Journal

Plant Biotechnology Journal (2016) 14, pp. 581–591 doi: 10.1111/pbi.12400

Compact tomato seedlings and plants upon overexpression of a tomato chromatin remodelling ATPase gene

Adam Folta[1], Joachim W. Bargsten[2,†], Ton Bisseling[1], Jan-Peter Nap[2,3] and Ludmila Mlynarova[1,*]

[1]Laboratory of Molecular Biology, Plant Sciences Group, Wageningen University and Research Centre, Wageningen, The Netherlands

[2]Applied Bioinformatics, Bioscience, Plant Research International, Plant Sciences Group, Wageningen University and Research Centre, Wageningen, The Netherlands

[3]Expertise Centre ALIFE, Institute for Life Science & Technology, Hanze University of Applied Sciences Groningen, Groningen, The Netherlands

Received 26 February 2015;
revised 13 April 2015;
accepted 16 April 2015.
*Correspondence (Tel +31 317 483273;
fax +31 317 418094
email ludmila.mlynarova@gmail.com)
†Present address: ENZA Zaden, The Netherlands.

Keywords: chromatin remodelling, growth, plant habit, SWI/SNF2, tomato.

Summary

Control of plant growth is an important aspect of crop productivity and yield in agriculture. Overexpression of the *AtCHR12/23* genes in *Arabidopsis thaliana* reduced growth habit without other morphological changes. These two genes encode Snf2 chromatin remodelling ATPases. Here, we translate this approach to the horticultural crop tomato (*Solanum lycopersicum*). We identified and cloned the single tomato ortholog of the two Arabidopsis Snf2 genes, designated *SlCHR1*. Transgenic tomato plants (cv. Micro-Tom) that constitutively overexpress the coding sequence of *SlCHR1* show reduced growth in all developmental stages of tomato. This confirms that *SlCHR1* combines the functions of both Arabidopsis genes in tomato. Compared to the wild type, the transgenic seedlings of tomato have significantly shorter roots, hypocotyls and reduced cotyledon size. Transgenic plants have a much more compact growth habit with markedly reduced plant height, severely compacted reproductive structures with smaller flowers and smaller fruits. The results indicate that either GMO-based or non-GMO-based approaches to modulate the expression of chromatin remodelling ATPase genes could develop into methods to control plant growth, for example to replace the use of chemical growth retardants. This approach is likely to be applicable and attractive for any crop for which growth habit reduction has added value.

Introduction

An important aspect of crop productivity and yield in agriculture is plant growth (Del Moral *et al.*, 1985; Özalkan *et al.*, 2010). Control of plant growth is therefore an important feature of proper crop management. Plant growth is affected by both internal genetic factors and external environmental conditions. Plants evolved finely orchestrated mechanisms to regulate growth either in response to short-term adverse environments or as programmed part of their life cycle (Claeys and Inze, 2013). A growing body of evidence indicates that epigenetic modifications provide mechanisms that help plants to integrate intrinsic and environmental signals (Gutzat and Mittelsten Scheid, 2012; Sahu *et al.*, 2013; Seffer *et al.*, 2013). In such epigenetic modifications, chromatin remodelling plays a major role. Chromatin remodelling is based on the activity of multiprotein enzymes that are conserved from yeast to man. These enzymes alter the accessibility of chromatin to the transcriptional machinery (Kennison, 1995; Vignali *et al.*, 2000), particularly in case of inducible or increased gene expression (Narlikar *et al.*, 2002; Sudarsanam and Winston, 2000; Tsukiyama, 2002). Prominent chromatin remodelling moieties are ATPase-dependent chromatin remodelling complexes such as SWI/SNF, which utilize ATP hydrolysis to generate the energy to restructure chromatin.

Previously, we have shown a functional relationship between the expression of the *Arabidopsis thaliana AtCHR12* and *AtCHR23* genes and the regulation of plant growth. These two genes are paralogs encoding chromatin remodelling ATPases of the SWI/SNF2-type. Overexpression of *AtCHR12* resulted in the growth arrest of primary buds, as well as reduced growth of the primary stem (Mlynarova *et al.*, 2007). Overexpression of *AtCHR23* led to the reduced growth of seedlings and vegetative rosette compared to the wild type (Folta *et al.*, 2014). Upon applying abiotic stress, overexpressing mutants were reduced in overall growth significantly more than wild-type Arabidopsis. Except for this reduction in growth, the overexpressing plants showed no other morphological changes. Another Arabidopsis Snf2-type chromatin remodeler BRAHMA (BRM) was shown to affect growth regulation. A loss-of-function mutant of this gene shows reduced growth and is early flowering (Tang *et al.*, 2008). BRM promotes vegetative growth by the suppression of PcG activities at the SHORT VEGETATIVE PHASE locus (Li *et al.*, 2015).

Modulated expression of chromatin remodelling genes, either through genetic engineering or by genetic selection, could therefore present an innovative technology for the control of plant growth in crops. However, it requires that Arabidopsis growth regulation is a good model for growth regulation in crops.

To be able to investigate whether comparable phenotypes are obtained in a crop upon overexpression of a SWI/SNF2 chromatin remodelling ATPase, we have analysed all putative Snf2 family members in all currently available plant genomes (Bargsten *et al.*, 2013). In tomato (*Solanum lycopersicum*), this analysis identified one gene that is the putative ortholog of both *AtCHR12* and *AtCHR23*. The two Arabidopsis paralogs are likely the result of a gene duplication specific to the Arabidopsis genus. In view of the evolutionary relationships, it was suggested that the one tomato

ortholog would combine the functions of both its Arabidopsis counterparts (Bargsten *et al.*, 2013).

Tomato is a major vegetable crop with increasing popularity over the last decades. Commercial production of tomato, either field- or greenhouse-grown, makes use of transplanting pre-grown seedlings. The major benefit of the use of such tomato transplants is uniformity, earlier production and it results in increases in crop yield and quality (Hochmuth and Hochmuth, 2012). In addition, smaller plants with equal yield and/or smaller fruits with better taste are attractive breeding targets.

Here, we show that overexpression of a single chromatin remodelling gene affects plant growth habit markedly. We have isolated and cloned the tomato ortholog of the Arabidopsis *AtCHR12* and *AtCHR23* genes. Transgenic tomato plants constitutively overexpressing this gene show reduced growth at all developmental stages. Compared to wild type, the transgenic seedlings have significantly shorter roots, hypocotyls and reduced cotyledon size. The growth reduction also affects vegetative growth, resulting in smaller, more compact, tomato plants with severely compacted reproductive structures and smaller flowers. These results show that modulating the expression of chromatin remodelling ATPase genes could develop into novel methods to control plant growth habit that may prove attractive for agricultural or horticultural practice.

Results

Characterization and isolation of the coding sequence of the tomato Snf2 ATPase gene *SlCHR1*

Detailed phylogenetic analyses have shown that the two *Arabidopsis thaliana* Snf2 ATPase in-paralogs, *AtCHR12* (*At3g06010*) and *AtCHR23* (*At5g19310*), have only one tomato (*Solanum lycopersicum*) ortholog (Bargsten *et al.*, 2013). In the ITAG1 tomato genome release (10 March 2010), this gene was annotated as *SL100sc05189_42.1.1* (http://solgenomics.net/), whereas in the more recent ITAG2.4 annotation release (23 February 2014), two genes are predicted in the same genomic region: *Solyc01g079690.2.1* and *Solyc01g079700.2.1* (Figure 1). ITAG2.4 *Solyc01g079700.2.1* is identical to the first two exons and first intron of *SL100sc05189_42.1.1* apart from an additional 5′-UTR and 16 additional bases including stop codon at the 3′-end. ITAG2.4 *Solyc01g079690.2.1* covers most of the ITAG1 *SL100sc05189_42.1.1* gene; it corresponds to ITAG1 *SL100sc05189_42.1.1* from its third exon, except for an additional exon at the 5′-end and 3′-UTR sequence (Figure 1). The ITAG2.4 *Solyc01g079700.2.1* gene does not contain any domain that is characteristic for chromatin remodelling ATPases (Bargsten *et al.*, 2013).

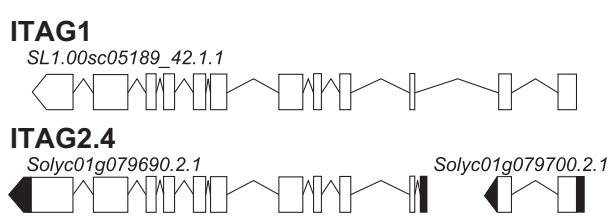

Figure 1 Layout of the structure of *SlCHR1* gene in two different tomato genome annotations, ITAG1 and ITAG2.4. Exons are illustrated as boxes, and lines represent introns. White-filled boxes show exons common to both annotations, and black boxes represent exons specific for the ITAG2.4 annotation.

To determine the correct configuration in the Heinz tomato genome, exploratory RT-PCR analyses were undertaken with several primer sets specific for each annotation (Figure S1a). Total RNA was isolated from both leaves and flowers. No PCR product was obtained with primer sets specific for cDNA according to the ITAG2.4 annotation (Figure S1b). In contrast, the expected PCR product was obtained with primer set specific for the ITAG1 annotation (Figure S1b). Moreover, in publicly available RNA-seq libraries for *S. lycopersicum* (Sato *et al.*, 2012), no reads covering the 16 bases present at the 3′end of the *Solyc01g079700.2.1*, nor reads mapping to the first exon of *Solyc01g079690.2.1*, are present. In contrast, a number of reads are present that start in the second exon of *Solyc01g079700.2.1* and continue into the second exon of *Solyc01g079690.2.1*. (Figure S2). These results show that the ITAG1 annotation is most close to the true situation in the tomato genome and we therefore based the isolation of the coding sequence on the ITAG1 annotation of *SL100sc05189_42.1.1*. In the remainder of this study, we refer to this tomato gene as *SlCHR1* and to its coding sequence as c*SlCHR1*.

The coding sequence of *SlCHR1* was amplified by RT-PCR from total RNA isolated from leaves of *in vitro*-grown tomato cv. Heinz 1706. The DNA sequence of c*SlCHR1* confirms the existence of a single (3321 bp) transcript, essentially matching the ITAG1 annotation, except for three changes on exon/intron boundaries that were correct in the ITAG2.4 annotation: a deletion of 57 b at position 1645, an insertion of one base at position 2763 and an insertion of 20 b at position 2871. The resulting sequence of the *SlCHR1* coding sequence and the derived protein sequence are given in Figure S3. The distribution of protein domains and elements in this sequence was presented earlier (Bargsten *et al.*, 2013).

Generation and first characterization of c*SlCHR1* overexpressing transgenic lines for Arabidopsis and tomato

To test whether the overexpression of c*SlCHR1* would affect plant growth, we generated transgenic Arabidopsis and tomato lines overexpressing c*SlCHR1*. The binary plasmid 35S:c*SlCHR1*-GFP contains the full-length coding sequence of *SlCHR1* with a C-terminal GFP-tag put under the control of the constitutive 35S CaMV promoter (Figure 2a). The T-DNA was transferred to *Arabidopsis thaliana* Col-0 using the floral dip method (Clough and Bent, 1998). Single-locus homozygous F3 lines were selected based on kanamycin segregation. Two such lines, At-c*SlCHR1*-ov1 and At-c*SlCHR1*-ov2, were selected for more detailed analyses.

Transgenic tomato lines were generated by transformation of the tomato cultivar Micro-Tom (Carvalho *et al.*, 2011) using the same binary plasmid and regenerating transgenic shoots from cotyledons (Qiu *et al.*, 2007). Single-locus homozygous F3 lines were selected as for Arabidopsis. From 10 transgenic lines obtained, two lines, Sl-c*SlCHR1*-ov1 and Sl-c*SlCHR1*-ov2, were randomly selected for more detailed analysis.

Analysis of *SlCHR1* expression levels by qRT-PCR in Arabidopsis and tomato lines showed the intended overexpression in both plant genera (Figure 2b,c). In Arabidopsis, the two lines differ in expression level (Figure 2b). The expression relative to the reference gene *UBC* was about 2.3 for At-c*SlCHR1*-ov1 and 1.0 for At-c*SlCHR1*-ov2. Relative to the endogenous Snf2 genes *AtCHR12* and *AtCHR23*,

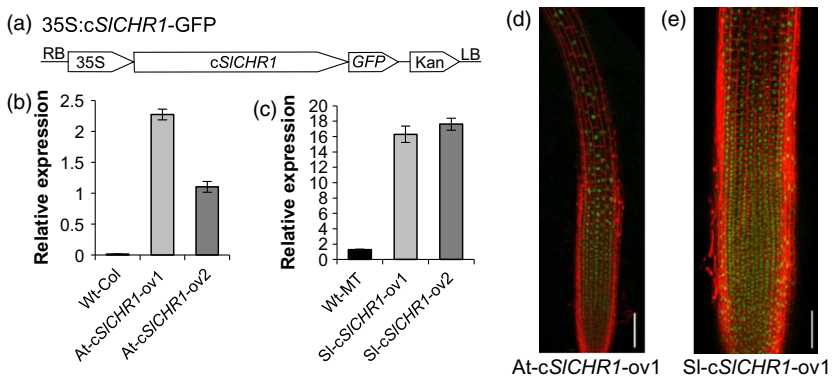

Figure 2 Expression and localization of c*SlCHR1* in transgenic lines. (a) Schematic layout of plasmid T-DNA region used to generate transgenic Arabidopsis and tomato lines overexpressing c*SlCHR1* gene. 35S, CaMV 35S promoter; *GFP*, green fluorescence protein gene; Kan, kanamycin resistance gene; RB, LB, right and left T-DNA borders. (b) Relative expression levels of *SlCHR1* mRNA in Arabidopsis At-c*SlCHR1*-ov1 and At-c*SlCHR1*-ov2 transgenic lines as measured by qRT-PCR. No expression was detected in Arabidopsis wild-type Col-0. The *UBC* gene was used as reference. The error bars represent standard deviation (SD). (c) Relative expression levels of *SlCHR1* mRNA in tomato Sl-c*SlCHR1*-ov1 and Sl-c*SlCHR1*-ov2 transgenic lines compare to the wild-type Micro-Tom (MT). The *L33* gene was used as a reference. The error bars represent SD. (d, e) Nuclear localization of cSLCHR1-GFP in roots of transgenic Arabidopsis line At-c*SlCHR1*-ov1 (d) and transgenic tomato line Sl-c*SlCHR1*-ov1 (e). Confocal images of 6-day-old seedlings taken with Leica confocal microscope. Propidium iodide (1 μg/mL) was used to colour the cell walls red. Bar = 100 μm.

expression of the transgene is about 8–10 fold higher (data not shown). Relative expression levels of the endogenous genes are not compromised (data not shown), so introduction of the tomato cDNA does not result in silencing of the endogenous Snf2 paralogs. In tomato, in both lines, the *SlCHR1* gene was about 15–17 fold higher expressed, relative to either the reference gene *L33* or the endogenous gene *SlCHR1* (Figure 2c).

In both Arabidopsis (Figure 2d) and tomato (Figure 2e), the GFP-tagged SLCHR1 protein was localized in root nuclei, as well as in nuclei of hypocotyls and leaves (data not shown). These data confirm the expected nuclear localization of a chromatin remodelling ATPase (Sang *et al.*, 2012; Sarnowski *et al.*, 2002).

Overexpression of c*SlCHR1* in Arabidopsis does not affect growth and development

The growth of the two transgenic Arabidopsis lines was monitored during early seedling and vegetative development. Transgenic seedlings did not differ from the wild type in the length of the hypocotyl or in cotyledon size when grown under optimal conditions (data not shown). To assess the impact of c*SlCHR1* overexpression on vegetative growth when exposed to environmental stress, the length of the primary root was compared between the wild type and transgenic plants under salt stress (75 mM NaCl). Salt stress reduces the length of the root of the wild type to about half, and the reduction of the root length was similar in the two transgenic lines (Figure 3a). Also the diameter of the leaf rosette of soil-grown plants was compared between standard conditions and salt stress. The rosette diameters were determined from digital images of 4-week-old plants as described previously (Folta *et al.*, 2014). No differences in rosette diameter between wild type and transgenic lines were observed (data not shown). To compare the phenotypic effects of c*SlCHR1* overexpression with the phenotypes obtained with *AtCHR12* (Mlynarova *et al.*, 2007), we measured the length of the primary stem of 40-day-old plants without (control) and with a heat stress treatment of 12 days as described previously. The results (Figure 3b) show that although the length of the primary stem of line At-c*SlCHR1*-ov1 was slightly reduced compared to wild type in control conditions, the length of the primary stem of both transgenic

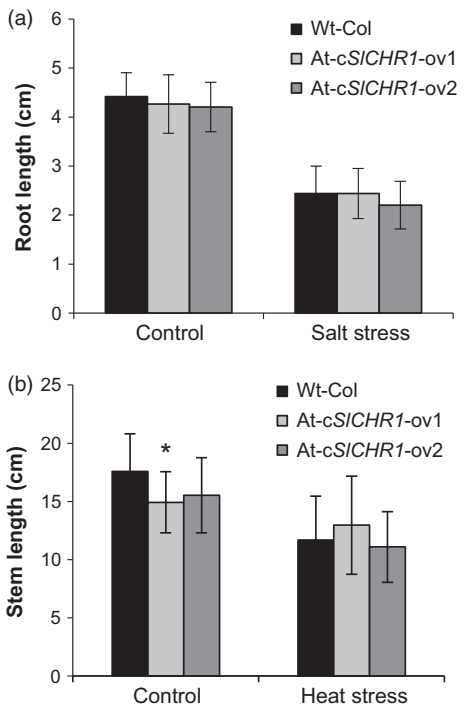

Figure 3 Overexpression of c*SlCHR1* does not affect the growth habit of Arabidopsis. (a) Mean length of the primary roots of 8-day-old seedlings grown in control and salt stress (75 mM NaCl) conditions. (b) Mean length of primary stem of 40-day-old control and heat-stressed plants. The heat stress (37 °C for 16 h) was applied to 28-day-old plants. Control, nontreated plants were grown and measured in parallel with stressed plants. The error bars represent SD. For each condition, asterisks indicate significant differences from wild type: $*P < 0.05$. For each line, at least 32 seedlings and 13 plants were measured.

lines upon stress was not different from the primary stem length of the wild type (Figure 3b). These results show that overexpression of the tomato c*SlCHR1* gene does not affect seedling and vegetative

growth in Arabidopsis as seen upon overexpression of the *AtCHR23* (Folta *et al.*, 2014) or the *AtCHR12* gene (Mlynarova *et al.*, 2007). Constitutive expression of the tomato c*SlCHR1* gene has no significant impact on vegetative growth and development of Arabidopsis plants and also does not seem to affect the response of Arabidopsis to adverse environmental conditions.

Overexpression of c*SlCHR1* in tomato results in considerably compacter growth

To quantify the effect of c*SlCHR1* overexpression on the growth of tomato seedlings, three parameters were measured: the cotyledon area, the length of the main root and the length of the hypocotyl. The two transgenic tomato lines overexpressing c*SlCHR1* showed significantly reduced growth compared to the wild type in all parameters measured (Figure 4). The average length of the root of 7-day-old seedlings was reduced from 6.7 cm in wild type to 5.1 cm and 5.7 cm in the Sl-c*SlCHR1*-ov1 and Sl-c*SlCHR1*-ov2 lines, respectively (Figure 4a). This is a reduction in growth of 23.9% and 14.9% relative to wild type. A similar reduction was observed for the length of the hypocotyl (Figure 4b). While the average length of the wild-type hypocotyl was 2.1 cm, in Sl-c*SlCHR1*-ov1 and Sl-c*SlCHR1*-ov2 lines, it was 1.6 cm (23.8% reduction) and 1.8 cm (14.3% reduction), respectively. The cotyledon area of 0.32 cm^2 in the wild type was reduced to 0.24 cm^2 (25% reduction) in both transgenic lines (Figure 4c). Upon overexpression of c*SlCHR1*, tomato seedlings become markedly more compact than the wild type. In contrast, transgenic tomato lines obtained via RNAi that had markedly reduced (~50%) levels of *SlCHR1* expression revealed no differences in growth habit relative to the wild type (data not shown).

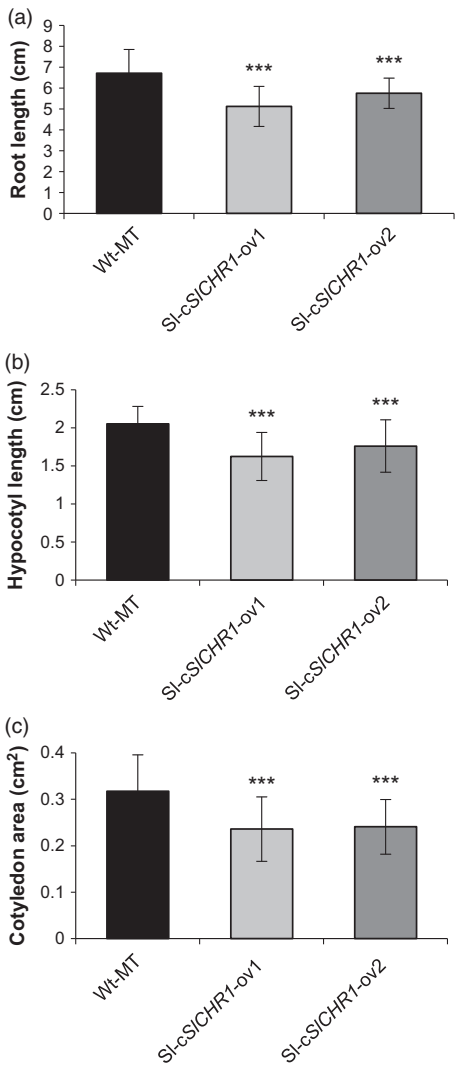

Figure 4 Overexpression of c*SlCHR1* in tomato results in reduced seedling growth. (a) Mean root length, (b) mean hypocotyl length and (c) mean cotyledon area of 7-day-old seedlings of wild-type MT and two transgenic lines grown in normal environmental conditions. The error bars represent SD. Asterisks indicate significant differences from wild type: ***$P < 0.001$. For each line, at least 15 seedlings were measured.

To evaluate how the overexpression of c*SlCHR1* and compactness of tomato seedlings translates to later stages of vegetative growth and development of tomato, height and diameter of 6-week-old greenhouse-grown plants were measured. Both height and diameter of the two transgenic lines were significantly reduced compared to the wild type (Figure 5). An example of the height difference is shown in Figure 5a. The average height of wild-type plants was 17.8 cm. It was 12.8 cm (28.1% reduction) in Sl-c*SlCHR1*-ov1 and 13.4 cm (24.7% reduction) in Sl-c*SlCHR1*-ov2 (Figure 5b). The reduced height is due to shorter internodes at the same number of nodes (data not shown). The diameter of the wild-type plants was 24.2 cm. It was reduced to 14.8 cm (reduction 38.8%) in Sl-c*SlCHR1*-ov1 and to 18.3 cm (reduction 24.4%) in Sl-c*SlCHR1*-ov2 (Figure 5c).

Also the individual leaves of the two transgenic lines show a more compact phenotype compared to the wild type (Figure 5d). The average length of the fourth leaf from the plant base was 12.5 cm in the wild type, while in the Sl-c*SlCHR1*-ov1 and Sl-c*SlCHR1*-ov2 lines, it was 9.6 cm (22.9% reduction) and 10.4 cm (16.4% reduction), respectively (Figure 5e). In addition, the top leaflet of the fourth compound leaf is smaller in the transgenic lines than in the wild type. The average length of the wild-type top leaflet was 6.3 cm; it was 4.9 cm (22.2% reduction) in Sl-c*SlCHR1*-ov1 and 5.5 cm (12.7% reduction) in Sl-c*SlCHR1*-ov2 (Figure 5f). All data demonstrate that overexpression of c*SlCHR1* in tomato leads to overall markedly reduced vegetative growth, resulting in more compact seedlings and plants.

A prominent feature of the phenotype associated with c*SlCHR1* overexpression was associated with flowering and reproduction organs. Flowering of the transgenic lines was on average six days delayed compared with nontransgenic wild-type plants (Figure S4). The reproductive structures of the two transgenic lines were severely compacted compared to the wild type (Figure 6a). The average diameter of the wild-type reproductive structure was 16.2 cm. It was reduced to only 3.6 cm (77.8% reduction) in Sl-c*SlCHR1*-ov1 and to 5.2 cm (67.9% reduction) in Sl-c*SlCHR1*-ov2 (Figure 6b). Closer examination of the inflorescence architecture revealed significantly shortened peduncles and pedicels in the transgenic lines. In addition, also the diameter of fully open individual flowers was significantly reduced in transgenic plants (Figure 6c). The diameter of wild-type flower was 2.5 cm; the transgenic lines have both a flower diameter of about 1.8 cm (28% reduction).

Although the transgenic lines set fruit that appeared to ripen normally, the fruits were considerably smaller in size (Figure 6d) and in weight (Figure 6f); the number of fruits per plants appeared smaller than for the corresponding wild-type tomato, but the compact nature of the transgenic plants did not allow proper quantification of the average number of fruits per plant.

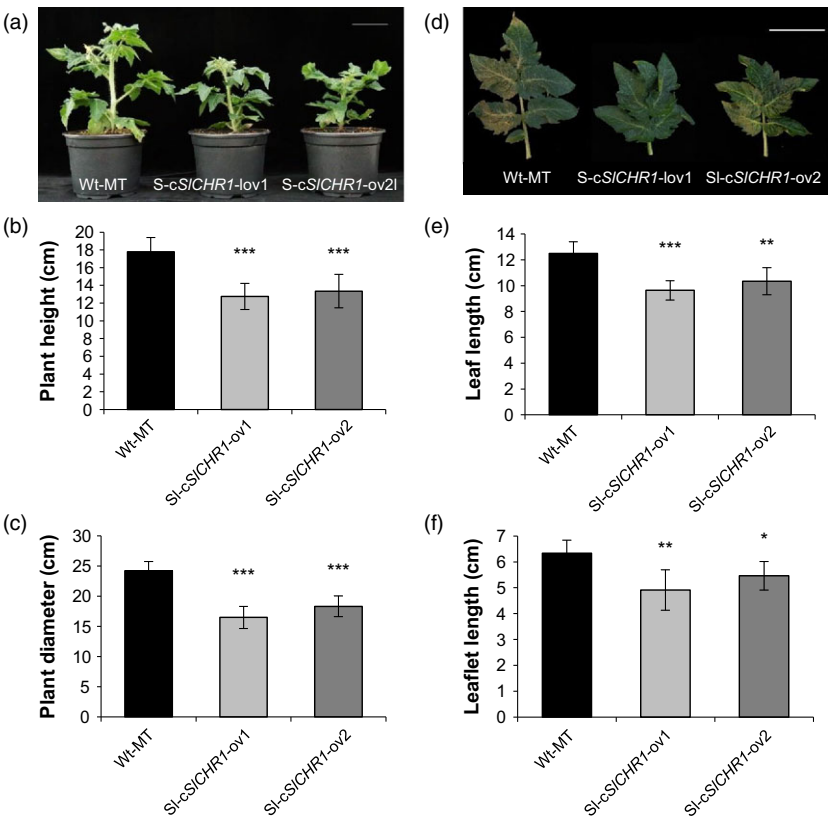

Figure 5 Tomato plants overexpressing c*SlCHR1* show reduced vegetative growth. (a) Phenotype of wild-type MT and two transgenic lines 6 weeks after sowing; bar = 5 cm. (b) Plant height and (c) plant diameter of 6-week-old plants grown in normal environmental conditions. (d) Phenotype of the fourth leaf of wild-type MT and overexpressing lines 6 weeks after sowing; bar = 5 cm. (e) Mean length of the fourth leaf and (f) mean length of the terminal leaflet on the same leaf. The error bars represent SD. Asterisks indicate significant differences from wild type: **P* < 0.05; ***P* < 0.01; ****P* < 0.001. For each line, at least 10 plants and six leaves were measured.

The number of seeds per fruit was smaller, and also the size of the seeds themselves was reduced in length (Figure 6e,g).

The effect of c*SlCHR1* overexpression on growth parameters of plants grown under stress conditions

In Arabidopsis, overexpression of the Snf2 chromatin remodelling genes *AtCHR12* and *AtCHR23* particularly affected the growth under adverse environmental conditions (drought, heat, salt) (Folta *et al.*, 2014; Mlynarova *et al.*, 2007). To check whether overexpression of c*SlCHR1* has similar effects in tomato, the c*SlCHR1* overexpressing transgenic tomato plants were subjected to drought and salt stress and compared to the wild type. Two-week-old plants were subjected to drought stress by withholding water supply. After 2 weeks of water shortage, plant height was measured after another 2 weeks of growth without stress and compared with wild-type plants that had undergone the same treatment. The wild-type plants showed a reduction in height from 17.1 cm to 13.3 cm (reduction 22.2%). The height of Sl-c*SlCHR1*-ov1 was reduced from 13.8 cm to 11.8 cm (14.5% reduction) and of Sl-c*SlCHR1*-ov2 from 13.2 cm to 11.2 cm (15.1% reduction) (Figure 7). The two transgenic tomato lines seem to be a bit more resistant to water shortage (less growth reduction). While in control conditions, the height of transgenic plants was significantly shorter than wild type, after drought stress the difference was not significant ($P < 0.05$).

Growth in the presence of salt stress was analysed by growing plants for 2 weeks under standard conditions followed by 2 weeks of watering with 150 mM NaCl. After another 2 weeks of growth without stress, plant height was measured. Salt stress reduced the height of wild-type plants from 17.1 to 13.6 cm (20.5% reduction). The average height of salt-treated Sl-c*SlCHR1*-ov1 was reduced to 11.1 cm (19.6% reduction), and of Sl-c*SlCHR1*-ov2, it was 10.9 cm (17.4% reduction) (Figure 7). These data show that in case of salt stress, both wild type and transgenic plants show the same reduction in growth relative to control conditions.

Discussion

We here present the cloning of the coding sequence of a tomato (*S. lycopersicum*) chromatin remodelling Snf2-type ATPase gene (*SL100sc05189_42.1.1./Solyc01g079690,* here designated *SlCHR1*) and the first phenotypic characterization of plants upon overexpression in both Arabidopsis and tomato. To our knowledge, this is the first tomato chromatin remodelling gene analysed this way. Two tomato genome annotations (ITAG1 and ITAG2.4) were contradictory with respect to the structure of this tomato gene. Such discrepancy between the two tomato genome annotations demonstrates the intrinsic difficulties for automated annotation in case of gene families and/or the presence of alternative transcripts (Fawal *et al.*, 2014). Detailed PCR analyses showed the earlier annotation (ITAG1) to be most close to the true genomic structure. This result emphasizes the importance of experimental confirmation and manual curation of automated gene prediction, especially in case of newly sequenced genomes. Based on phylogenetic analyses, *SlCHR1* is thought to combine in tomato the functions of its two Arabidopsis paralogs (Bargsten *et al.*, 2013), which upon overexpression will affect the growth habit of tomato. Constitutive overexpression of the coding sequence of *SlCHR1* indeed resulted in significant reduction of growth and development of tomato plants. Compared to the wild type, transgenic tomato lines have smaller seedlings,

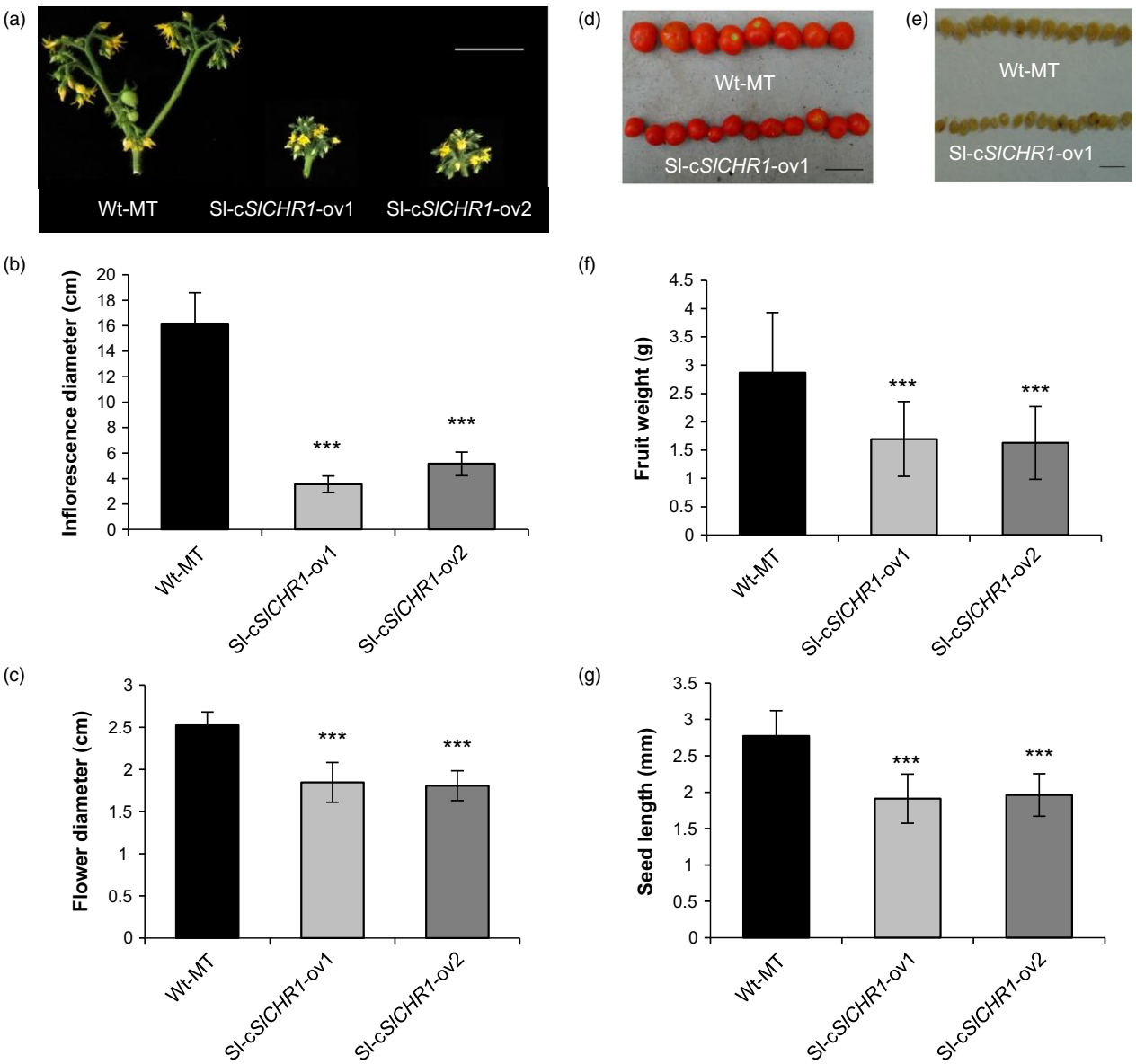

Figure 6 Overexpression of c*SlCHR1* in tomato results in compact reproductive structures and smaller flowers and fruits. (a) Phenotype of the reproductive structure of wild-type MT and overexpressing mutants; bar = 5 cm. (b) The mean diameter of the reproductive structures and of fully open flowers (c) in 6-week-old wild type and two transgenic lines. (d, e) Phenotype of the fruit (d) and seed (e) of wild-type MT and Sl-c*SlCHR1*-ov1 line; bar = 2.5 cm for fruits (d) and 3 mm for seeds (e). (f) Mean weight of the ripe fruit and the seed length (g) in wild type and mutant. The error bars represent SD. Asterisks indicate significant differences from wild type: ***$P < 0.001$. For each line, at least 10 reproductive structures, 15 flowers and 30 fruits and seeds were measured.

much more compact vegetative growth habit, and severely compacted reproductive structures.

Overexpression of a tomato chromatin remodelling ATPase gene does not affect the growth habit of Arabidopsis

The finding that overexpression of c*SlCHR1* in Arabidopsis did not impact plant growth as expected based on the overexpression of either *AtCHR12* or *AtCHR23* was quite surprising. Transgenic Arabidopsis plants overexpressing *SlCHR1* could not be distinguished phenotypically from the wild type, neither in standard growth conditions, nor in environmentally adverse conditions (Figure 3). It seems sufficiently unlikely that the lack of phenotype is due to too low expression levels. The relative level of overexpression accomplished seems high enough, and as ATCHR12-GFP fusion protein gives the same phenotype in Arabidopsis as ATCHR12 (Folta, unpublished data), the small GFP tail is not likely to affect the chromatin remodelling function of the fusion protein.

One of the possible explanations for the lack of growth phenotype in Arabidopsis could be that the structure of the single tomato gene deviates to such an extent that it cannot take the functions of the two Arabidopsis genes. Both *AtCHR12* and *AtCHR23* carry at their C-terminal end an unfolded region that is not present in the tomato *SlCHR1* gene (Bargsten *et al.*, 2013). Subtle differences in domain architecture may change the function of orthologous proteins (Gabaldon and Koonin, 2013). Possibly the lack of the C-terminal unfolded region in SLCHR1 is

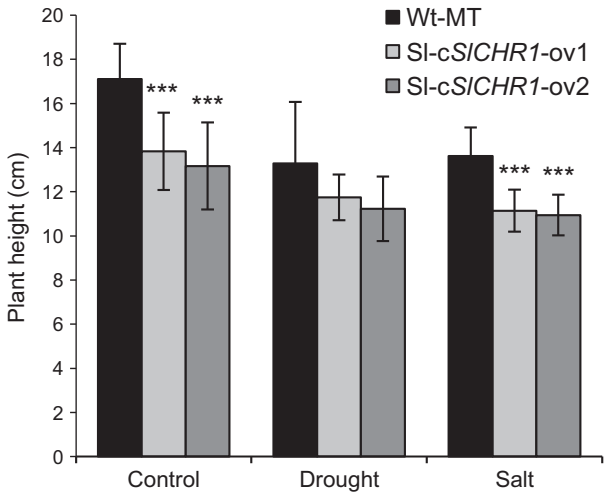

Figure 7 The height of wild type and transgenic tomato plants after stress. Plants were grown for 2 weeks without stress followed by 2 weeks of drought or salt stress. The plant height was measured after another 2 weeks of growth without stress. Control, nonstressed plants were grown and measured in parallel with stressed plants. The error bars represent SD. For each condition, asterisks indicate significant differences from wild type: ***$P < 0.001$.

crucial for the apparent lack of function in Arabidopsis. Such unfolded or disordered regions help or guide protein–protein or protein–DNA interactions (Uversky and Dunker, 2010; Uversky *et al.*, 2000). Disordered regions could potentially adopt different conformations that allow interactions with multiple binding partners (Grau *et al.*, 2011). SWI/SNF2 ATPases function in the context of protein complexes, and the recruitment of one of the components of the remodelling complex in Arabidopsis may become affected. More detailed analyses are required to show whether this part of the Snf2 protein family has indeed such an influence on function. Alternatively, the two species may be evolutionary too far apart for proper gene function analysis. Arabidopsis and tomato belong to two different clades of the eudicots, the *Cruciferae* and the *Solanaceae*, respectively. However, Arabidopsis has been used to characterize the function of tomato genes (Fradin *et al.*, 2011; Li *et al.*, 2013, 2014).

Vice versa, Arabidopsis genes have been successfully used to modify tomato (Zhang *et al.*, 2004). When introduced into the solanaceous tobacco (*Nicotiana tabacum*), overexpression of the Arabidopsis *AtCHR12* gene did result in more compact plants (data not shown). These results indicate that the evolutionary distance is not necessarily a bottleneck for functional characterization. The lack of phenotype here obtained for Arabidopsis only implies that Arabidopsis cannot be used as model species for this type of growth-related genes, possibly because the detailed regulation of growth in the two species differ subtly. Arabidopsis can be considered a pioneer species used to encounter adverse environments (Chew and Halliday, 2011), whereas tomato has been subject to many years of selection and breeding for uniformity and stability of growth.

Overexpression of *SlCHR1* results in more compacted tomato seedlings and plants

Transgenic seedlings of the tomato cultivar Micro-Tom overexpressing c*SlCHR1* driven by the near-constitutive CaMV 35S promoter were more compact than the untransformed controls. They showed up to 25% reduction of growth compared to the wild type. Also during vegetative growth, the plants have more compact growth habit (Figures 4 and 5). The most severe effect of *SlCHR1* overexpression was observed for the reproductive organs. The average diameter of the reproductive structures was reduced up to one-fifth of the wild type (Figure 6). Micro-Tom is already one of the smallest tomato cultivars known (Marti *et al.*, 2006). It is remarkable that overexpression of a single gene can reduce plant habit so much further.

In Arabidopsis, the phenotype upon overexpression of *AtCHR12* could only be distinguished from the wild type in case of mild stress conditions. It resulted in growth arrest of primary buds and reduced growth of the primary stem that recovered in the absence of the environmental stress. In mature plants, notably the growth after the transition to the reproductive development was affected (Mlynarova *et al.*, 2007). Overexpression of *AtCHR23* in Arabidopsis resulted in reduced growth of seedlings and more compacted vegetative rosette (Folta *et al.*, 2014). Tomato *SlCHR1* is considered to be the single ortholog of *AtCHR12* and *AtCHR23* and supposed to combine their functions (Bargsten *et al.*, 2013). This is indeed reflected in the phenotype obtained. Upon overexpression, tomato shows a compact vegetative growth habit (*AtCHR23* overexpression-like) and considerably smaller reproductive organs (*AtCHR12* overexpression-like). However, the compact growth habit is seen without the need for applying additional stress conditions. This suggests that in this respect, the ATCHR23 function of the SLCHR1 protein overrides the ATCHR12 function. The concept of priming the plants for growth arrest upon actual environmental stress associated with *AtCHR12* overexpression (Mlynarova *et al.*, 2007) is either less important in Arabidopsis than in tomato or is taken over by other protein or mechanisms.

The c*SlCHR1* overexpressing tomato lines do, however, differ markedly from the *AtCHR12/23* overexpressing Arabidopsis lines with respect to their reaction to environmental stress. In Arabidopsis, environmental stress results in stronger growth reduction (Folta *et al.*, 2014; Mlynarova *et al.*, 2007), irrespective of the type of environmental stress applied (drought, heat, salt). In tomato, the growth reduction was not significantly different between overexpressing lines and the wild type when subjected to salt stress. When subjected to drought stress, tomato overexpressing c*SlCHR1* showed even less growth reduction, hence more stress tolerance, than wild-type plants (Figure 7). We speculate that these differences may be related to the intrinsic differences between Arabidopsis and tomato, their natural habit and habitat as well as human selection in tomato breeding. The better performance of the transgenic tomato plants under drought stress may be a side effect of the reduced plant size and slower growth rate that result in decreased water evaporation (Blum, 2005). The *SlCHR1* overexpression phenotype may be related to hormone signalling. In Arabidopsis, chromatin remodelling plays a role in growth regulation and hormone signalling (Archacki *et al.*, 2013; Sarnowska *et al.*, 2013). However, overexpression of *AtCHR12/AtCHR23* in Arabidopsis was not associated with notable differences in expression of any of the known phytochrome-related genes (Folta *et al.*, 2014; Mlynarova *et al.*, 2007). More data are required to speculate about a relationship between chromatin remodelling, growth regulation and hormone signalling in tomato.

Potential applications of modulated expression of chromatin remodelling genes in crops

The markedly reduced growth habit of tomato as result of the overexpression of c*SlCHR1* could be exploited in several ways. The compact growth habit is advantageous for the production of field-grown tomatoes. It could reduce production costs because of diminished labour costs for stalking, tying and pruning. The latter account to up to 55% of the field-grown tomato production cost (Davis and Estes, 1993; Kemble *et al.*, 1994). Alternatively, it could help develop cultivars with smaller, more cherrylike tomatoes from larger-fruit cultivars. Possibly the smaller tomatoes have a shape or taste that is more appreciated by consumer panels (Jones Jr, 2008; Rocha *et al.*, 2013).

In view of the current controversy about transgenic approaches, notably in Europe, these potential applications should and can be translated into non-GMO strategies based on breeding and selection (e.g. marker-assisted selection, MAS). Promoter activity will be critical for the targeted modification of tomato growth through chromatin remodelling. Methods such as TILLING, EcoTILLING or CRISPR/Cas (Barkley and Wang, 2008; Belhaj *et al.*, 2013) can be used to induce or identify mutations in the *SlCHR1* promoter sequence to generate plant lines which produce higher levels of the chromatin remodelling protein SLCHR1. As it is only one particular promoter that must be targeted, such approaches will become more straightforward in the future.

When it becomes feasible to modulate the specificity of an endogenous promoter, new options for application arise. The use of an endogenous promoter redesigned to be specifically active in the seedling stage would allow targeted adjustments of tomato growth habit. Possibly a tuneable transcriptional factor could provide the desired regulation for inducible, spatial or temporal expression (Liu *et al.*, 2013). This may give better control of the growth of tomato seedlings used as transplants. Commercially grown tomatoes are generally produced from transplanted seedlings previously grown in greenhouses. Short, uniform and sturdy seedlings are required to enable the use of mechanical transplanting machinery. Seedlings can become tall and leggy prior to field establishment and good control of notably the height of tomato transplants is important. Nowadays, transplant growth rate is regulated in nurseries through nutrient and water management, as well as temperature control, clipping shoots and mechanical treatment (brushing) (Garner and Björkman, 1996), but nurseries have not always the desired flexibility. In industry, the use of plant growth retardants (PGRs) is explored (Choudhury *et al.*, 2013; Nickell and McLaren, 1982). PGRs are synthetic chemicals, which temporarily inhibit the elongation of stem and shoots, without irreversible blocking of vital metabolic and developmental processes. The use of PGRs, when used appropriately at the correct stage of development and in the required concentration, enables to get shorter, sturdier and possibly healthier transplants (Biles and Cothren, 2001)**.** To date, only Sumagic (Valent Professional Products, Morrisville, North Cariolina) is registered for use of height control of tomato transplants in greenhouse production (Runkle and Blanchard, 2012). The active compound of this very potent growth retardant, Uniconazole, suppresses stem elongation by the inhibition of gibberellin acid biosynthesis (Zandstra *et al.*, 2007). However, the use of such a PGR in plant production is not without controversy or risk. Misapplication can result in phytotoxicity, delayed flowering and stunned growth (Whipker *et al.*, 2001). In addition, there is the possibility of undesired persistence in plant material or in the environment (Wu *et al.*, 2013). Therefore, alternative methods for temporary growth retardation of seedlings are still desired (Gargul *et al.*, 2015). Growth retardation of tomato seedlings based on overexpression of the *SlCHR1* gene in specifically the seedling stage could develop into a promising and environmentally friendlier alternative for the use of chemical plant growth retardants. In all applications, possibly compromised fruit yield will have to be assessed for economic feasibility and sustainability.

All crop species carry genes orthologous to *SlCHR1* (Bargsten *et al.*, 2013). Therefore, the use of chromatin remodelling genes to reduce plant height is likely to be applicable to and attractive for any crop for which height reduction could have added value. This applies to edible crops such as vegetables and herbs. In grasses such as wheat or barley, shorter-stemmed plants will be more resistant to wind and rain, therefore reducing the lodging losses before the harvest (Jones *et al.*, 2013). Growth control would also be beneficial for horticultural uses, such as the reduction of vegetative growth in turf, fruit trees, grapes and other woody plant species. Improvements in ornamental floral crops and bedding plants are also feasible (Chandler and Sanchez, 2012). Short, compact ornamental plants look more balanced and are less likely to be damaged during shipping. Our data do indicate, however, that the phenotype conferred by this type of genes after interspecies transfer cannot be predicted easily and should be cautiously managed and/or interpreted. This way, modulation of the expression of chromatin remodelling genes could develop into a widely applicable approach to control the growth of plants for agronomic and commercial purposes.

Experimental procedures

RNA isolation and RT-PCR analysis

Total RNA and RT-PCR analysis were performed as previously described (Bargsten *et al.*, 2013; Folta *et al.*, 2014). Quantitative RT-PCR was performed at least in triplicate with 2.5 μL of 10-times-diluted cDNA using iQ™ SYBR® Green Supermix (Bio-Rad Laboratories, Inc., Hercules, California) in a CFX Connect™ Real-Time PCR Detection System (Bio-Rad Laboratories, Inc.). Genes *L33* (*Solyc01 g007450.1.1*) (Schijlen *et al.*, 2007) and *UBC* (*At5 g25760*) (Czechowski *et al.*, 2005) were used for normalization of tomato and Arabidopsis samples, respectively. Primers were designed with Primer3Plus (Untergasser *et al.*, 2007) and are listed in Table S1.

Cloning of the coding sequence of the tomato *SlCHR1* gene

To obtain the cDNA sequence of the *SlCHR1* gene, RNA from leaves of tomato cultivar Heinz 1706 was isolated as described above. The cDNA was prepared from one microgram of total RNA using SuperScript® III First-Strand Synthesis System (Invitrogen) with oligo$(dT)_{20}$ primers, and 1 μL of the first-strand cDNA was used as a template for PCR with Phusion® High-Fidelity DNA Polymerase (Finnzymes, Finland) with the primer pair SlCHR1-F1 and SlCHR1-R1 (Table S1). The conditions used for PCR were 98 °C for 4 min; 35 cycles: 98 °C for 30 s, 61 °C for 30 s and 72 °C for 150 s; 72 °C for 7 min. The PCR product was cloned into pENTR™/D-TOPO® vector (Invitrogen Corporation, Waltham, Massachusetts), and its integrity was verified by DNA sequencing. Next, the *SlCHR1* coding sequence was cloned by an LR Gateway (Life Technologies) recombination reaction into the destination vector pK7FWG2.0, obtained from VIB Gent, Belgium

(Karimi *et al.*, 2002). This generated a fusion gene with a C-terminal GFP moiety driven by the (near-)constitutive CaMV 35S promoter (35S:c*SlCHR1*-GFP, Figure 2a). The final plasmid was transferred into *Agrobacterium tumefaciens* strain C58C1 with the freeze–thaw method (Weigel and Glazebrook, 2006).

Plant growth conditions

Arabidopsis thaliana seedlings and plants were grown in control (without stress) and stress conditions as previously described (Folta *et al.*, 2014; Mlynarova *et al.*, 2007). To analyse the growth of tomato (*Solanum lycopersicum*) seedlings, seeds of the cultivar Micro-Tom were surface sterilized and grown on 0.5 x MS agar plates. For salt stress treatment, the agar plates were supplemented with 75 mM NaCl. Seedlings were grown vertically in fully controlled growing chambers lit by Philips TD 32W/84HF lamps at 25 °C in long-day conditions (16-h light/8-h dark). The tomato cultivar Heinz used for RNA isolation was grown in the same conditions in pots containing 0.5 x MS agar. Tomato plants were grown in standard potting soil in a controlled greenhouse at 21 °C with supplemental light provided by four Son-T (Philips Greenpower, 400 W) lamps when required, in long-day conditions (16-h light/8-h dark). To apply salt stress, 2-week-old greenhouse-grown plants were watered for two weeks with 150 mM NaCl. To apply drought stress, water supply of 2-week-old greenhouse-grown plants was stopped for 2 weeks. Wild type and transgenic plants were grown and treated in parallel. In all cases, also untreated plants were grown in parallel.

Generation of transgenic plants

Transgenic Arabidopsis plants (ecotype Col-0) were obtained by the floral dip method (Clough and Bent, 1998) using C58C1 agrobacteria bearing the 35S:c*SlCHR1*-GFP binary plasmid. To obtain transgenic tomato lines, cultivar Micro-Tom was transformed with the same binary plasmid using a method described previously (Qiu *et al.*, 2007) with minor modifications. Cotyledons of 10-day-old seedlings were used, when the first true leaves were only 2–3 mm long. During regeneration, 50 mg/L of vancomycin was used instead of carbenicillin, and in all media, 0.5 g/L of MES (2-N-morpholinoethanesulfonic acid) was used to buffer the pH. Transgenic lines were selected based on kanamycin resistance and segregation. Homozygous F3 transgenic plants of both Arabidopsis and tomato were used.

Analysis of growth

Arabidopsis seedlings, vegetative and reproductive growth parameters were analysed as described previously (Folta *et al.*, 2014; Mlynarova *et al.*, 2007). Tomato growth parameters, such as the length of the main root, the length of the hypocotyl and the area of the cotyledon, were analysed in a similar way. Seven-day-old seedlings grown vertically as described above were photographed, and the root and hypocotyl length were measured using ImageJ (http://imagej.nih.gov/ij/). The cotyledon area was determined from a photograph of flattened cotyledons in ImageJ. The growth of the tomato plants was determined on 6-week-old plants. Plant height was measured by a ruler from the stem base till the top of the plant. The length of the fourth leaf and the terminal leaflet on fourth leaf was determined from a photograph of flattened leaves using ImageJ. The leaf length was measured from the axil till the tip of terminal leaflet, the terminal leaflet length from the rachis till the tip of the leaflet. To analyse the growth during reproductive development, 6-week-old plants were photographed from the top and the reproductive structure diameter was measured after enclosing in a square section using ImageJ. The individual flowers were also flattened and photographed. Using ImageJ software, the flowers were enclosed in a square section and the diameter was determined. The fully ripe fruits were weighted on a laboratory weight, and the seed length was determined from a photograph using ImageJ software. The significance of differences was determined with the Student's *t*-test assuming unequal variances in Excel.

GFP imaging and photography

The location of the cSLCHR1-GFP fusion protein in 6-day-old Arabidopsis and tomato seedlings grown vertically was determined with a Leica TCS SP2 confocal microscope (Leica Microsystems B.V., Rijswijk, The Netherlands) with a 16x objective. To visualize the cell walls, the tissue was incubated for 1 min in a solution of 1 μg/mL of propidium iodide and washed in water before inspection. The photographs were obtained by Olympus SZ-30MR camera against a black background.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

We acknowledge the Centre for BioSystems Genomics 2012 (CBSG2012) which was part of the Netherlands Genomics Initiative (NGI) for financial support. AF was supported by the Netherlands Organization for Scientific Research (NWO), through its ALW/TTi Green Genetics Program. JPN was supported by the RaakPRO BioCOMP Project coordinated by Hanze University of Applied Sciences Groningen.

References

Archacki, R., Buszewicz, D., Sarnowski, T.J., Sarnowska, E., Rolicka, A.T., Tohge, T., Fernie, A.R., Jikumaru, Y., Kotlinski, M., Iwanicka-Nowicka, R., Kalisiak, K., Patryn, J., Halibart-Puzio, J., Kamiya, Y., Davis, S.J., Koblowska, M.K. and Jerzmanowski, A. (2013) BRAHMA ATPase of the SWI/SNF chromatin remodeling complex acts as a positive regulator of gibberellin-mediated responses in Arabidopsis. *PLoS ONE*, **8**, e58588.

Bargsten, J.W., Folta, A., Mlynarova, L. and Nap, J.P. (2013) Snf2 family gene distribution in higher plant genomes reveals DRD1 expansion and diversification in the tomato genome. *PLoS ONE*, **8**, e81147.

Barkley, N.A. and Wang, M.L. (2008) Application of TILLING and EcoTILLING as reverse genetic approaches to elucidate the function of genes in plants and animals. *Curr. Genomics*, **9**, 212–226.

Belhaj, K., Chaparro-Garcia, A., Kamoun, S. and Nekrasov, V. (2013) Plant genome editing made easy: targeted mutagenesis in model and crop plants using the CRISPR/Cas system. *Plant Methods*, **9**, 39.

Biles, S.P. and Cothren, J.T. (2001) Flowering and yield response of cotton to application of Mepiquat chloride and PGR-IV. *Crop Sci.* **41**, 1834–1837.

Blum, A. (2005) Drought resistance, water-use efficiency, and yield potential—are they compatible, dissonant, or mutually exclusive? *Aust. J. Agric. Res.* **56**, 1159–1168.

Carvalho, R.F., Campos, M.L., Pino, L.E., Crestana, S.L., Zsogon, A., Lima, J.E., Benedito, V.A. and Peres, L.E. (2011) Convergence of developmental mutants into a single tomato model system: 'Micro-Tom' as an effective toolkit for plant development research. *Plant Methods*, **7**, 18.

Chandler, S.F. and Sanchez, C. (2012) Genetic modification; the development of transgenic ornamental plant varieties. *Plant Biotechnol. J.* **10**, 891–903.

Chew, Y.H. and Halliday, K.J. (2011) A stress-free walk from Arabidopsis to crops. *Curr. Opin. Biotechnol.* **22**, 281–286.

Choudhury, S., Islam, N., Sarkar, M.D. and Ali, M.A. (2013) Growth and yield of summer tomato as influenced by plant growth regulators. *Int. J. Sustain. Agric.* **5**, 25–28.

Claeys, H. and Inze, D. (2013) The agony of choice: how plants balance growth and survival under water-limiting conditions. *Plant Physiol.* **162**, 1768–1779.

Clough, S.J. and Bent, A.F. (1998) Floral dip: a simplified method for Agrobacterium-mediated transformation of *Arabidopsis thaliana*. *Plant J.* **16**, 735–743.

Czechowski, T., Stitt, M., Altmann, T., Udvardi, M.K. and Scheible, W.R. (2005) Genome-wide identification and testing of superior reference genes for transcript normalization in Arabidopsis. *Plant Physiol.* **139**, 5–17.

Davis, J.M. and Estes, E.A. (1993) Spacing and pruning affect growth, yield, and economic returns of staked fresh-market tomatoes. *J. Am. Soc. Hortic. Sci.* **118**, 719–725.

Del Moral, L.F.G., Ramos, J.M. and Recalde, L. (1985) Relationships between vegetative growth, grain yield and grain protein content in six winter barley cultivars. *Can. J. Plant Sci.* **65**, 523–532.

Fawal, N., Li, Q., Mathe, C. and Dunand, C. (2014) Automatic multigenic family annotation: risks and solutions. *Trends Genet.* **30**, 323–325.

Folta, A., Severing, E.I., Krauskopf, J., van de Geest, H., Verver, J., Nap, J.P. and Mlynarova, L. (2014) Over-expression of Arabidopsis AtCHR23 chromatin remodeling ATPase results in increased variability of growth and gene expression. *BMC Plant Biol.* **14**, 76.

Fradin, E.F., Abd-El-Haliem, A., Masini, L., van den Berg, G.C., Joosten, M.H. and Thomma, B.P. (2011) Interfamily transfer of tomato Ve1 mediates Verticillium resistance in Arabidopsis. *Plant Physiol.* **156**, 2255–2265.

Gabaldon, T. and Koonin, E.V. (2013) Functional and evolutionary implications of gene orthology. *Nat. Rev. Genet.* **14**, 360–366.

Gargul, J.M., Mibus, H. and Serek, M. (2015) Manipulation of MKS1 gene expression affects *Kalanchoe blossfeldiana* and Petunia hybrida phenotypes. *Plant Biotechnol. J.* **13**, 51–61.

Garner, L.C. and Björkman, T. (1996) Mechanical conditioning for controlling excessive elongation in tomato transplants: sensitivity to dose, frequency, and timing of brushing. *J. Am. Soc. Hortic. Sci.* **121**, 894–900.

Grau, D.J., Chapman, B.A., Garlick, J.D., Borowsky, M., Francis, N.J. and Kingston, R.E. (2011) Compaction of chromatin by diverse Polycomb group proteins requires localized regions of high charge. *Genes Dev.* **25**, 2210–2221.

Gutzat, R. and Mittelsten Scheid, O. (2012) Epigenetic responses to stress: triple defense? *Curr. Opin. Plant Biol.* **15**, 568–573.

Hochmuth, G.J. and Hochmuth, R.C. (2012) Production of Greenhouse tomatoes. In *Florida Greenhouse Vegetable Production Handbook*. pp. 1–18. Gainesville, Florida: University of Florida.

Jones, J.B. Jr. (2008) *Tomato Plant Culture: In the Field, Greenhouse, and Home Garden*, 2nd ed. Boca Raton, Fl, USA: CRC Press.

Jones, R., Ougham, H., Thomas, H. and Waaland, S. (2013) *The Molecular Life of Plants*. Oxford, GB: Wiley-Blackwell.

Karimi, M., Inzé, D. and Depicker, A. (2002) GATEWAY™ vectors for Agrobacterium-mediated plant transformation. *Trends Plant Sci.* **7**, 193–195.

Kemble, J.M., Davis, J.M., Gardner, R.G. and Sanders, D.C. (1994) Spacing, root cell volume, and age affect production and economics of compact-growth-habit tomatoes. *HortScience*, **29**, 1460–1464.

Kennison, J.A. (1995) The Polycomb and trithorax group proteins of Drosophila: trans-regulators of homeotic gene function. *Annu. Rev. Genet.* **29**, 289–303.

Li, C., Chang, P.P., Ghebremariam, K.M., Qin, L. and Liang, Y. (2014) Overexpression of tomato SpMPK3 gene in Arabidopsis enhances the osmotic tolerance. *Biochem. Biophys. Res. Commun.* **443**, 357–362.

Li, C., Chen, C., Gao, L., Yang, S., Nguyen, V., Shi, X., Siminovitch, K., Kohalmi, S.E., Huang, S., Wu, K., Chen, X. and Cui, Y. (2015) The Arabidopsis SWI2/SNF2 chromatin Remodeler BRAHMA regulates polycomb function during vegetative development and directly activates the flowering repressor gene SVP. *PLoS Genet.* **11**, e1004944.

Li, Z., Zhang, L., Wang, A., Xu, X. and Li, J. (2013) Ectopic overexpression of SlHsfA3, a heat stress transcription factor from tomato, confers increased thermotolerance and salt hypersensitivity in germination in transgenic Arabidopsis. *PLoS ONE*, **8**, e54880.

Liu, W., Yuan, J.S. and Stewart, C.N. Jr. (2013) Advanced genetic tools for plant biotechnology. *Nat. Rev. Genet.* **14**, 781–793.

Marti, E., Gisbert, C., Bishop, G.J., Dixon, M.S. and Garcia-Martinez, J.L. (2006) Genetic and physiological characterization of tomato cv. *Micro-Tom*. *J. Exp. Bot.* **57**, 2037–2047.

Mlynarova, L., Nap, J.P. and Bisseling, T. (2007) The SWI/SNF chromatin-remodeling gene AtCHR12 mediates temporary growth arrest in *Arabidopsis thaliana* upon perceiving environmental stress. *Plant J.* **51**, 874–885.

Narlikar, G.J., Fan, H.Y. and Kingston, R.E. (2002) Cooperation between complexes that regulate chromatin structure and transcription. *Cell*, **108**, 475–487.

Nickell, L.G. and McLaren, J.S. (1982) 13 – Plant growth regulators in the sugarcane industry. In *Chemical Manipulation of Crop Growth and Development*. (McLaren, J.S., ed.) pp. 167–189. Oxford, United Kingdom: Butterworth-Heinemann.

Özalkan, Ç., Sepetoglu, H.T., Daur, I. and Sen, O.F. (2010) Relationship between some plant growth parameters and grain yield of chickpea (*Cicer arietinum* l.) during different growth stages. *Turk. J. Field Crops*, **15**, 79–83.

Qiu, D., Diretto, G., Tavarza, R. and Giuliano, G. (2007) Improved protocol for Agrobacterium mediated transformation of tomato and production of transgenic plants containing carotenoid biosynthetic gene CsZCD. *Sci. Hortic.* **112**, 172–175.

Rocha, M.D.C., Deliza, R., Corrêa, F.M., Carmo, M.G.F.d. and Abboud, A.C.S. (2013) A study to guide breeding of new cultivars of organic cherry tomato following a consumer-driven approach. *Food Res. Int.* **51**, 265–273.

Runkle, E. and Blanchard, M. (2012) *Sumagic on Vegetable Transplants*. Michigan State University: Greenhouse Product News.

Sahu, P.P., Pandey, G., Sharma, N., Puranik, S., Muthamilarasan, M. and Prasad, M. (2013) Epigenetic mechanisms of plant stress responses and adaptation. *Plant Cell Rep.* **32**, 1151–1159.

Sang, Y., Silva-Ortega, C.O., Wu, S., Yamaguchi, N., Wu, M.F., Pfluger, J., Gillmor, C.S., Gallagher, K.L. and Wagner, D. (2012) Mutations in two non-canonical Arabidopsis SWI2/SNF2 chromatin remodeling ATPases cause embryogenesis and stem cell maintenance defects. *Plant J.* **72**, 1000–1014.

Sarnowska, E.A., Rolicka, A.T., Bucior, E., Cwiek, P., Tohge, T., Fernie, A.R., Jikumaru, Y., Kamiya, Y., Franzen, R., Schmelzer, E., Porri, A., Sacharowski, S., Gratkowska, D.M., Zugaj, D.L., Taff, A., Zalewska, A., Archacki, R., Davis, S.J., Coupland, G., Koncz, C., Jerzmanowski, A. and Sarnowski, T.J. (2013) DELLA-interacting SWI3C core subunit of switch/sucrose nonfermenting chromatin remodeling complex modulates gibberellin responses and hormonal cross talk in Arabidopsis. *Plant Physiol.* **163**, 305–317.

Sarnowski, T.J., Swiezewski, S., Pawlikowska, K., Kaczanowski, S. and Jerzmanowski, A. (2002) AtSWI3B, an Arabidopsis homolog of SWI3, a core subunit of yeast Swi/Snf chromatin remodeling complex, interacts with FCA, a regulator of flowering time. *Nucleic Acids Res.* **30**, 3412–3421.

Sato, S., Tabata, S., Hirakawa, H., Asamizu, E., Shirasawa, K., Isobe, S., Kaneko, T., Nakamura, Y., Shibata, D. and Aoki, K. (2012) The tomato genome sequence provides insights into fleshy fruit evolution. *Nature*, **485**, 635–641.

Schijlen, E.G., de Vos, C.H., Martens, S., Jonker, H.H., Rosin, F.M., Molthoff, J.W., Tikunov, Y.M., Angenent, G.C., van Tunen, A.J. and Bovy, A.G. (2007) RNA interference silencing of chalcone synthase, the first step in the flavonoid biosynthesis pathway, leads to parthenocarpic tomato fruits. *Plant Physiol.* **144**, 1520–1530.

Seffer, I., Nemeth, Z., Hoffmann, G., Matics, R., Seffer, A.G. and Koller, A. (2013) Unexplored potentials of epigenetic mechanisms of plants and animals-theoretical considerations. *Genet. Epigenet.* **5**, 23–41.

Sudarsanam, P. and Winston, F. (2000) The Swi/Snf family nucleosome-remodeling complexes and transcriptional control. *Trends Genet.* **16**, 345–351.

Tang, X., Hou, A., Babu, M., Nguyen, V., Hurtado, L., Lu, Q., Reyes, J.C., Wang, A., Keller, W.A., Harada, J.J., Tsang, E.W. and Cui, Y. (2008) The Arabidopsis BRAHMA chromatin-remodeling ATPase is involved in repression of seed maturation genes in leaves. *Plant Physiol.* **147**, 1143–1157.

Tsukiyama, T. (2002) The in vivo functions of ATP-dependent chromatin-remodelling factors. *Nat. Rev. Mol. Cell Biol.* **3**, 422–429.

Untergasser, A., Nijveen, H., Rao, X., Bisseling, T., Geurts, R. and Leunissen, J.A. (2007) Primer3Plus, an enhanced web interface to Primer3. *Nucleic Acids Res.* **35**, W71–W74.

Uversky, V.N. and Dunker, A.K. (2010) Understanding protein non-folding. *Biochim. Biophys. Acta*, **1804**, 1231–1264.

Uversky, V.N., Gillespie, J.R. and Fink, A.L. (2000) Why are "natively unfolded" proteins unstructured under physiologic conditions?. *Proteins: Struct. Funct. Bioinform.* **41**, 415–427.

Vignali, M., Hassan, A.H., Neely, K.E. and Workman, J.L. (2000) ATP-dependent chromatin-remodeling complexes. *Mol. Cell. Biol.* **20**, 1899–1910.

Weigel, D. and Glazebrook, J. (2006) Transformation of agrobacterium using the freeze-thaw method. *CSH Protoc.* **2006**, pdb.prot4666.

Whipker, B., Gibson, J. and Cavins, T. (2001) *Diagnosing Problems Due to Plant Growth Regulators*. pp. 1–5. Raleigh, North Carolina NC State University Floriculture Research: Floriculture Rx.

Wu, C., Sun, J., Zhang, A. and Liu, W. (2013) Dissipation and enantioselective degradation of plant growth retardants paclobutrazol and uniconazole in open field, greenhouse, and laboratory soils. *Environ. Sci. Technol.* **47**, 843–849.

Zandstra, J.W., Squire, R.C. and Watt, G.J. (2007) Managing transplant size and advancing field maturity of fresh tomatoes and peppers. In: *Ontario Vegetable Crop Research*, pp. 1–16. Guelph, Canada: University of Guelph Ridgetown Campus.

Zhang, J.Z., Creelman, R.A. and Zhu, J.K. (2004) From laboratory to field. Using information from Arabidopsis to engineer salt, cold, and drought tolerance in crops. *Plant Physiol.* **135**, 615–621.

Supporting information

Additional Supporting information may be found in the online version of this article:

Figure S1 RT-PCR analysis of the structure of *SlCHR1* in two tomato annotations.
Figure S2 Tomato RNA-seq libraries from different developmental stages used to map the RNA-seq reads to the genomic region of *SlCHR1* gene.
Figure S3 The coding sequence of *SlCHR1* gene and the derived protein sequence of SLCHR1 in one-letter amino acid abbreviations.
Figure S4 Flowering time of wild type and the two transgenic lines as the number of days from sowing till the first flower opens.
Table S1 List of primers used in this study.

