. 152, no. 2, pp. 487-499.

Reinhardt, ., Pesce, E., Stieger, P., Mandel, T., Baltensperger, K., Bennett, M., Traas, J., Friml, J., Kuhlemeier,C. 2003, "Regulation of phyllotaxis by polar auxin transport", *Nature,* vol. 426, pp. 255-260.

Reitz, M.U., Bissue, J.K., Zocher, K., Attard, A., Hückelhoven, R., Becker, K., Imani, J., Eichmann, R. & Schäfer, P. 2012, "The subcellular localization of Tubby-like proteins and participation in stress signaling and root colonization by the mutualist Piriformospora indica", *Plant Physiology,* vol. 160, no. 1, pp. 349-364.

Rerie, W.G., Feldmann, K.A. & Marks, M.D. 1994, "The GLABRA2 gene encodes a homeo domain protein required for normal trichome development in Arabidopsis.", *Genes & development,* vol. 8, no. 12, pp. 1388-1399.

Rieu, I. & Laux, T. 2009, "Signaling pathways maintaining stem cells at the plant shoot apex", *Seminars in cell & developmental biology*Elsevier, pp. 1083.

Sabatini, S., Beis, D., Wolkenfelt, H., Murfett, J., Guilfoyle, T., Malamy, J., Benfey, P., Leyser, O., Bechtold, N., Weisbeek, P. & Scheres, B. 1999, "An Auxin-Dependent Distal Organizer of Pattern and Polarity in the Arabidopsis Root", *Cell,* vol. 99, no. 5, pp. 463-472.

Sabatini, S., Heidstra, R., Wildwater, M. & Scheres, B. 2003, "SCARECROW is involved in positioning the stem cell niche in the Arabidopsis root meristem", *Genes & development,* vol. 17, no. 3, pp. 354-358.

Sablowski, R. 2009, "Cytokinin and WUSCHEL tie the knot around plant stem cells", *PNAS,* vol. 106, no. 38, pp. 16016-16017.

Sarkar, A.K., Luijten, M., Miyashima, S., Lenhard, M., Hashimoto, T., Nakajima, K., Scheres, B., Heidstra, R. & Laux, T. 2007, "Conserved factors regulate signalling in Arabidopsis thaliana shoot and root stem cell organizers", *Nature,* vol. 446, no. 7137, pp. 811-814.

Scharf, K.D., Berberich, T., Ebersberger, I. & Nover, L. 2011, "The plant heat stress transcription factor (Hsf) family: structure, function and evolution", *Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms,* .

Scheres, B. & Benfey, P.N. 1999, "Asymmetric cell division in plants", *Annual review of plant biology,* vol. 50, no. 1, pp. 505-537.

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

Scheres, B., Di Laurenzio, L., Willemsen, V., Hauser, M.T., Janmaat, K., Weisbeek, P. & Benfey, P.N. 1995, "Mutations affecting the radial organisation of the Arabidopsis root display specific defects throughout the embryonic axis", *Development,* vol. 121, no. 1, pp. 53-62.

Scheres, B. & Kornet, N. 2009, "Members of the GCN5 Histone Acetyltransferase Complex Regulate PLETHORA-Mediated Root Stem Cell Niche Maintenance and Transit Amplifying Cell Proliferation in Arabidopsis", *Plant cell,* vol. 21, no. 4, pp. 1070-1079.

Scheres, B., Wolkenfelt, H., Willemsen, V., Terlouw, M., Lawson, E., Dean, C. & Weisbeek, P. 1994, "Embryonic origin of the Arabidopsis primary root and root meristem initials", *Development,* vol. 120, no. 9, pp. 2475-2487.

Schiefelbein, J., Kwak, S.H., Wieckowski, Y., Barron, C. & Bruex, A. 2009, "The gene regulatory network for root epidermal cell-type pattern formation in Arabidopsis", *Journal of experimental botany,* vol. 60, no. 5, pp. 1515-1521.

Schlereth, A., Möller, B., Liu, W., Kientz, M., Flipse, J., Rademacher, E.H., Schmid, M., Jürgens, G. & Weijers, D. 2010, "MONOPTEROS controls embryonic root initiation by regulating a mobile transcription factor", *Nature,* vol. 464, no. 7290, pp. 913-916.

Schöffl, F., Prändl, R. & Reindl, A. 1998, "Regulation of the heat-shock response", *Plant Physiology,* vol. 117, no. 4, pp. 1135-1141.

Schoof, H., Lenhard, M., Haecker, A., Mayer, K.F.X., Jürgens, G. & Laux, T. 2000, "The Stem Cell Population of Arabidopsis Shoot Meristems Is Maintained by a Regulatory Loop between the CLAVATA and WUSCHEL Genes", *Cell,* vol. 100, no. 6, pp. 635-644.

Scortecci, K.C., Michaels, S.D. & Amasino, R.M. 2001, "Identification of a MADS-box gene, FLOWERING LOCUS M, that represses flowering", *The Plant Journal,* vol. 26, no. 2, pp. 229-236.

Sena, G., Jung, J.W. & Benfey, P.N. 2004, "A broad competence to respond to SHORT ROOT revealed by tissue-specific ectopic expression", *Development,* vol. 131, no. 12, pp. 2817-2826.

Sena, G., Wang, X., Liu, H.Y., Hofhuis, H. & Birnbaum, K.D. 2009, "Organ regeneration does not require a functional stem cell niche in plants", *Nature,* vol. 457, no. 7233, pp. 1150-1153.

Smith, Z.R. & Long, J.A. 2010, "Control of Arabidopsis apical–basal embryo polarity by antagonistic transcription factors", *Nature,* vol. 464, no. 7287, pp. 423-426.

Smyth, G. 2005, "Limma: linear models for microarray data", *Bioinformatics and computational biology solutions using R and Bioconductor,* pp. 397-420.

Smyth, G.K. 2004, "Linear models and empirical bayes methods for assessing differential expression in microarray experiments.", *Statistical applications in genetics and molecular biology,* vol. 3, no. 1, pp. 3.

Sozzani, R., Cui, H., Moreno-Risueno, M., Busch, W., Van Norman, J., Vernoux, T., Brady, S., Dewitte, W., Murray, J. & Benfey, P. 2010, "Spatiotemporal regulation of cell-cycle genes by SHORTROOT links patterning and growth", *Nature,* vol. 466, no. 7302, pp. 128-132.

Sprinzak, E., Sattath, S. & Margalit, H. 2003, "How reliable are experimental protein–protein interaction data?", *Journal of Molecular Biology,* vol. 327, no. 5, pp. 919-923.

Stahl, Y., Wink, R.H., Ingram, G.C. & Simon, R. 2009, "A Signaling Module Controlling the Stem Cell Niche in Arabidopsis Root Meristems", *Current Biology,* vol. 19, no. 11, pp. 909-914.

Stahl, Y., Grabowski, S., Bleckmann, A., Kühnemuth, R., Weidtkamp-Peters, S., Pinto, K.G., Kirschner, G.K., Schmid, J.B., Wink, R.H. & Hülsewede, A. 2013, "Moderation of Arabidopsis Root Stemness by CLAVATA1 and ARABIDOPSIS CRINKLY4 Receptor Kinase Complexes", *Current Biology,* vol. 23, no. 5, pp. 362-371.

Stahl, Y., Wink, R.H., Ingram, G.C. & Simon, R. 2009, "A Signaling Module Controlling the Stem Cell Niche in Arabidopsis Root Meristems", *Current Biology,* vol. 19, no. 11, pp. 909-914.

Steeves, T.A. & Sussex, I.M. 1989, *Patterns in plant development,* Cambridge University Press.

Stepanova, A.N., Robertson-Hoyt, J., Yun, J., Benavente, L.M., Xie, D.Y., Doležal, K., Schlereth, A., Jürgens, G. & Alonso, J.M. 2008, "TAA1-Mediated Auxin Biosynthesis Is Essential for Hormone Crosstalk and Plant Development", *Cell,* vol. 133, no. 1, pp. 177-191.

Storey, J.D. & Tibshirani, R. 2003, "Statistical significance for genomewide studies", *Proceedings of the National Academy of Sciences,* vol. 100, no. 16, pp. 9440-9445.

Su, Y.H., Cheng, Z.J., Su, Y.X. & Zhang, X.S. 2010, "Pattern analysis of stem cell differentiation during in vitro Arabidopsis organogenesis", *Frontiers in Biology,* vol. 5, no. 5, pp. 464-470.

Swindell, W.R., Huebner, M. & Weber, A.P. 2007, "Transcriptional profiling of Arabidopsis heat shock proteins and transcription factors reveals extensive overlap between heat and non-heat stress response pathways", *BMC genomics,* vol. 8, no. 1, pp. 125.

Szemenyei, H., Hannon, M. & Long, J.A. 2008, "TOPLESS mediates auxin-dependent transcriptional repression during Arabidopsis embryogenesis", *Science Signalling,* vol. 319, no. 5868, pp. 1384.

Takada, S. & Jürgens, G. 2007, "Transcriptional regulation of epidermal cell fate in the Arabidopsis embryo", *Development,* vol. 134, no. 6, pp. 1141-1150.

ten Hove, C.A. & Heidstra, R. 2008, "Who begets whom? Plant cell fate determination by asymmetric cell division", *Current opinion in plant biology,* vol. 11, no. 1, pp. 34-41.

ten Hove, C.A., Willemsen, V., de Vries, W.J., van Dijken, A., Scheres, B. & Heidstra, R. 2010, "SCHIZORIZA Encodes a Nuclear Factor Regulating Asymmetry of Stem Cell Divisions in the Arabidopsis Root", *Current Biology,* vol. 20, no. 5, pp. 452-457.

To, J.P.C., Haberer, G., Ferreira, F.J., Deruère, J., Mason, M.G., Schaller, G.E., Alonso, J.M., Ecker, J.R. & Kieber, J.J. 2004, "Type-A Arabidopsis response regulators are partially redundant negative regulators of cytokinin signaling", *The Plant Cell Online,* vol. 16, no. 3, pp. 658-671.

Tominaga-Wada, R., Ishida, T. & Wada, T. 2011, "New insights into the mechanism of development of Arabidopsis root hairs and trichomes", *Int Rev Cell Mol Biol,* vol. 286, pp. 67-106.

Tonaco, I.A.N., Borst, J.W., de Vries, S.C., Angenent, G.C. & Immink, R.G.H. January 2006, "In vivo imaging of MADS-box transcription factor interactions", *Journal of experimental botany,* vol. 57, no. 1, pp. 33-42.

Torii, K.U., Mitsukawa, N., Oosumi, T., Matsuura, Y., Yokoyama, R., Whittier, R.F. & Komeda, Y. 1996, "The Arabidopsis ERECTA gene encodes a putative receptor protein kinase with extracellular leucine-rich repeats.", *The Plant Cell Online,* vol. 8, no. 4, pp. 735-746.

Ueda, M. & Laux, T. 2012, "The origin of the plant body axis", *Current opinion in plant biology,* vol. 15, no. 6, pp. 578-584.

Umen, J.G. & Goodenough, U.W. 2001, "Control of cell division by a retinoblastoma protein homolog in Chlamydomonas", *Genes & development,* vol. 15, no. 13, pp. 1652-1661.

van der Graaff, E., Dulk-Ras, A., Hooykaas, P. & Keller, B. 2000, "Activation tagging of the LEAFY PETIOLE gene affects leaf petiole development in Arabidopsis thaliana", *Development,* vol. 127, no. 22, pp. 4971.

van der Graaff, E., Laux, T. & Rensing, S.A. 2009, "The WUS homeobox-containing (WOX) protein family", *Genome biology,* vol. 10, no. 12, pp. 248.

Verdeil, J.L., Alemanno, L., Niemenak, N. & Tranbarger, T.J. 2007, "Pluripotent versus totipotent plant stem cells: dependence versus autonomy?", *Trends in plant science,* vol. 12, no. 6, pp. 245-252.

Von Mering, C., Krause, R., Snel, B., Cornell, M., Oliver, S.G., Fields, S. & Bork, P. 2002, "Comparative assessment of large-scale data sets of protein-protein interactions", *Nature,* vol. 417, pp. 399-404.

Von Schaewen, A., Sturm, A., O'neill, J. & Chrispeels, M. 1993, "Isolation of a mutant Arabidopsis plant that lacks N-acetyl glucosaminyl transferase I and is unable to synthesize Golgi-modified complex N-linked glycans", *Plant Physiology,* vol. 102, no. 4, pp. 1109.

Walter, M., Chaban, C., Schütze, K., Batistic, O., Weckermann, K., Näke, C., Blazevic, D., Grefen, C., Schumacher, K. & Oecking, C. 2004, "Visualization of protein interactions in living plant cells using bimolecular fluorescence complementation", *The Plant Journal,* vol. 40, no. 3, pp. 428-438.

Ward, J.M., Smith, A.M., Shah, P.K., Galanti, S.E., Yi, H., Demianski, A.J., Van Der Graaff, E., Keller, B. & Neff, M.M. 2006, "A new role for the Arabidopsis AP2 transcription factor, LEAFY PETIOLE, in gibberellin-induced germination is revealed by the misexpression of a homologous gene, SOB2/DRN-like", *The Plant Cell Online,* vol. 18, no. 1, pp. 29.

Weijers, D., Benkova, E., Jäger, K.E., Schlereth, A., Hamann, T., Kientz, M., Wilmoth, J.C., Reed, J.W. & Jürgens, G. 2005, "Developmental specificity of auxin response by pairs of ARF and Aux/IAA transcriptional regulators", *The EMBO journal,* vol. 24, no. 10, pp. 1874-1885.

Weijers, D., Schlereth, A., Ehrismann, J.S., Schwank, G., Kientz, M. & Jürgens, G. 2006, "Auxin Triggers Transient Local Signaling for Cell Specification in Arabidopsis Embryogenesis", *Developmental cell,* vol. 10, no. 2, pp. 265-270.

Weimer, A.K., Nowack, M.K., Bouyer, D., Zhao, X., Harashima, H., Naseer, S., De Winter, F., Dissmeyer, N., Geldner, N. & Schnittger, A. 2012, "RETINOBLASTOMA RELATED1 Regulates Asymmetric Cell Divisions in Arabidopsis", *The Plant Cell Online,* .

Weinthal, D. & Tzfira, T. 2009, "Imaging protein–protein interactions in plant cells by bimolecular fluorescence complementation assay", *Trends in plant science,* vol. 14, no. 2, pp. 59-63.

Welch, D., Hassan, H., Blilou, I., Immink, R., Heidstra, R. & Scheres, B. 2007, "Arabidopsis JACKDAW and MAGPIE zinc finger proteins delimit asymmetric cell division and stabilize tissue boundaries by restricting SHORT-ROOT action", *Genes & development,* vol. 21, no. 17, pp. 2196-2204.

Whitford, R., Fernandez, A., De Groodt, R., Ortega, E. & Hilson, P. 2008, "Plant CLE peptides from two distinct functional classes synergistically induce division of vascular cells", *Proceedings of the National Academy of Sciences,* vol. 105, no. 47, pp. 18625-18630.

Wildwater, M., Campilho, A., Perez-Perez, J.M., Heidstra, R., Blilou, I., Korthout, H., Chatterjee, J., Mariconti, L., Gruissem, W. & Scheres, B. 2005, "The RETINOBLASTOMA-RELATED Gene Regulates Stem Cell Maintenance in Arabidopsis Roots", *Cell,* vol. 123, no. 7, pp. 1337-1349.

Willemsen, V., Bauch, M., Bennett, T., Campilho, A., Wolkenfelt, H., Xu, J., Haseloff, J. & Scheres, B. 2008, "The NAC Domain Transcription Factors FEZ and SOMBRERO Control the Orientation of Cell Division Plane in Arabidopsis Root Stem Cells", *Developmental cell,* vol. 15, no. 6, pp. 913-922.

Willemsen, V., Wolkenfelt, H., de Vrieze, G., Weisbeek, P. & Scheres, B. 1998, "The HOBBIT gene is required for formation of the root meristem in the Arabidopsis embryo", *Development,* vol. 125, no. 3, pp. 521-531.

William, D.A., Su, Y., Smith, M.R., Lu, M., Baldwin, D.A. & Wagner, D. 2004, "Genomic identification of direct target genes of LEAFY", *Proceedings of the National Academy of Sciences of the United States of America,* vol. 101, no. 6, pp. 1775-1780.

Wodarz, A. & Huttner, W.B. 2003, "Asymmetric cell division during neurogenesis in Drosophila and vertebrates", *Mechanisms of development,* vol. 120, no. 11, pp. 1297-1309.

Wright, A. & Smith, L. 2008, "Division plane orientation in plant cells", *Cell Division Control in Plants,* , pp. 33-57.

Wu, X., Chory, J. & Weigel, D. 2007, "Combinations of WOX activities regulate tissue proliferation during Arabidopsis embryonic development", *Developmental biology,* vol. 309, no. 2, pp. 306-316.

Wysocka-Diller, J.W., Helariutta, Y., Fukaki, H., Malamy, J.E. & Benfey, P.N. 2000, "Molecular analysis of SCARECROW function reveals a radial patterning mechanism common to root and shoot", *Development,* vol. 127, no. 3, pp. 595-603.

Xu, J., Hofhuis, H., Heidstra, R., Sauer, M., Friml, J. & Scheres, B. 2006, "A molecular framework for plant regeneration", *Science Signalling,* vol. 311, no. 5759, pp. 385.

Yang, Z., Zhou, Y., Wang, X., Gu, S., Yu, J., Liang, G., Yan, C. & Xu, C. 2008, "Genomewide comparative phylogenetic and molecular evolutionary analysis of tubby-like protein family in Arabidopsis, rice, and poplar", *Genomics,* vol. 92, no. 4, pp. 246.

Yeung, E.C. & Meinke, D.W. 1993, "Embryogenesis in angiosperms: development of the suspensor.", *The Plant Cell,* vol. 5, no. 10, pp. 1371.

Zheng, Q., Ryvkin, P., Li, F., Dragomir, I., Valladares, O., Yang, J., Cao, K., Wang, L. & Gregory, B.D. 2010, "Genome-wide double-stranded RNA sequencing reveals the functional significance of base-paired RNAs in Arabidopsis", *PLoS genetics,* vol. 6, no. 9, pp. e1001141.

Zhu, X., Thalor, S.K., Takahashi, Y., Berberich, T. & Kusano, T. 2012, "An inhibitory effect of the sequence-conserved upstream open-reading frame on the translation of the main open-reading frame of HsfB1 transcripts in Arabidopsis", *Plant, Cell & Environment,* vol. 35, no. 11, pp. 2014-2030.

Chapter 9

Dankwoord

DANKWOORD/ ACKNOWLEDGEMENTS

En dan is nu de tijd gekomen om te bedanken / The time has come to say thank you.

Prof. dr. T. Mariani, dr. M. Pernas, prof. dr. R. Koes, prof. dr. S. van den Heuvel, dr. M. Boxem and prof. dr. H. Wösten, thank you for judging my thesis. I am honored to discuss my research with you on June 12th.

When I was still a master student at the Dutch Cancer Institute, I attended a seminar held by a guest speaker at the institute. This speaker was professor Ben Scheres, showing the scientist at the Dutch Cancer Institute the beauty of plant biology. My curiosity was raised and six months later I started my PhD in his group. Dear Ben, thank you for giving me the opportunity to do my PhD in the Molecular Genetics group. Your group consist of extraordinary scientist, unique people and I am happy to have been part of this inspiring environment.

Renze, terugkijkend op mijn promotie is er in ieder geval één aspect dat ik, ook in een herhaalexperiment, beslist niet zou veranderen: mijn co-promotor. Je hebt in deze jaren enorm veel betekend voor mij, mijn ontwikkeling als wetenschapper en voor dit proefschrift. Onderzoek volgens de methode Heidstra bleek een waar avontuur: wetenschap-in-staccato, wars van 'wauwelen', jouw 'niewaar?' achter elke veronderstelling, ambitieuze cloneertechnieken, maar ook tijd voor Google searches naar (te) dure auto's, voor schieten met (plastic) machinegeweren of voor een persoonlijk woord: soms afsluitdijken overbruggend, maar meestal soepel samenwerkend. Ik voel me vereerd jouw eerste AiO te zijn met de naam *SCHIZORIZA* in de titel van haar proefschrift, het gen dat onze fascinatie heeft weten te vangen. Ik hoop dat er nog velen volgen. Renze, bedankt!

Viola, ik heb genoten van onze talloze samenzweringen. De MolGenners hebben het geweten! Twee zeer geslaagde 1 Aprilgrappen (Renze ziet nog steeds wat pips bij de gedachte) en natuurlijk de Sinterklaasviering 2011, waar wij als strenge Sinterklaas (ik) en een voorbeeldige Zwarte Piet (jij) onze collega's versteld hebben doen staan. Ik zal onze pizza-vrijdagavonden in een donker Kruytgebouw missen. Behalve voor lol en luchtigheid, kon ik bij jou terecht voor wijze raad of een lief woord, een combinatie die cruciaal is in het leven van een AiO. Ik ben heel erg blij dat ik mij ook tijdens de verdediging van mijn proefschrift door jou gesteund zal weten, deze keer in de rol van mijn paranimf.

Violaine, 'the pinnacle of the celebration has come'. Both of us arrived at MolGen in autumn 2008. I appreciate your sense of humor, razor-sharp comments and your warm-heartedness as a colleague and as a friend. The memory of all great fun we had, still makes me smile: (Snack) worms will never look the same to us. Bisous!

Klaartje (Klarieta), mijn uitzicht van achter de computer. Meermaals hebben we geprobeerd De Mol in onze promoties aan te wijzen; met wisselend succes. Eén ding weet ik wel heel zeker: jij bent een te gekke collega en lieve vriendin geworden.

Maaike, stop een Prutser en een Hooligan samen in één lab en je bent verzekerd van chaos, slechte grappen en een heleboel plezier. Zonder 'Reasons to stay positive' en kilo's chocola had dit proefschrift hier niet gelegen. Dank voor je steun.

Albert, ongeacht de vraag, altijd bereid te helpen. Voor een onderzoeker en collega zoals jij neem ik mijn sombrero af. Ik hoop je snel als doctor van den Toorn aan te kunnen spreken. Sabine, sinds twee jaar weet jij ook hoe het is om aan een gen met een onmogelijke naam (is het wel alleen de naam?) te werken. Dat het een cruciale factor is weten wij allang. Nu nog even uitzoeken hoe het precies werkt. Er ligt weer een aantal mooie lijnen om op door te borduren. Heel veel succes, ik hoop dat Schizoriza je veel goeds brengt. Anja, Inez, Maartje en Colette, bij mijn aankomst: Renze's Posse. (Mevrouwtje) Anja, mijn spin-maatje, betrokken en altijd een helpende hand biedend. Ons Groote Takkenwerk heeft toch maar mooi de allerbeste Taq-voorraad ooit opgeleverd! En het door ons georganiseerde ligfietsuitje van Renze's Posse (zonder Renze, want die zat aan de andere kant van een aswolk in Portugal) was te gek! Inez, immer positief en altijd beschikbaar voor koffie, koekjes en een vrolijke noot. Later mentor-op-afstand in talloze mailtjes en wanneer ik na onze verhuizing, weer eens in het Kruytgebouw was. Maartje, samen begonnen we op het ambitieuze Horizon project. Jij besloot de stamcellen te laten voor wat ze zijn, helaas, want ik heb het fijn gevonden om met jou als tweeling-AiO te werken. Colette, Schizoriza-voorganger, er lag voor mij een behoorlijke uitdaging, maar ook een heleboel materiaal klaar. Ik denk dat een aantal componenten van mijn proefschrift je zeker bekend voor zal komen.

Rene (doctor Benjamins), achter jouw brommerige opmerkingen schuilt een zeer betrokken collega. Onze IKEA-Sligro kerstinkopen (waarbij we ons zó verbaasden over de veelzijdigheid van de IKEA-serviesafdeling, dat we niet meer konden stoppen met items in de winkelwagen gooien) hebben geresulteerd in het gezelligste kerstdiner met de allerbeste wijn ooit. (Dokter) Bas, eindelijk hoef ik je niet langer als zuster te woord staan. Jouw humor en het vermogen om écht belangrijke zaken daarbij niet te verliezen, werkte prettig relativerend; Dare to say NO to Bokito! Hugo, man van binnensmondse antwoorden en createur van de chaos op de labtafel achter mij. Maar ook altijd zeer geïnteresseerd en klaar met een goed advies. Fenne, eindeloos rijtjes zaden uitplaten voor de RLK-screen was met jou samen helemaal niet erg. Ronald en Frits, 'een beeld zegt meer dan duizend woorden', dank voor jullie hulp hierin. Annie, bedankt voor onze samenwerking in het promoterfusie project. Chapter 2 van mijn proefschrift laat zien dat het een succes was. Herman, met jouw hulp bleef geen antilichaam, prep-kit of oligo onvindbaar. Roeska, Evert en Jos ik ben blij jullie te hebben kunnen adviseren over half dode goudvissen en het uitzoeken van nieuwe huisdieren. Jullie brachten een mooie dynamiek in het lableven.

Dongping(-etje), my dear Chi-Nederlandse colleague. Our stay in Vienna was a pleasure, your enthusiasm for art, fashion ('mooie hoed mevrouw') and music was contagious. Bea, thanks for always being warm and considerate. Ming, my sweet, quiet office neighbor and Du, always kind and interested, both of you often surprised me with sudden sharp-minded remarks. Smita and Klementina, our girltalk and coffee-drinking were a welcome break from work. Ikram and Sarah, two fine ladies in a very messy office, thanks for your help. Don Alfredo, the greatest Mexican man I know and science role model for me. Remember: Stay calm and assertive. Gabino, BioinforMagician, thanks for helping to decode the Schizoriza array. AP, office neighbor upon my arrival, you made me feel very welcome in the group. Please don't forget to look out for the fat horse! (Houtje-) Hongtao(-tje), my lab neighbor, thank you for always lightening the day with a humorous comment. Guy, discovering Japan with you and Alfredo was a real adventure. Mister Long, I will miss your enthusiastic greetings ('Mevrouw Jansweijer!!') in the morning. Stephen, underneath beard and hoodie, there turned out to hide a very nice guy. I will never forget your karaoke performances. Christophe, Kay and Jurgen, my students: teaching you was an educational experience for me as well.

Finally, to all MolGen colleagues, formerly and present, thank you for all inspiring scientific discussions, your advice and the so needed distractions from labwork: coffee, borrels, skiing, kerstdiners, beers, barbecues and more.

Beste Utrechtse Suikergroep, onze gedeelde Wortel-Suiker koffiepauze is me altijd heel goed bevallen. Beste Wageningse Molbi, PCB, Marie-José en Maria bedankt voor jullie warme welkom en jullie hulp bij allerlei praktische zaken rondom de verhuizing.

En dan mijn lieve vrienden en familie, als er één reden is om een PhD te gaan doen, dan is dat omdat je de mensen om je heen op hun mooist leert kennen. Met name de laatste fase van mijn promotie had ik niet zo goed kunnen doen zonder alle begrip en steun die ik kreeg van vrienden en familie, duizend maal dank daarvoor!

Hilde en Karin, wat jullie steun de afgelopen tijd voor mij heeft betekend is met geen pen te beschrijven. Jullie haalden alles uit de kast; luxe lunches, diners, 'stress relief serum', ontspannende doucheoliën, prachtige bloemen, bonbons, chocolademelk, martini en wijn. Maar bovenal was het de wetenschap te allen tijde bij jullie terecht te kunnen, wat mij het meest geholpen heeft.

Liesbeth, Riti, Joep en Johan, hoe wreed een weddenschap af te sluiten over drie arme AiO's! Ik wil hier niet onvermeld laten dat degene die in beide componenten van de weddenschap op mij gestemd zou hebben, de maximale score zou hebben gehaald… Maar eerlijk is eerlijk: mijn succes is te danken aan jullie steun. Zonder onze etentjes, wijnavonden en jullie herhaaldelijk uitgesproken vertrouwen in mijn kunnen, had ik niet zo netjes binnen mijn eigen gestelde tijdslimiet kunnen blijven. Maartje, heel bijzonder om zo synchroon onze promoties te beleven. Dat we zelfs in dezelfde week ons proefschrift verdedigen vind ik geweldig. Ik denk dat we dit maar tot een jaarlijks

terugkerende feestweek moeten uitroepen. Janwillem, een quote van jou uit een email aan mij, illustreert je steun het best: 'Kom lekker langs; we hebben bier, worst en koffie om de winter door te komen'. Komende winter zal ik deze zin aan jou herhalen en dan wordt 2014 jouw oogstjaar.

Yvonne, Ellen, Anne en Maarten, onze barre tocht naar de top van de Kota Kinabalu op Borneo bleek onverwachte paralellen te vertonen met momenten in mijn promotie. Dank voor deze goede repetitie, die me heeft geleerd dat met voldoende eet-en drinkstops, adempauzes en de juiste balans tussen vloeken en zingen je sámen de top wel bereikt.

Francine en Pieter, 'We zullen dóóóórgaan!', dank voor jullie interesse en jullie stroom van lieve berichten. Samen koken, eten en logeren bij jullie in Wageningen was ontspannen als vanouds en 3x30 worden met jullie was geweldig. Jeike, dank voor je goede zorgen voor je promoverende/werkende oud-huisgenoten met een lekkere lunch of eten van tijd tot tijd.

In Baarn staat een paard met een bovengemiddelde kennis van de ontwikkeling van planten. Niemand heeft mijn 'science-slam' presentatie zo vaak gehoord als dit paard. Ook formuleringen en figuren uit mijn proefschrift kregen vorm en betekenis in het bos vanaf haar rug. Tegelijkertijd voelt niemand zo feilloos aan wanneer het tijd is voor een pittige galop om het hoofd weer leeg te maken. Omdat onze gezamenlijke tochten mijn geest helder hebben gehouden, wil ik het paard Peseta en haar eigenaresse Rickie hier ook bedanken.

Lieve Oma en Opa Bril, de kiem van mijn interesse voor de biologie is voor een belangrijk deel gelegd tijdens mijn logeerpartijen bij jullie in Markelo. Mijn allereerste fotorolletje schoot ik helemaal vol met foto's van konijnenholen in het stuk bos dat bij jullie huis daar hoorde. Opa's unieke manier van kijken naar de omgeving, planten, dieren en mensen heeft mij leren observeren en de schoonheid van de natuur leren herkennen. Er bestond voor mij dan ook geen twijfel over hoe de omslag van mijn proefschrift eruit moest zien. Het schilderij dat mijn proefschrift siert, is van de oude beuk in jullie tuin en is geschilderd door Opa; een eerbetoon aan jullie bijdrage.

Lieve Nikki, mijn wijze zusje! Jij liet geen gelegenheid onbenut om op te scheppen over je zus als wetenschapper (Jouw favoriete vraag de afgelopen vier jaar: 'Wat vind jij van asymmetrische celdeling?'). Maar tegelijkertijd ben jij degene die ik bel als ik me even geen raad meer weet. Met jou als paranimf naast mij kan ik de verdediging van mijn proefschrift met een gerust hart ingaan.

Lieve MaPa, fenotype is het resultaat van genotype en omgeving. Jullie hebben mij opgevoed in het vertrouwen dat ik alles kan en mag doen wat ik wil. Mij zo veel ruimte te gunnen en me tegelijkertijd onvoorwaardelijk te steunen, heeft ervoor gezorgd dat ik mijn aangeboren voorliefde voor de natuur nu kan bekronen met een doctor-titel. I love You!

Vera.

Chapter 10

Curriculum Vitae

CURRICULUM VITAE

Vera Maria Angenesa Jansweijer werd geboren op 23 september 1982 in Amsterdam als dochter van Duveke de Vries en Bart Jansweijer. Vera groeide op in Weesp. Het nieuwe millennium vierde zij met het behalen van haar diploma aan het Gemeentelijk Gymnasium in Hilversum. Na een jaar gewerkt te hebben bij Dierenpension Naarden, begon zij in 2001 met de studie biologie aan de Wageningen Universiteit. In het collegejaar 2004-2005 nam zij zitting in het studentenbestuur van de Wageningse Studenten Roeivereniging ARGO. In 2006, rondde zij haar opleiding aan de Wageningen Universiteit af met een bachelorsdiploma biologie, specialisatierichting celbiologie. Aansluitend begon zij aan de Universiteit van Utrecht met een speciaal op onderzoek gerichte master: biomedical sciences, bioveterinary sciences. De volgende twee collegejaren deed ze allereerst een major onderzoeksproject met een studie naar de moleculair genetische achtergrond van dwerggroei bij Friese Paarden onder supervisie van dr. W. Back bij de faculteit Diergeneeskunde in Utrecht. Dit onderzoek werd gedaan in samenwerking met het Van Haeringen Laboratorium in Wageningen en het Friesch Paarden Stamboek. Haar minor onderzoeksproject deed ze vervolgens bij het Nederlands Kanker Instituut in Amsterdam. Hier deed ze een celbiologisch- en biochemisch onderzoek, onder begeleiding van prof. dr. W.H. Moolenaar en dr. M. Jongsma, naar een eerste remmende LPA receptor en via welke signaaltransductieketens deze receptor de tumorcelmigratie remt. In augustus 2008 studeerde zij af aan de Universiteit Utrecht met een major moleculaire genetica en een minor cellulaire biochemie. In september 2008 begon zij aan haar promotieonderzoek bij de groep moleculaire genetica van de faculteit Bètawetenschappen van de Universiteit van Utrecht, onder supervisie van dr. Renze Heidstra. Het proefschrift dat hier voor u ligt is het resultaat hiervan.