# TITLE PAGE

## Title: *SHR* overexpression induces the formation of supernumerary cell layers with cortex cell identity in rice

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## Abstract

The number of root cortex cell layers varies among plants, and many species have several cortical cell layers. We recently demonstrated that the two rice orthologs of the Arabidopsis *SHR* gene, *OsSHR1* and *OsSHR2,* could complement the *A. thaliana shr* mutant. Moreover, *OsSHR1* and *OsSHR2* expression in *A. thaliana* roots induced the formation of extra root cortical cell layers. In this article, we demonstrate that the overexpression of *AtSHR* and *OsSHR2* in rice roots leads to plants with wide and short roots that contain a high number of extra cortical cell layers. We hypothesize that SHR genes may share a conserved function in the control of cortical cell layer division and the number of ground tissue cell layers in land plants.

## Introduction

A vast majority of plant species, with the exception of some halophyte species, presents only one layer of endodermis (Inan et al., 2004), and the number of root cortex cell layers varies greatly among species as well as between root types in the same plant. For instance, *A. thaliana* roots have two cortex cell layers, whereas rice contains one to more than 10 cell layers, depending on the root type and its development (Coudert et al., 2010; Pauluzzi et al., 2012). In the Arabidopsis root, endodermis and cortex tissues arise from an asymmetric periclinal division of a single initial cell that is called cortical endodermal initial daughter (CEID) (Dolan et al., 1993). In rice and several species with multiple cortex cell layers, the formation of the cortex follows a process fairly similar to that described for *A. thaliana*, except for the occurrence of multiple cell divisions of a single or several CEIDs. For example, in rice, cortex cell layers may be produced following the repetitive periclinal cell divisions of a single CEID or by the divisions of several independent CEIDs (Coudert et al., 2010; Pauluzzi et al., 2012).

At the molecular level, CEID division is controlled by *SHR* and *SCR* genes, which encode two transcription factors of the GRAS family (Cui et al., 2007; Helariutta et al., 2000; Wysocka-Diller et al., 2000). *SHR* is expressed in the stele, and its protein moves outward to the CEID where it activates *SCR*. Both proteins induce CEID division through the activation of CYCLIND6;1 to separate the clonally related cortex and endodermis cell layers (Sozzani et al., 2010). Whereas SCR and SHR are both needed to induce CEID division, SHR is also needed to specify endodermis identity (Helariutta et al., 2000). SHR is believed to be both necessary and sufficient for endodermal cell specification, and its interaction with SCR prevents the extra-movement of SHR, thereby limiting the number of endodermal cell layers to one (Cui et al., 2007). *SHR* overexpression experiments in *A. thaliana* and the observation of increased SHR movement resulting from the reduction of *SCR* expression demonstrate that SHR controls the rate of CEID division in *A. thaliana* (Helariutta et al., 2000; Nakajima et al., 2001). Moreover, based on an SCR endodermis marker, the presence of suberin or immunolocalization of cell wall antibodies in extra cell layers, these experiments concluded that these supernumerary cell layers have an endodermis cell identity (Helariutta et al., 2000; Nakajima et al., 2001), suggesting that SHR may control the endodermis cell layer number. In our work, root expression of *OsSHR1* or *OsSHR2,* which are the two rice *SHR* orthologs, increased the root ground tissue cell layer number; this phenomenon was observed to be correlated with SHR movement (Wu et al., 2014). We demonstrated that, in all cases, the overexpression of *AtSHR* or expression of *OsSHR1* and *OsSHR2* in *A. thaliana* induced the formation of supernumerary cortex cell layers but not, as previously suggested, the formation of extra endodermis cell layers. These results confirmed the hypothesis that the movement/ectopic expression of SHR controls cortex formation (Wu et al., 2014). Therefore, we proposed a model of the regulation of the formation of multiple layers of cortex in plants that relies on SHR movement.

In this paper, we tested the predictions of our model by increasing the expression of *AtSHR* and *OsSHR2* in rice to confirm the likelihood that *SHR* regulates the cortex cell layer number in plants.

## Materials and methods

### Plant material and growth conditions

The growth conditions of *Nipponbare* (*Oryza sativa* L.) plants are described in Sallaud et al. (2003). The transgenic lines used in this study (OE *AtSHR* (n=265), OE *OsSHR2* (n=290) and the control lines (n=90)) were obtained in the laboratory and analyzed at T0 generation.

### Constructs and plant transformation

The *Agrobacterium tumefaciens* EHA105 strain, which harbors the pCAMBIA 5300 vector (CAMBIA, Canberra, Australia) as a binary vector, was used for the transformations. The binary vectors comprised a T-DNA cassette containing CDS sequence of the SHR gene driven by the ubiquitin promoter of maize. The CDS sequence (1596 bp) of the *SHR* gene (At4g37650) was cloned in the pCAMBIA 5300 vector (CAMBIA, Canberra, Australia) by the Genscript company (Piscataway, NY state, US) using XmaI/KpnI restriction sites, and the CDS sequence of the OsSHR2 gene (OsO3g31880) (1812 pb) was cloned in the lab using XmaI restriction sites. Empty vector and both *AtSHR*- and *OsSHR2*-containing vectors were then transferred into *Agrobacterium tumefaciens* EHA 105 strain (Hood et al.) by the freeze–thaw transformation method (Chen et al., 1994). The three constructs were used to transform embryogenic calli prepared from rice mature seed embryos (Sallaud et al., 2003). The regenerated hygromycin-resistant plants, overexpressing *AtSHR*, *OsSHR2* and control lines were cultivated on Murashige and Skoog agar medium containing 50-mg/l hygromycin. Transgenic T0 seedlings were phenotyped directly in tubes. Samples of roots and leaves were then fixed, and the plants were transplanted in soil in a greenhouse.

### Fresh root sectioning

Root tips were cut with a sharp blade and placed parallel, as described in Henry et al. (2016); then, they were embedded in a drop of 3% melted agarose (50°C). Patches containing root tips were inserted into 3x1x1-cm wells filled with 3% melted agarose. After solidification, the blocks were resized and glued onto a vibratome plate to be sliced. The parameters of the vibratome (Hm650v (Thermo Scientific Microm)) were as follows: speed: 30; frequency: 70; amplitude: 0.8; and thickness: 60 µm. Sections were transferred into chamber slides (Lab-teak 177402) for immunostaining or onto slides humidified with 1X phosphate-buffered saline (PBS, Sigma-Aldrich P3813) for direct observation.

### Immunolocalization with cell wall antibodies

Sections placed into chamber slides (3 per chamber) were first rinsed with 0.1-M glycine supplemented with 1X PBS and then twice with 1X PBS for 10 minutes each. A PBS solution containing 5% bovine serum albumin (BSA, Thermo Fisher 37520) was added overnight at 4°C under agitation. Primary antibodies diluted 1/10 in blocking solution were applied overnight at 4°C under agitation. Before the application of the secondary antibody, the sections were rinsed 3 times with PBS 1X for 10 minutes each. The secondary antibody was coupled to a fluorophore (Alexa 546 anti-mouse antibody (Invitrogen A11060)) and diluted 1/500 in blocking buffer. Root sections were incubated

for 2 hours under agitation and then rinsed 3 times with 1X PBS under agitation for 10 minutes each. The chambers were then removed from the glass substrate, and a few drops of Mowiol mounting media (Sigma-Aldrich 81381) were added. Finally, a coverslip was added to each glass slide, and the slides were allowed to dry for 36 hours at 4°C in the dark; numbers of lines analyzed for OE *AtSHR* (n=12), OE *OsSHR2* (n=9) and control (n=10).

**Immunolocalization with AtSHR- and OsSHR2-specific antibodies**

Radicles were grown on petri dishes, as described above. Roots were sampled at the 6-day-old stage, fixed in 4% paraformaldehyde in 1X PBS (pH=7.4) overnight at 4°C, and rinsed twice with 1X PBS. The fixed tissues were dehydrated in ethanol, cleared in Histochoice Clearing Agent (HistoClear, Sigma Aldrich), and embedded in Paraplast (Fisher). Tissue sections (6 µm thick) were obtained using a Leica RM2255 microtome and mounted on SuperfrostPlus slides (Fisher). Immunolocalizations were performed at Histalim Company (Montpellier, France) using a Ventana Medical Systems Discovery XT automated immunostainer. After deparaffinization, the samples were treated with trypsin-based antigen retrieval solution. Then, primary antibodies were applied at 1:100 dilution. Antibodies for actin (AS13 2640, AGRISERA) and the Golgi apparatus (AS08 325, AGRISERA) antibodies were used to control the sample quality. AtSHR and OsSHR2 were detected using specific antibodies produced in sheep and rabbit, respectively. The AtSHR antibody was kindly provided by R. Swarup (Nottingham University UK), whereas the OsSHR2 antibody was obtained from rabbit by Eurogenetec Company using the synthetic peptide CSPPDQPHKSYPSSRG. The pre-diluted universal secondary antibody (n. 760–4205; Ventana Medical Systems) was then added. Hematoxylin was used as a counterstain. Immunoreactive sites were visualized using a diaminobenzidine tetrahydrochloride preparation (DAB, Sigma). Control experiments were performed similarly, but without the first antibody step and diluted, as described earlier. Immunolocalization was performed in two independent lines for OE OsSHR2 and AtSHR lines and 3 for Nipponbarre control lines.

**Quantitative reverse-transcriptase polymerase chain reaction (RT-PCR) analysis**

Total RNA was extracted from 200 mg of 5-week-old roots and leaves of transgenic plants (RNA Isolation Kit, Macheray and Nagel, Germany). cDNA synthesis was performed with M-MLV RT (Pomega, US) with oligo dT, according to the manufacturer's recommendations. Primer pairs were designed to amplify fragments of *AtSHR* and the *OsEXP* housekeeping gene (Os06g11070). The following primers were used for real-time PCR: AtSHR_Fwd GTTTTCCAAGGACGAGCAAC; AtSHR_Rev: CACAAGCCACAAGATCAACG; Exp_Fwd: CGGTTAGCTAGAGTTCATGTGAGA; Exp_Rev: ATTGGAGTAGTGGAGTGCCAAA. Quantitative RT-PCR was performed using SYBR green master mix (Applied Biosystems), and the results were normalized against EXP expression. The experiment was repeated twice using two biological replicates.

**Confocal microscopy**

Immunolocalized sections were observed with a confocal microscope. Confocal images were obtained using a Zeiss LSM 510 multiphoton microscope or confocal Leica SP8 at a

magnification of 40x for radicles. The cell walls were first visualized via autofluorescence using a 720-nm (Chameleon) or 405-nm laser. The secondary antibody—Alexa 546 anti-mouse—was then visualized using a He/Ne laser with a wavelength of 543 nm or a laser with a wavelength of 561 nm.

**Bright-field and autofluorescence microscopy**

Bright-field and autofluorescence observations were collected from root sections using a Leica DM4500 microscope at a magnification of 40x. For autofluorescence observations, images were taken with the "A" filter cube (excitation range: ultraviolet [UV]; excitation filter: BP 340–380; suppression filter: LP 425). This microscope was coupled to a color Retiga 2000R camera (QIMAGING, Canada) running Volocity image acquisition software (Improvision, UK).

## Results

### *AtSHR* and *OsSHR2* overexpression in rice induces thicker and shorter roots containing supernumerary ground tissue cell layers

We overexpressed *AtSHR* and *OsSHR2* under the control of the maize ubiquitin promoter in the *Oryza sativa* var. *Nipponbare* rice cultivar. Compared to control seedlings, more than 60% of the transgenic seedlings obtained presented wider roots (**Figure 1a**). In the greenhouse, transgenic plants overexpressing *AtSHR* and *OsSHR2* had a reduced height and rolled-up leaves (data not shown) and were completely sterile (**Figure 1b**). We cross-sectioned the wider roots to evaluate the effects of SHR overexpression at the cellular level. Compared to control lines, the formation of a large number of supernumerary ground tissue cell layers is clearly visible in the radial sections (**Figure 1c**). On average, the root diameter increased twofold, and the ground tissue layer number increased 6-fold (**Table 1**). The wide root phenotype of AtSHR-overexpressing lines is attributable to the strong expression of *AtSHR* at the transcript level (**Figure S1**), as observed by qRT-PCR. We also looked for AtSHR and OsSHR2 protein accumulation in rice roots. As expected, AtSHR protein was absent in the control lines (**Figure S2b**). Interestingly, OsSHR2 protein is present in the nucleus and cytoplasm of almost all root cells in the control lines, from the stele tissues to the exodermis (**Figure S2c**). Immunolocalization with AtSHR and OsSHR2 antibodies revealed protein accumulation in the nucleus and cytoplasm of all cell layers in the wider roots (Figures S2e and S2h).

### Extra ground tissue cell layers have cortex cell identity

We hypothesized that the supernumerary cell layers present in *SHR*-overexpressing lines can be any type of ground tissue (e.g., sclerenchyma, exodermis, inner cortex/outer cortex, or endodermis) and attempted to clarify their identity using tissue-specific markers. Cross-sections were first used to determine the cell identity using cell morphological traits (**Figure 2**). Single typical endodermis/exodermis cell layers are easily observable in the transverse sections of overexpressing lines. These cell types are characterized by a strong decrease in cell wall UV fluorescence along the radial walls because of the presence of the Casparian strip (**Figures 2a, 2b, and 2d**). Single typical endodermis cell layers can be easily identified in AtSHR- and OsSHR2-overexpressing cross sections (**Figures 2f and 2i**). Moreover, the schizogenous aerenchyma, which

develops as cell wall separation in the cortex (see **Figure 2c** in the control), can be identified in all the supernumerary cell layers (**Figures 2g and 2j**).

We recently screened a large library of plant cell wall antibodies and identified cortex- and sclerenchyma-specific markers (Henry S, 2016). Cortex identity was then confirmed by the immunolocalization of M14, a cortex-specific marker that stained all the supernumerary cell layers for *AtSHR* (**Figures 3a and 3b**) and *OsSHR2* (**Figure 3c**) overexpressing lines. We then stained transverse sections for FASGA (Tolivia and Tolivia, 1987) and immunolocalized M150, which are markers of sclerenchyma. A single irregular sclerenchyma cell layer is visible in **Figures 3e and 3f** for M150 and in **Figures 3h and 3i** for FASGA**,** excluding a possible sclerenchyma origin of these extra cells and providing strong support for their identity as cortex cells. We previously demonstrated the presence of two subcortical domains in rice roots (Henry S, 2016) with a single cell layer called the inner cortex surrounding the endodermis and stele and tissue composed of multiple cell layers that we termed the outer cortex. We examined the immunolocalization of M107 and M133, which are markers that stain only the outer cortex in ground tissues and some vascular tissue inside the stele on wider *AtSHR*- and *OsSHR2*-overexpressing transverse sections. Both markers stained all the supernumerary cell layers for *AtSHR* (**Figures 3k, 3l, 3o, and 3p**) and *OsSHR2* (**Figures 3m and 3q**) overexpressing lines. The single inner cortex cell layer visible in the control lines (**Figure 3r**) is not distinct in *AtSHR* and *OsSHR2*, suggesting that all the cortex cell layers have an outer cortex cell identity. In summary, *AtSHR*- and *OsSHR2*-overexpressing roots have a similar internal structure as the control lines with a single layer of endodermis, multiple outer cortex cell layers, single cell layers of exodermis, sclerenchyma and epidermis. The only differences are the absence of an inner cortex and the presence of supernumerary cell layers with an outer cortex identity.

## Discussion

### SHR controls cortex formation in rice and *A. thaliana*

The enhancement of protein movement or the overexpression of *SHR* leads to the formation of supernumerary cortex cell layers in *A. thaliana* (Wu et al., 2014) and rice (based on our results for *AtSHR* and *OsSHR2*). Increased divisions of the initial cells in *A. thaliana* can be observed upon the down-regulation of genes involved in SHR movement restriction, such as *JACKDAW* and *BALD IBIS* (Long et al., 2015), or the expression of rice *SHR* orthologs, for which cell-to-cell movement is enhanced (Helariutta et al., 2000; Nakajima et al., 2001; Wu et al., 2014). This outcome is consistent with previously published results and provides a model in which SHR modulates ground tissue divisions, thereby generating clonally related endodermis and cortex cell layers. In *A. thaliana*, the *shr* mutant fails to induce CEID division (Helariutta et al., 2000). In rice, however, we do not know if *OsSHR1* or *OsSHR2* or both are essential for CEID division. More data will be needed to draw a definitive and consistent conclusion regarding CEID division. In all cases, a single functional endodermis cell layer is formed when *SHR* is overexpressed, confirming that *AtSHR* is necessary, but not sufficient to specify an endodermis identity (our results) in rice and that an independent stele-derived signal is also required in rice and *A. thaliana* (Wu et al., 2014). Recently, *SCR* and *SCL23* were demonstrated to be redundantly required for endodermis specification with *SHR* (Long et al., 2015). SCL23 moves rootward from the stele tissue to specify an endodermis identity with SCR and SHR (Long et al., 2015) and could be the stele-derived signal needed, together with SHR and SCR, to specify an endodermis identity, as postulated in our previous paper (Wu et al., 2014).

### Cortex cell layer number variation: Is *A. thaliana* the rule or the exception?

A large body of evidence demonstrates that the regulation of AtSHR movement in *A. thaliana* is complex. For instance, several redundant members of the BIRD family, among others, function to restrict AtSHR mobility by promoting the nuclear localization of AtSHR, presumably blocking its movement (Moreno-Risueno et al., 2015). Based on limited evidence, mostly relating to rice SCR ortholog interacting with OsSHR1 and the conserved expression pattern of *OsSHR1* ortholog in rice, the SCR/SHR model described in *A. thaliana* was suggested to be conserved in rice and probably in all land plants. This model was also used to explain why a single endodermis cell layer is always formed (Cui et al., 2007). We recently demonstrated that compared to AtSHR, OsSHR1 and OsSHR2 can move much further in *Arabidopsis* and induce several additional cortex cell layers, suggesting that the model should be amended (Wu et al., 2014). The overexpression of *AtSCR* also blocks AtSHR movement but not OsSHR2 movement thought AtSCR promotes the nuclear localization of both SHR proteins. This effect suggests that nuclear localization itself is not sufficient to prevent SHR mobility, as previously suggested (Cui et al., 2007). Finally, OsSHR2 is present in all cell layers in rice roots with the exception of epidermis in WT (**Figure S2**). This observation confirms that the movement or expression of SHR2 is not restricted to the endodermis in rice roots and reminiscent of the extra-movement of OsSHR2 in *A. thaliana*. These findings raise questions about (i) the complex network that regulates the movement of SHR in *A. thaliana*, which may be the exception rather than the rule, and (ii) the complex control mechanism for the movement of SHR, which may be present only in *A. thaliana* (Wu et al., 2014). The variation of the root diameter

and number of cortex cell layers in rice roots seems to be a consequence of the variation in the number of outer cortex cell layers. In the three types of rice lateral roots, which differ in their diameter, we previously demonstrated that this phenomenon can also be attributed to a variation in the number of outer cortex cell layers (Henry S, 2016).

It cannot be excluded that the regulation of cortex cell layer formation may involve other factors, such as the regulation of CYCLIN D activity (Pauluzzi et al., 2012; Wu et al., 2014). A deeper functional analysis of rice *SHR* and *SCR* orthologs is needed to gain further insight regarding the SHR/SCR model of cortex formation in land plants, especially in species having multiple cortex cell layers.

**Acknowledgements**

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## Figure legends

**Figure 1: Phenotype of *AtSHR- and OsSHR2*-overexpressing rice plants**
(a) Transgenic *AtSHR*-overexpressing plant (left) and *OsSHR2*-overexpressing plant (right) with wider roots compared to an empty vector control line (middle); bars=0.5 cm.
(b) *AtSHR*- (left) and *OsSHR2*-overexpressing (right) plants are smaller compared to the empty vector control (middle).
(c) Root radial cross sections observed under UV light. Cross sections of *AtSHR*- (left) and *OsSHR2*-overexpressing (right) rice roots containing a large number of extra ground tissue cell layers compared to the empty vector control root cross section (middle) at the same scale; bars=20 µm.

**Figure 2: *AtSHR*- and *OsSHR2*-overexpressing lines are characterized by one layer of endodermis and exodermis and several presumptive cortex layers**
Polar-transformed view of the transverse sections of 6-day-old *Nipponbare* roots (a), *AtSHR*-overexpressing roots (e) and *OsSHR2*-overexpressing roots (h). (b-d), (f-g) and (i-j) are magnified images of (a), (e) and (h), respectively.
The tissue layers are labeled follows: stele (st), a large central metaxylem (mx) surrounded by metaxylem (x) vessels, and ground tissue (gt) composed of several cell layers: one layer of endodermis (en), one layer of inner cortex (ic), several layers of outer cortex (oc), one layer of sclerenchyma (sc), and one layer of exodermis (ex). The endodermal and exodermal layers can be easily identified because of the strong reduction of UV fluorescence visible in their radial cell walls (red arrowheads). The outermost cell layer is the protective tissue, which is called the epidermis (ep). Two types of cortical layers can be identified: the inner cortex (ic), which is next to the endodermis (ed), and the outer cortex (oc), which will eventually form the schizogenous aerenchyma (ae); these layers are characterized by their cell wall separation (red diamonds). Supernumerary cell layers found in *AtSHR*- and *OsSHR2*-overexpressing roots are similar to the outer cortex of the control line. Bars=20 µm

**Figure 3: *AtSHR* and *OsSHR2* overexpression induces supernumerary cell layers of outer-cortex**
The immunolocalization in radial sections of *AtSHR, OsSHR2* overexpressing lines and the control (Nipponbare) have been done with tissue-specific antibodies. For each antibody, merged images of transverse sections were obtained under a confocal microscope after immunohistological staining: yellow: antibody staining at 561 nm; blue: cell wall autofluorescence under UV light.
(a) Radial sections of enlarged roots of the *AtSHR*- and (c) *OsSHR2*-overexpressing lines stained with M14 antibody, which is specific to both outer and inner cortex tissues. (b) Magnified image of the central part of the section (a). (d) Antibody immunolocalization of a radial section of a 6-day-old Nipponbare root as a control.
(e-f) Radial sections of *AtSHR*- and *OsSHR2*-overexpressing lines stained with the M150 antibody, which is specific to sclerenchyma tissue (white arrow). (g) Antibody staining of a radial section of a control root.
(h-i) Radial sections of *AtSHR*- and *OsSHR2*-overexpressing lines stained with FASGA, which is also specific to sclerenchyma tissue (black arrow). (j) FASGA staining of a radial section in the control.
(k and m) Radial sections of *AtSHR*- and *OsSHR2*-overexpressing lines stained with M107 antibody, which is specific to outer cortex tissue within ground tissue but also stains some

stele tissues. (l) Magnified image of the central parts of the sections in (k). (n) Antibody staining of a radial section in a control root.
(o and q) Radial sections of *AtSHR*- and *OsSHR2*-overexpressing lines stained with M133 antibody, which is also specific to outer cortex tissue. (p) Magnified image of the central parts of the sections in (o). (r) Antibody staining of a control radial section. en, endodermis; c, cortex; ic, inner-cortex; oc, outer cortex; and sc, sclerenchyma. Bars=20 μm.

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## Highlights

>Cortex and endodermis cells form the ground tissue (GT) in roots
>The number of root cortex cell layers varies among plant species
>Rice is a species with multiple ground cell layers
>SHR (SHORT-ROOT) control cortex cell layer number

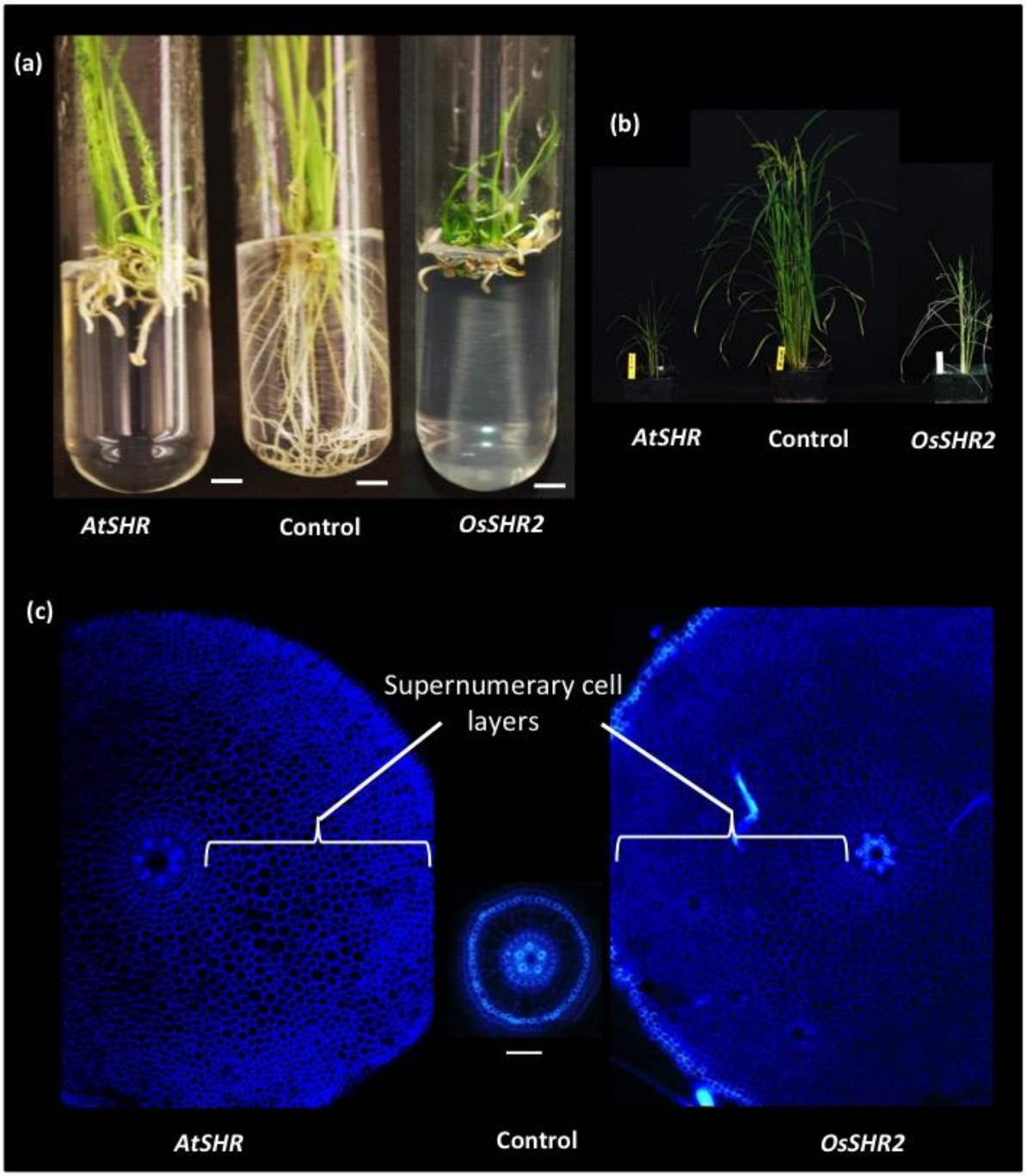
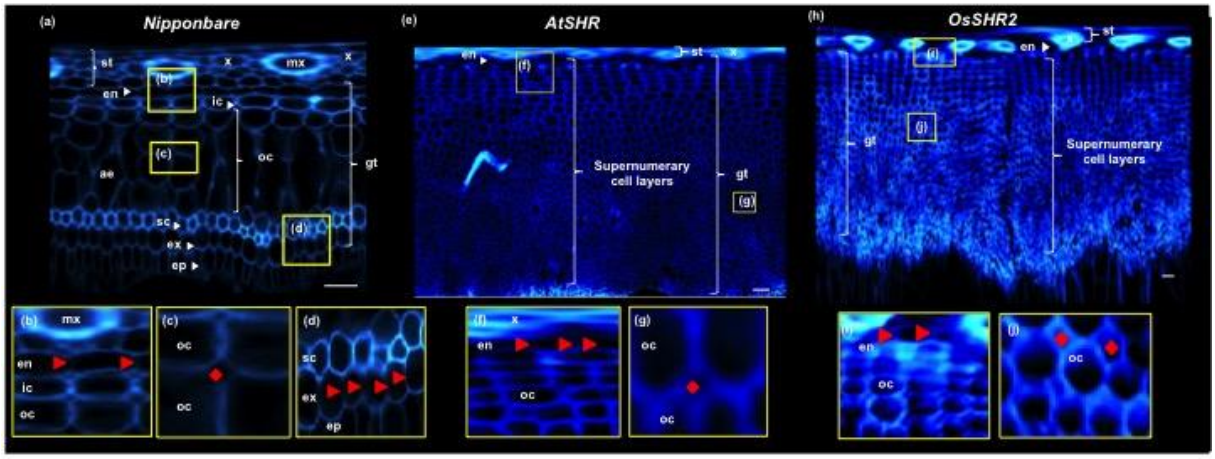

(a)
AtSHR
Control
OsSHR2
(b)
AtSHR
Control
OsSHR2
(c)
Supernumerary cell layers
AtSHR
Control
OsSHR2

**Figure 1**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Supernumerary cell layers
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OsSHR2
st
en
(i)
gt
(j)
Supernumerary cell layers
(b)
mx
en
ic
oc
(c)
oc
oc
(d)
sc
ex
ep
(f)
x
en
oc
(g)
oc
oc
(i)
en
oc
(j)
oc

**Figure 2**

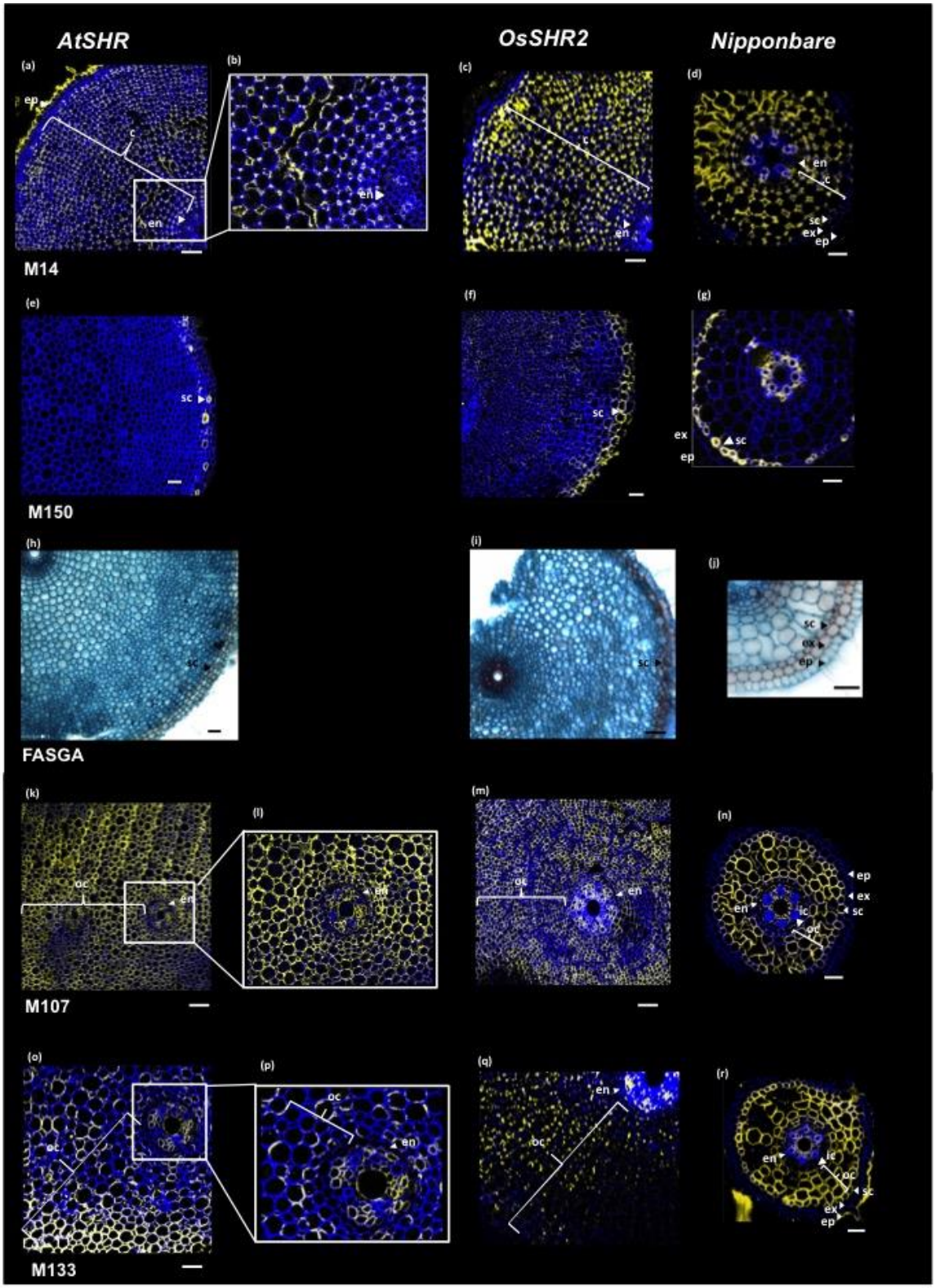
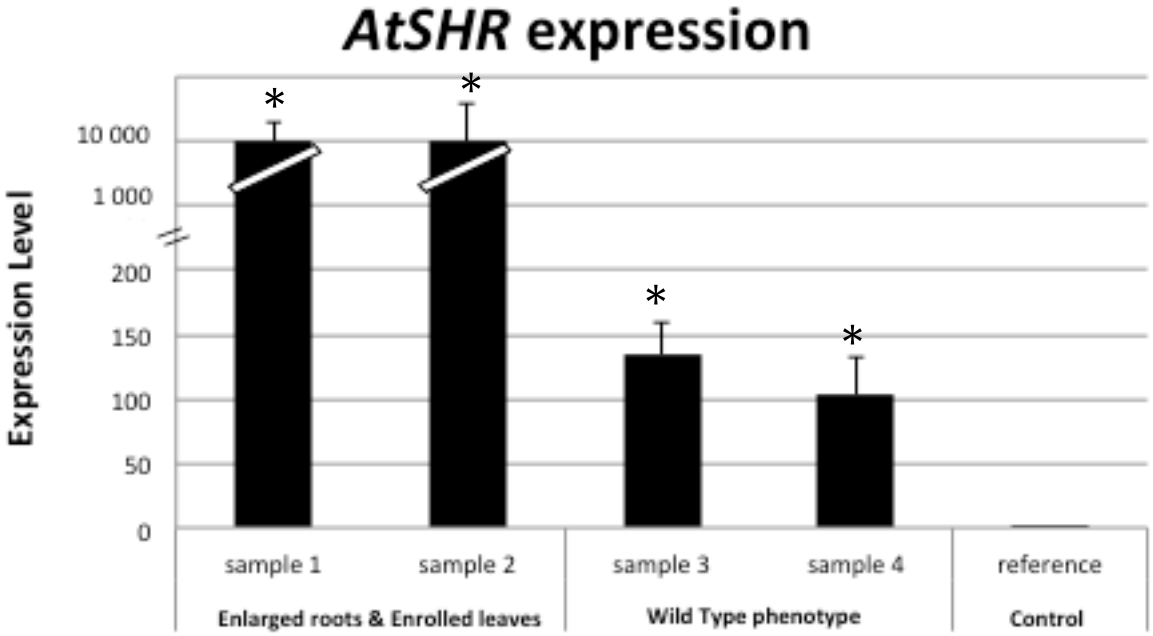

AtSHR
OsSHR2
Nipponbare
(a)
(b)
(c)
(d)
ep
c
en
sc
ex
M14
(e)
(f)
(g)
M150
(h)
(i)
(j)
FASGA
(k)
(l)
(m)
(n)
oc
ic
M107
(o)
(p)
(q)
(r)
M133

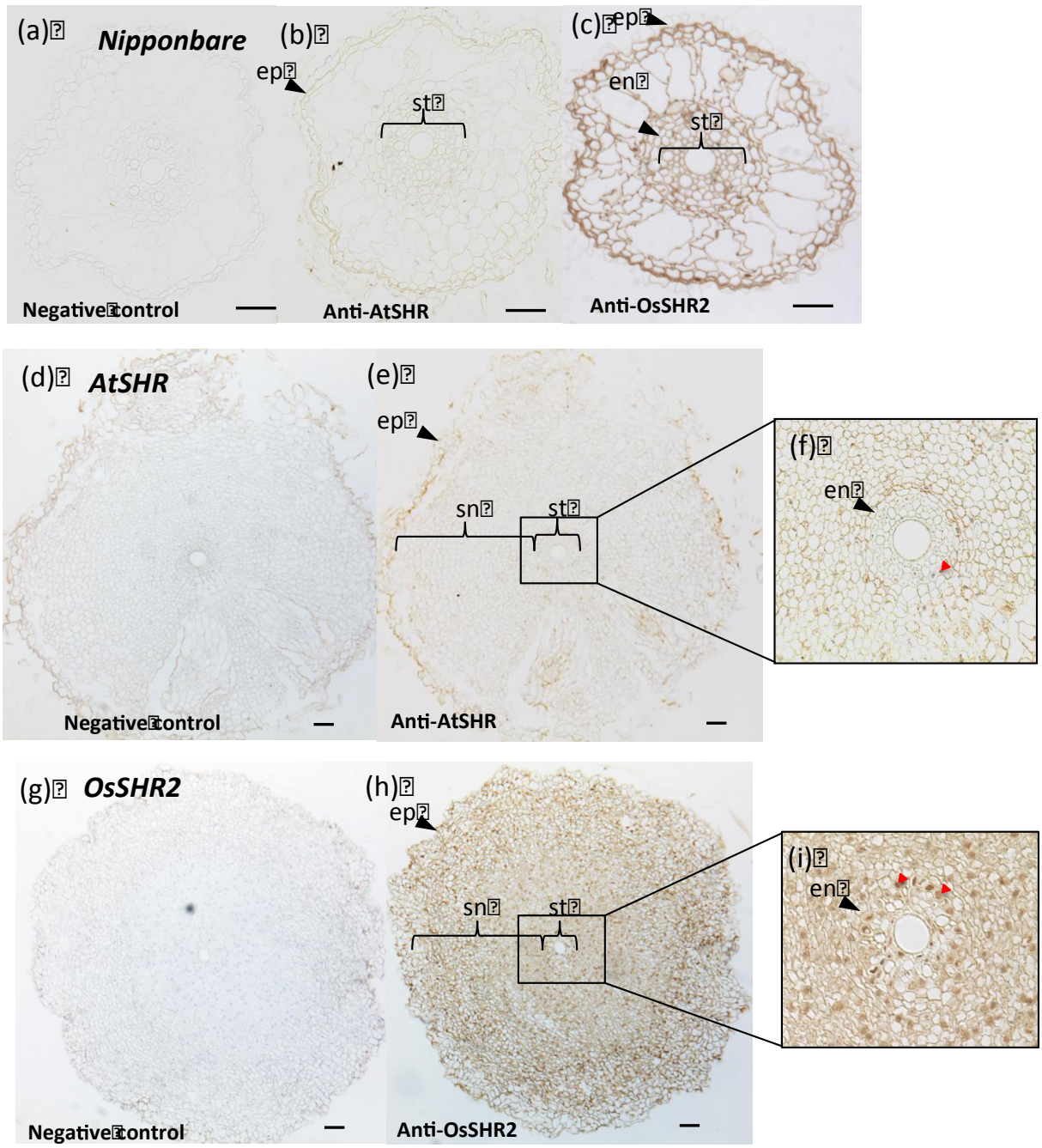
**Figure 3**




**Supplemental Figure 1: mRNA level analysis for *AtSHR* by quantitative real time PCR in lines overexpressing *AtSHR*.**

One month old transgenic plants and controls (empty vector lines) were used for investigating AtSHR expression. Samples 1 and 2 represent plants with enlarged roots and rolled-up leaves; Samples 3 and 4 represent a plant with a wild-type phenotype; and the reference sample is an empty vector control.

Values are the means of two biological replications ± standard error. Student t-test, p-value < 0,05 (*)




**Supplemental Figure 2: Immunolocalization of AtSHR & OsSHR2 proteins in control and overexpressing lines.**

Immunolocalisations of AtSHR (courtesy of R. Swarup) and OsSHR2 antibodies (designed in the lab) have been done on one-month old roots of overexpressing and control plants.

In Nipponbare roots (a-c), AtSHR protein is lacking as expected and do not cross-react.

OsSHR2 protein localize in all cell layers with the exception of epidermis.

In overexpressing *AtSHR* lines (d-f) and OsSHR2 lines (g-i), SHR proteins localize in nucleus and cytoplasm of all cells including supernumerary cells.

(st) stele; (ep) epidermis; (en) endodermis; (sn) supernumerary cell layers.

Negative control: immunolocalization with secondary antibody only; f) and g) are close-up view of stele of e) and h) respectively. Red arrowheads: antibody stained nuclei

**Table 1: *AtSHR* & *OsSHR2* overexpression induce enlarged roots**

Root diameter and number of ground tissue cell files are compared between overexpressed lines with enlarged roots (*AtSHR,* n=10; OsSHR2, n=8) and empty vector lines (Control, n=10). Student t-test, p-value < 0.001

| Lines | Root diameter (µm) | Ground tissue cell files number |
|---|---|---|
| *AtSHR* | 964,8 ± 25 (***) | 33 ± 6 (***) |
| *OsSHR2* | 628± 92 (***) | 30.60± 1.12 (***) |
| Control | 327 ± 25 | 7,5 ± 0,5 |