Genetic and molecular analysis of aerial plant architecture in tomato

Inaugural-Dissertation

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1 Introduction

1.A Aerial architecture of seed plants

The plasticity of architectures found in seed plants is enormous. It ranges from rather simple, forming only one axis of growth with simple leaves, to highly complex architectures, with manifold of branches, varying internode establishment, different types of leaves and leaflets and a tremendous variation in inflorescence architecture.

Although the potential to achieve different forms seems infinite, the aerial architecture of seed plants appears to be made up of single repeating modules, the phytomers or metamers. These basic modules consist of an internode, a leaf, and an axillary meristem (Sussex 1989; Lyndon, 1990). However, not the entire plant kingdom has evolved this kind of modules. Of the ten to twelve living plant phyla known, only five, comprising the seed plants (spermatophytes), form axillary meristems (Tomescu, 2006).

Depending on the plant species, developmental phase and growth conditions, all elements of the phytomers can be modified (Steeves and Sussex, 1989; Sussex and Kerk, 2001). Specifically the development of the axillary meristems has an important impact on the plant habitus, as AMs serve as multiplier of plant growth, providing new axis of growth. E.g. in *Arabidopsis* the formation of AMs is different in two phases of shoot development (Hempel and Feldman, 1994; Grbic and Bleecker, 1996). During the vegetative phase of development the initiation and morphological appearance of AMs happens in some distance to the shoot apical meristem (SAM) in an acropetal gradient. However, at the reproductive transition this pattern is changed and AMs are initiated closer to the SAM in a basipetal gradient (Hempel and Feldmann, 1994; Grbic and Bleecker, 1996). In reproductive development, axillary meristems can give rise to inflorescences, inflorescence branches or flowers. Which kind of lateral shoot is formed by a lateral meristem, i. e the so-called meristem identity, greatly influences inflorescence architecture (Prusinkiewicz *et al.*, 2007). When axillary meristems during the reproductive phase emerge as floral meristems and the primary apical meristem continues growth, this leads to the formation of an inflorescence type called raceme (e.g. in *Arabidopsis*). When axillary meristems have the identity of inflorescence meristems, while the apical meristem terminates by switching to floral meristem identity, this generates an inflorescence called cyme (e.g. in tomato, see also Fig. 1.D-1). Finally, if both, axillary meristems and the apical meristem can act as inflorescence meristems and then all terminate into floral meristems, this leads to the formation of a panicle (like in grasses).

Morphologists and other plant biologists have invented manifold of terms to describe the different parts derived by the variable manifestations of phytomer modification. For example leaf-homologous organs were named cataphylls, bracts, prophylls, sepals, lateral organs, scale -, basal -, juvenile -, true – or vegetative leaves, just to mention a few. Unfortunately, these terms are often very difficult to define and therefore often used in a contradictory way in scientific literature. Especially when crossing species borders, general definitions are repeatedly difficult to adopt. Therefore, frequently just new terms are invented. One reason for the difficulty with morphological categories might be that the modifications of the basic elements of aerial plant architecture, internodes, leaves and axillary meristems, often have resulted in developmental continuities rather than categories. Many plants display gradual patterns in the plasticity of phytomer elements. For instance, many plants establish continuous modification of leaf forms, from simple basal leaves over more complex adult vegetative leaves to again simpler bracts and then sepals (e.g. rose plants).

The presented reverse genetics project, analysing the function of selected MYB and bHLH transcription factors of tomato, revealed the identity of crucial regulators of the fate of phytomer elements influencing serval aspects of tomato architecture. Therefore, tomato architecture development and some related regulatory genes will be introduced here.

1.B The vegetative phytomer in tomato

Tomato leaves

The leaves in tomato phytomers can adopt three different fates: basal leaves develop only a few leaflets, adult compound leaves consist of up to 40 leaflets, whereas leaf development in the reproductive phytomers is completely suppresses (see chapter 1.D). Tomato plants clearly undergo a gradual development, from germination until the formation of the primary inflorescence. The basal leaves display less complexity than the subsequently formed leafs. There is a increase in complexity until at least the sixth leaf. This pattern is reiterated, although less pronounced, in each vegetative side-shoot.

Table 1.B-1 Tomato mutants developing reduced leaf complexity

gene / mutant	phenotype note	protein class	references	remark
potato leaf (c)	less leaflets, entire margins	-	Price and Drinkard, 1908; Kessler *et al.*, 2001	
goblet (gob)	similar to c	**NAC**	Berger *et al.*, 2009; Blein *et al.*, 2008	ortholog of CUC
Gob$^{4\text{-}D}$	less leaflets, deeply lobed and serrated	"	Berger *et al.*, 2009	miRNA164 resistant allele
procera (pro)	similar to c	**GRAS/ DELLA**	Jasinski *et al.*, 2008	constitutive gibberrellin response
lanceolate (la)	leaf blade formation along whole rachis, entire margins	**TCP**	Ori *et al.*, 2007	
entire (e)	leaflets adnate to each other, outgrowth along the whole rachis	**AUX/ IAA**	Zhang *et al.*, 2007	activated auxin response
entire^{2}	"	"	Stubbe, 1971b	allele in S. pimpinellifolium
trifoliate (tf)	reduced to three to five leaflets, lobed margins	-	Robinson and Rick 1954; Stubbe, 1957 and 1963;	*tf*^{2} and *tf*^{3} are former *tricuspis*
lyrate (lyr)	less leaflets, lobed margins	-	Soressi *et al.*, 1974	
solanifolia (sf)	similar to c	-	Kessler *et al.*, 2001	not allelic to c
jugata² (jug)	reduced leaflets, lobes and serration; inflorescence fusions	-	Stubbe, 1963	jug^{1} is a weak allele
rustica (rust)	blunt leaflets	-	Stubbe, 1957	dwarfish
inordinata (ida)	mild reduction in leaflet number, leaves in small angle to stem	-	Stubbe, 1971	double mutant with *praematura*
gibberosa (gi)	mild reduction in leaflet number, broad leaf blades, rarely wiry leaflets	-	Stubbe, 1971	double mutant with *praematura*
grossa (gro)	mild reduction in leaflet number	-	Stubbe, 1971	double mutant with *eluta*
side shoots repressed (sre)	reduced leaflets, lobes and serration	-	G. Schmitz personal communication	
complicata (com)	dwarf	-	Stubbe, 1958 and 1959	synonyme indiga

Table 1.B-1 Tomato mutants developing increased leaf complexity

gene / mutant	phenotype note	protein class	references	remark
mouse ears (me)	highly complex leaves	**KNOX**	Parnis *et al.*, 1997	gain of function allele of TKn2 second allele: curl
bipinnata (bi)	leaflets can resemble whole leaves	**BELL-like**	Kimura *et al.*, 2008	
Petro-selinum (Pts)	leaflets can resemble whole leaves	**KNOX**	Kimura *et al.*, 2008	allele from Solanum cheesmanii
clausa (clau)	continuously initiates „shooty“ leaflets	-	Stubbe, 1958	five alleles exist
multifolia (muf)	more leaflets, irregular	-	Stubbe, 1959	
polyphylla (pp)	leaflets can resemble whole leaves	-	Stubbe, 1963	
dupla (du)	more second order leaflets	-	Stubbe, 1971	double mutant with *splendens*
repetita (rpa)	leaflets like whole leaves, dwarf	-	Stubbe, 1971	double mutant with splendens, currently not available
tripinnate (tp)	mild phenotypic deviations	-	Sinha, 1999	
suffulta (su)	deeply cut, long petioles	-	Stubbe, 1957 and TGRC	synonyme nitida
transgenic line:				
LeKn1-over-expression	highly complex	KNOX	Haveren *et al.*, 1996	overexpression lines in wt, la, tf and c

The development of the leaf complexity in tomato has gained increasing interest in recent years. While many mutants affecting the complexity of tomato leaves were described in the last hundred years, it was only recently that several of the underlying genes were identified. Table 1.B-1 and Table 1.B-2 give an overview on mutants with altered leaf complexity and the underlying genes, if known. A new source of tomato mutants was generated by an EMS mutagenesis project in the cv. M82. These mutants are searchable in a phenotype database called "Genes that make tomatoes" (Menda *et al.*, 2004) and were not included in Table 1.B-1 and Table 1.B-2.

Knotted-like homeobox genes play an important role in the formation of compound leaves. While in the development of the simple *Arabidopsis* leaves, expression of the KNOX gene *STM* is excluded from leaf tissues, KNOX expression was found to trigger leaflet formation in compound leaves (Barkoulas *et al.*, 2007; Parnis *et al.*, 1997; Kimura *et al.*, 2008). Another important discovery is the presence of auxin response maxima and the establishment of "inverted fountain" fluxes, at the tips outgrowing leaflets and leaf lobes (Barkoulas *et al.*, 2007 and 2008). Also in tomato, auxin activated pathways are supposed to act as triggers of outgrowth from the flanks leaf primordia. In the mutant *entire*, an *AUX/IAA* gene is mutated leading to an auxin independent activation of the auxin response pathway and to enhanced outgrowth along the leaf rachis (Zhang *et al.*, 2007). Recently another important regulator of leaf complexity was identified in tomato. *procera* mutants carry a mutation in the tomato ortholog of the *Arabidopsis GAI* gene causing a simpler leaf phenotype (Jasinski *et al.*, 2008). *GAI* is known to negatively regulate gibberrellin response pathways. Therefore, it can be concluded that suppression of gibberrellin response is essential to establish the compound leaves of tomato.

Axillary meristems

Primary axillary meristems in the vegetative phase of tomato development are formed as protrusions in the axil of a leaf about four to five plastochrons later then the leaf itself (Gregor Schmitz, personal communication). A plastochron is the time elapsing between the formation of two consecutive phytomers. As described for other species (Hempel and Feldmann, 1994; Grbic and Bleecker, 1996) the last AMs before reproductive transition are formed faster than other vegetative AMs (see also chapter Fig. 1.C-1). Depending on the cultivar and growth condition, an accessory vegetative AM can be initiated in the axil between a primary side-shoot and the subtending leaf.

Although branching is an agronomically important trait in tomato breeding, only two genes regulating AM initiation are known in tomato yet. The *Lateral suppressor* (*Ls*) gene was identified to encode a member of the VHIID protein family (Schumacher *et al.*, 1999). *ls* mutant plants are characterized by the almost complete lack of AM initiation during the vegetative phase. However, the vegetative sympodial AM and the AM in the phytomer before are often formed in *ls* mutants.

The second important regulator of AM initiation in tomato is *Blind*. *blind* mutant plants show defects in vegetative and reproductive branching (Stubbe, 1959; Stubbe, 1964 Schmitz *et al.*, 2002). In the vegetative phase 40 % to 90 % of the phytomers lack AM initiation, while wild-type plants produce AM in nearly 100 % of vegetative phytomers (Schmitz *et al.*, 2002). *blind*

are distributed along the shoot axis in a specific pattern. Predominantly phytomers number two to five and the two phytomers below the inflorescence initiated axillary meristems (Mapelli and Kinet, 1992). Besides the function in vegetative development, *Blind* also regulates inflorescence architecture (see also Table 1.D 1). Flower number per inflorescence is strongly reduced compared to wild-type. Furthermore, the flowers often exhibit severe fusions (Schmitz *et al.*, 2002). The gene product of *Blind* was identified as an R2R3 MYB transcription factor (Schmitz *et al.*, 2002).

Also in other species, genes regulating AM formation were identified in the recent years. The function of *Blind* was described to be conserved in the orthologous gene family in *Arabidopsis*. *rax1 rax2 rax3* triple mutants almost completely lack vegetative AM initiation (Müller *et al.*, 2006). Interestingly, the paralogous genes act partly redundant, but also control AM initiation in different phases of vegetative development (Fig. 1.B-1, Müller *et al.*, 2006). Due to these results, paralogous genes of *Blind* were subject of the present work and were characterized for their function in tomato development (chapter 3.A).

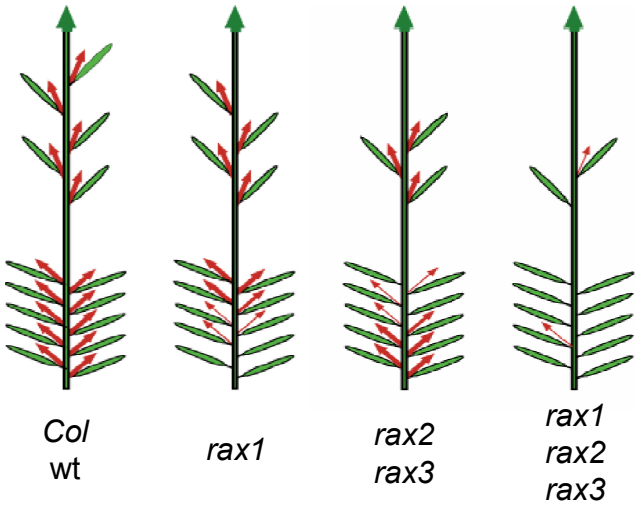


Fig. 1.B-1 Schematic illustration of the branching defects in *rax1*, *rax2* and *rax3*
The drawings represent *Arabidopsis* wild-type and mutant plants developing under short-day conditions. Red arrows indicate side-shoot formation in rosette or cauline leaf axils. The width of a red arrow indicates the proportion of plants developing a side shoot in a specific zone along the shoot axis (modified from Müller *et al.*, 2006).

Another pair of orthologous genes controlling AM formation was identified in grass species. The bHLH transcription factors LAX PANICLE and barren stalk1 were shown to regulate the formation of AMs in rice and maize respectively (Fig. 3.B-1, Komatsu *et al.,* 2003 and

Gallavotti *et al.*, 2004). In the second part of the present work, the function of an *LAX*-orthologous gene in tomato is characterized (chapter 3.B).

Furthermore, the function of *Lateral suppressor* was shown to be conserved in *Arabidopsis* (LAS, Greb *et al.*, 2003) and in rice (MOC, Li *et al*. 2003). Several other genes have been identified to influence AM formation in monocots and dicots. E.g. in *Arabidopsis* the class III HD-ZIP genes *REV*, *PHV* and *PHB* (McConnell and Barton, 1998), the PAZ/PIWI genes *PNH* and *AGO1* (Lynn *et al*., 1999; Kidner and Martienssen, 2004) and the NAC domain genes *CUC1, CUC2* and *CUC3* (Hibara *et al*. 2006, Raman *et al.*, 2008) are crucial players in the process of axillary meristem formation (reviewed in Bennett and Leyser, 2006 and Schmitz and Theres, 2005).

Development of internodes

Almost all internodes in tomato are elaborated and elongated. In contrast, in wild relatives of tomato the first and sometimes second internode of side-shoots are suppressed and the primary leaves of the side-shoots, the so-called prophylls, locate at the initiation site of the side-shoot and are attached to the leaf axil. As they are located at a similar position as stipules, but indeed are not formed by the leaf, but by the side-shoot, they are referred to as pseudo-stipules (Sawhney and Greyson, 1972).

Another important modification of aerial plant architecture connected to internode development is caused by fasciations. Solanaceae are well known to develop several kind of fusion of internodes with other organs or with each other. Tomato shoot architecture is modified by a fusion of the sympodial side-shoot with its subtending leaf, called recaules-cence (for a detailed description see chapter 3.A.3.2 and Fig. 3.A-4).

1.C Tomato phytomers at reproductive transition

In tomato, the change in phytomer architecture upon flowering transition is abrupt. While the last vegetative phytomer forms a fully compound leaf, the inflorescence phytomers do not develop any visible leaves (bracts). Furthermore, compared to vegetative AMs, reproductive AMs are formed without delay and develop with high velocity. However, with respect to AM formation and development at least one intermediate phytomer develops between true vegetative and reproductive stages in tomato plants. The last vegetative phytomer formed harbours an AM with a specific fate. This AM is formed earlier than normal vegetative AMs and grows out faster. As the primary meristem terminates by forming the inflorescence (see

next section), this last vegetative axillary meristem continues the vegetative growth of the tomato plant. The side-shoot resulting from this AM obtains the leading position of apical growth. Because later on this side-shoot forms part of the main plant axis, it is called sympodial shoot. In most cases, the sympodial shoot itself generates three vegetative phytomers before it terminates into the next inflorescence. The AM of the last of the three phytomers develops the next sympodial shoot continuing the sympodial growth pattern of tomato (Fig. 1.C-1) Sympodial development found in tomato is contrasting to the monopodial development in *Arabidopsis thaliana* and many other model organisms. In the monopodial growing species *Arabidopsis*, the primary shoot apical meristem maintains the pole position of indeterminate plant growth, while floral termination of meristem occurs only for reproductive AMs (Long and Barton 2000).

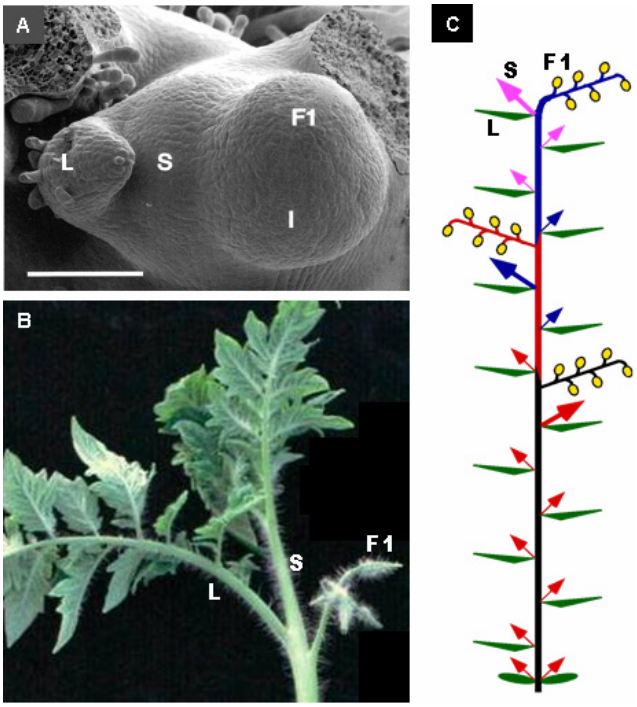


Fig. 1.C-1 Sympodial shoot development of tomato

(A) SEM image of a reproductive shoot tip of wild-type tomato (from Allen and Sussex, 1996). (B) Tomato plant with a young inflorescence and a sympodial shoot (C) Schematic illustration of sympodial shoot development of tomato. L: last leaf generated by the shoot apical meristem, S: sympodial shoot (meristem), F1: first flower (meristem), I: inflorescence meristem.

In the history of tomato breeding an important modifier of sympodial shoot development was discovered. Current field tomato varieties carry a mutation in the gene *Self pruning* (*Sp*). *Sp* suppresses reproductive identity in the vegetative sympodial axillary meristem. *sp* mutants develop increasing levels of reproductive identity in successive vegetative sympodial AMs, finally terminating the tomato sympodium due to the immediate formation of an inflorescence from a sympodial meristem (Pnueli *et al.*, 1998).

1.D The reproductive phytomer in tomato

After initiating the last leaf, the primary shoot, apical meristem of wild-type tomatoes generates one last phytomer before terminating into the first flower of the tomato inflorescence (Fig. 1.D-1). In this last phytomer, the development of a morphologically distinguishable leaf primordium is suppressed, while the axillary meristem develops with high velocity. This meristem forms the first branch of the tomato cymose inflorescence and therefore was named sympodial inflorescence meristem (SIM, Lippman *et al.*, 2008).

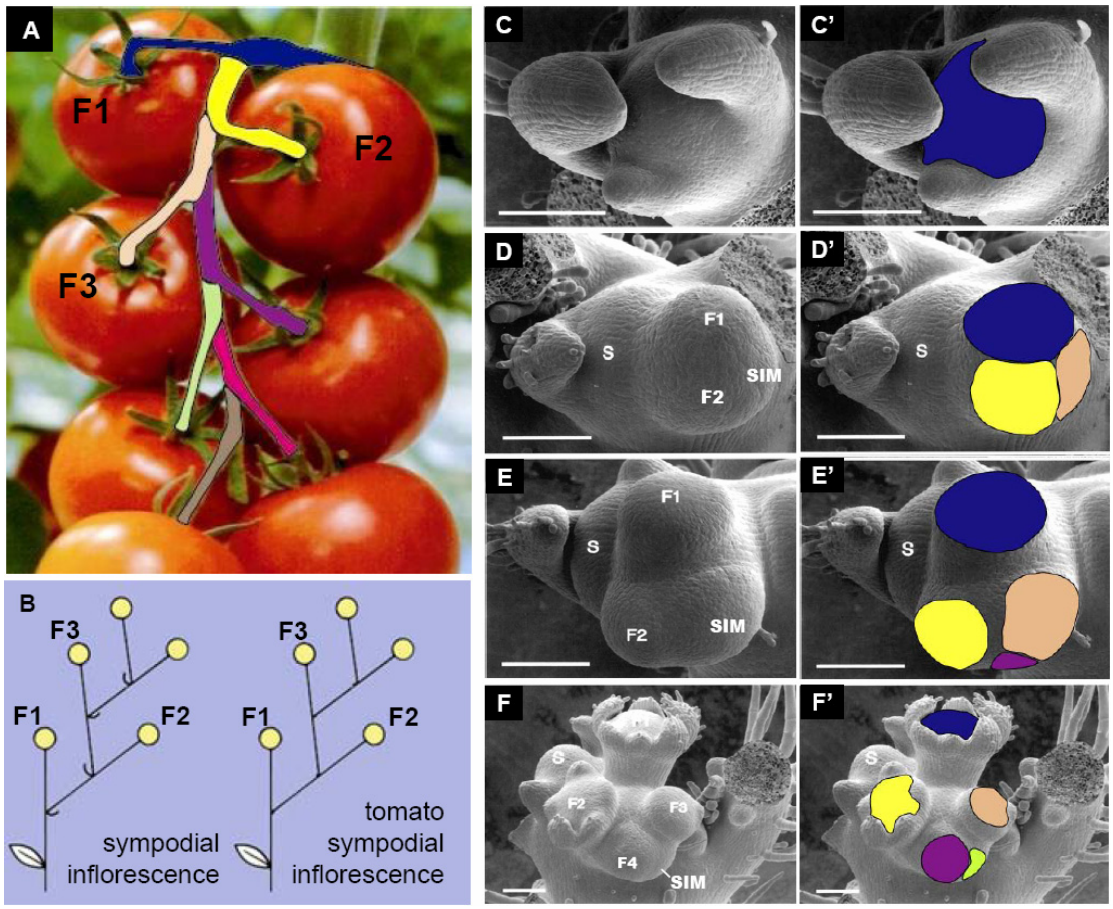


Fig. 1.D-1 Development of the tomato cymose inflorescence

(A) Wild-type tomato truss. Colours indicate the primary shoot (blue) and the successive reproductive side-shoots (colours correlate with colours in (C'-F')). (B) Schematic drawing of a sympodial inflorescence with alternating initiation sides of branches (scorpioid cyme) with bracts (left) and without bracts (right, tomato inflorescence type). (C-F) SEM images of wild-type tomato apices (modified from Allen and Sussex, 1996). (C'-F') Illustration of the fate of the primary shoot apical meristem (blue) and the successive sympodial inflorescence meristems (yellow, pink, purple) at early stages of reproductive development (colours correlate with colours in (A)). F … fruit (A), flower (B) or floral meristem (C-F); SIM … sympodial inflorescence meristem.

Due to the suppression of leaf development in tomato reproductive phytomers, the SIM does not obviously originate from an axil, however similar reproductive phytomer development can be observed in many species, e.g. in grasses the reproductive axillary meristems, namely branch meristems, spikelet pair meristems and spikelet meristems, develop in the axils of highly reduced bracts (McSteen, 2009).

Continuing the tomato inflorescence development, the SIM generates a single phytomer, again devoid of a visible leaf primordium but displaying an immediately emerging axillary meristem, the second SIM. Consecutively, the first SIM terminates forming the second floral meristem. This pattern reiterates producing the typical tomato scorpioid cyme inflorescence (Fig. 1.D-1, Helm, 1951; Danert, 1958; Sawhney and Greyson, 1972 and Lippman *et al.*, 2008). Finally, the inflorescence terminates after producing six or more flowers, normally by ceasing growth and development of the last phytomer and flower bud.

Notably, wild-type plants do not always achieve this ideal pattern of development. Some wild-type inflorescences generated vegetative structures like leaves or shoots in the cultivars and conditions investigated in this study. Furthermore, wild-type inflorescences can form branched cymes, i.e. that the primary shoot apical meristem or any sympodial inflorescence meristem does not terminate immediately after initiating one new SIM, but forms a second one before being transformed into a flower meristem. Consequently, two inflorescence meristems exist, both continuing cymose growth (Danert 1958).

In the last decades, several tomato mutants with altered inflorescence growth patterns were identified. Although some genes underlying these phenotypic deviations could be identified, many remain unknown yet. Table 1.D 1 gives an overview over the most important tomato mutants with altered inflorescence architectures and indicates the developmental aberrations and the responsible proteins, where known. Mutations in *SINGLE FLOWER TRUSS*, the tomato ortholog of the *Arabidopsis* gene *FLOWERING LOCUS T*, lead to late flowering and to the generation of inflorescences with mixed vegetative and reproductive characters, named pseudoshoots (Lifschitz *et al.*, 2006). Recently two genes, *compound inflorescence* (*s*) and *anantha* (*an*), controlling inflorescence and floral meristem identity were identified to encode orthologs of the *Arabidopsis* genes *WOX9* and *UFO* (Lippman *et al.*, 2008). *s* mutants develop highly branched inflorescences, resulting from serial initiation of SIMs by reproductive apical meristems prior to floral termination. *an* mutants fail to establish floral meristem identity and exhibit indeterminate SIM initiation (Helm, 1951; Lippman *et al.*, 2008).

Table 1.D-1 Tomato mutants exhibiting altered inflorescence architecture

mutant	inflorescence development	remarks	reference	protein
uniflora (uf)	suppressed branching, single flower inflorescences, pseudoshoot* formation	late flowering, depending on growth conditions	Fehleisen, 1967; Dielen *et al.*, 2004; Lifschitz *et al.*, 2006	
single flower truss (sft)	leafy, pseudoshoot formation, often solitary flowers	late flowering	Lifschitz *et al.*, 2006	CETS protein, ortholog of *FT*
falsiflora (fa)	leafy, inflorescence meristems initiate several SIMs, no flower formation	*fa²* is an allele in S. pimpinel-lifolium	Molinero-Rosales *et al.*, 1999	ortholog of *FLORICAULA* and *LEAFY*
leafy inflorescence (lfi)	weak allele of falsiflora, produces flowers		Kato *et al.*, 2005	
jointless (j)	jointless, inflorescence reverts to vegetative development		Mao *et al.*, 2000; Szymkowiak and Irish 2006	MADS box protein, ortholog of *SVP*
macrocalyx (mc)	leafy, jointless	similar to j, not allelic	Rick and Butler, 1956, Vrebalov *et. al.*, 2002	MADS box protein ortholog of *AP1*
macrosepala (mcs)	jointless, enlarged sepals	similar to j	Stubbe 1971	
composita (cpa)	branched, jointless, single flowers are subtended by reduced leaves		Stubbe 1963	
frondea (fro)	leafy, branched, jointless	other plant development normal	Stubbe 1971	
compound inflorescence (s)	branched (initiates serial SIMs before FM transition)		Lippman *et al.*, 2008	homeobox ortholog of *WOX9*
multifurcata (mua)	branched	similar to s	Stubbe 1963	
multiplicata (mup)	branched, king flowers, elongated internodes		Stubbe 1963	
anantha	continuously initiates SIMs but no FMs		Lippman *et al.*, 2008	F-box protein, ortholog of *UFO*
blind (bl)	reduced flower numbers, fusions, king flowers	reduced vegetative branching	Schmitz *et al.*, 2002	MYB protein
terminata (te)	king flowers, fusions	vegetative sympodial shoot suppressed;	Stubbe 1963	
multiplex (mux)	jointless, fusions (e.g. sepals with petals)	terminating shoot development	Stubbe 1963	
terminating flower (tmf)	single abnormal flower in primary inflorescence	lateral branches develop normal inflorescences	Hareven *et al.*, 1994	
bushy (bu)	joints dislocated to flower base	all internodes shortened, increased shoot branching	Stubbe 1957 and 1958	
conjunctiflora (cjf)	fusions		Fehleisen 1967	

*pseudoshoot: see text, SIM: sympodial inflorescence meristem, FM: floral meristem

2 Materials and Methods

2.A Materials

2.A.1 Chemicals

The following were main sources of supply for chemicals used in this work:

Ambion, Austin, USA

Amersham Pharmacia Biotec, Braunscheig, Germany

Biozym, Hess.Oldendorf, Germany

Carl Roth GmbH, Karlsruhe, Germany

Invitrogen GmbH, Karlsruhe, Germany

MBI Fermentas GmbH, St. Leon-Rot, Germany

Merck KgaA, Feinchemikalien und Laborbedarf Deutschland, Darmstadt

New England BioLabs GmbH, Schwalbach/Taunus, Germany

Operon, Cologne, Germany

PIERCE, Rockford, USA

QIAGEN, Hilden, Germany

Roche, Basel, Switzerland

Sigma Chemical Co., St.Lois, USA

2.A.2 Expendable materials and reagents

The following were the main suppliers of laboratory expendables used during this work: Incubation tubes and Petri-dishes: Greiner Lobortechnik; Eppendorf-Netheler-Hiny GmbH, Hamburg; Sarstedt AG & Co, Nümbrecht Membran for Southern hybridisation: Hybond XL, Amersham Biosciences, Braunscheig Kits for DNA and RNA extraction and purification:

Qiagen, Hildesheim Kits for total RNA extraction from plant: Qiagen, Hildesheim cDNA synthesis kit: MBI, GmbH, Fermentas RNA probe transcription kit: AMBION Austin, USA pCR®-Blunt-II-TOPO® and pGEM-Teasy for cloning and RNA probe transcription: Invitrogen, GmbH, Karlsruhe, Germany Gateway cloning kit: Invitrogen, GmbH, Karlsruhe, Germany

2.A.3 Enzymes and antibodies

Enzymes used during the course of this work were from following suppliers: Invitrogen GmbH, Karlsruhe, Germany New England BioLabs GmbH, Schwalbach/Taunus, Germany MBI Fermentas GmbH, St. Leon-Rot, Germany Roche, Basel, Switzerland Sigma Chemical Co., St.Lois, USA KOD hot start DNA polymerase, Novagen, Toyobo, Japan. Anti-Digoxigenin-AP Fab-Fragments (from sheep), Roche, Basel, Switzerland

2.A.4 DNA vectors

The following vectors were used to clone specific DNA fragements during this work:

pCR®-Blunt-II-TOPO®, Invitrogen, for cloning and transcription of DNA under the T7 promotor.

pGEM-Teasy Vector for cloning of PCR products and their Promega transcription under the T7 Promotor.

pDONR201 Vector for cloning of DNA-Fragmenten for use Invitrogen in Gateway System

pJawohl17 RNAi vector suitable for Gateway® cloning (Bekir Ulker, MPIZ DNA vector database)

pPZP212, binary plant transformation vector (GenBank accession U10462, Hajdukiewicz *et al.*, 1994)

pJaZP cloned in this project, see 2.B.10

2.A.5 Antibiotics selection

Antibiotics final concentrations

Ampicillin (Amp) 100 µg/L

Carbenicillin (Carb) 100 µg/L

Gentamycin (Gent) 50 µg/L

Kanamycin (Kan) 50 µg/L

Rifampicin (Rif) 100 µg/L

Spectinomycin (Spec) 100 µg/L

2.A.6 Bacteria

The following *Escherichia coli* strains were used during the course of this work. For cloning specific DNA fragements into vectors, DH5α (Hanahan, 1983) was transformed. The chemical competent cells were prepared as described by Sambrook and Russell (2001). DH5 α F-, end A1, hsdR17 (rk-, mk+), gyrA96, relA1, Hanahan, 1983 supE44, L-, recA1, 80dlacZM15, Δ(lacZYAargF) U196 DB3.1 B F– *ompT hsdS*(rB– mB–) *dcm*+ Tetr *gal l* (DE3) Stratagene *endA* Hte *metA::Tn5*(KanSr) *[argU proL* Camr] For plant transformation, *Agrobacterium tumefaciens* strain GV3101 with virulence plasmid pMP90 was used (Koncz and Schell, 1986).

2.A.7 Plant material

Arabidopsis seeds from *cycB1;1* mutants (ecotype Columbia) were kindly provided by Arp Schnittger. *lrp1* seeds (ecotype Nossen) were kindly provided by Eva Sundberg and wild-type seeds from ecotype Nossen were kindly provided by Maarten Koornneef. Potato genomic DNA from cv. *Desiree* was kindly provided by Christiane Gebhardt. *Solanum melongena* seeds were obtained from seed store (accession Madonna 2621 from F1 Hybrid). *Solanum linnaeanum* seeds were kindly provided from Heinz Saedler (accessions PI 388846, PI 388847 and PI 420415). *Solanum lycopersicon* seeds cv. Moneymaker, Kiepenkerl®, 2001,

were obtained from seed store. For transformation and genomic sequencing cv. Moneymaker was utilized.

uniflora lines were used as follows: *uf* ^{1} original mutant line from TGRC (accession LA1200 yg $^{-/-}$, cv. Platense). *uf* ^{1} Ailsa Craig near isogenic line MLE567 (Genebank IPK Gatersleben). *uf* ^{Y} cv. M82 (sp$^{-/-}$ and sp$^{+/+}$) kindly provided by Yuval Eshed, lines e2082m2 and e9312m1 from the mutant collection "genes that make tomatoes" (http://zamir.sgn.cornell.edu/mutants/-index.html) (Menda *et al.*, 2004). *uf*^{1} *sp* double mutant in cv. ACxHz were kindly provided by Muriel Quinet (Quinet *et al.*, 2006). Seeds of the tomato mutant *goblet* were kindly provided by Naomi Ori (Berger *et. al.*, 2009).*potato leaf* lines see Table 2.A-1. Other *S. lycopersicon* lines were obtained from TGRC, UC Davis and Genebank IPK, Gatersleben.

Table 2.A-1 Table with accessions and backgrounds of *c* alleles

current symbol	*previous name*	allelic variation	back-ground	control	accession	source	note
c^{1}		insertion	AC	NIL	LA3168	TGRC	
c^{2}	*c^prov2*	deletion	MM	IL	3-345	TGRC	
c^{3}	*c^prov3*	SNP	X	IL	3-604	TGRC	
c^{4}	*c^prov4*	SNP	VCH	IL	3-609	TGRC	
c^{5}	*c^prov5*	SNP	VCH	IL	3-626	TGRC	
c^{int}	*integerrima*^{1}	SNP	CR	IL	LA0611	TGRC	
c^{int}	*integerrima*^{1}	SNP	AC	NIL	LA3728A	TGRC	
c^{clt}	*coalita*	SNP	LU	IL	LA2026	TGRC	splendens$^{-/-}$
c^{bli2-1}	*blind-like2*^{1}	SNP	M82	IL	e2978	A. Bendhamane	sp$^{-/-}$
c^{bli2-2}	*blind-like2*^{2}	SNP	M82	IL	e2986	A. Bendhamane	sp$^{-/-}$
c^{int2}	*integerrima*^{2}	n.d.	S.pimp.	IL	MLP 97	Genebank IPK, Gatersl.	

Abbreviations are used as in TGRC databases.

2.B Methods

All general molecular biology laboratory methods not mentioned here are as described by Sambrook and Russell (2001).

2.B.1 Isolation of genomic DNA

Isolation of genomic DNA from plants for genotyping and segregation analyses was done using the quick-prep protocol (Edwards *et al.,* 1991) with slight adaptation for tomato leaf tissue. Extraction buffer (100mM TRIS-HCl pH8, 10mM EDTA pH8, 500mM NaCl, 0.7 % SDS); tissue + 500 µl extraction buffer + 150 µl 5M KAc. High quality genomic DNA for mapping, cloning and genotyping was extracted using Phenol/Chloroform extraction method (Sambrook and Russell, 2001) or using the *DNeasy® 96 Plant Kit* (Qiagen, Hilden,) and *BioSprint® 96* automated DNA extraction apparatus (Qiagen, Hilden).

2.B.2 Mutant and transgenic plant identification

Offspring generations of primary transgenic plants were analysed by germination on medium containing Kanamycin or spraying of seedlings with Kanamycin. However, transgenic plants could not be reliably identified by antibiotic resistance selection, putatively due to silencing of the transgene locus in the RNAi lines. Therefore, PCR tests had to be performed to confirm transgenic identity of individual plants (see Table 2.B-1).

Table 2.B-1 Molecular methods for identification of mutants and transgenic plants

locus	PCR-primers	polymorphism detection	restriction enzym	wt [bp]	mutant [bp]	heterozygot [bp]
mutants						
sp	Sp-1 Sp-2	CAPS	EcoRII or MvaI	~500 + 400	~900	~900 + 500 + 400
c^b2-2	2f10 2r11	CAPS	BccI	409+253	662	662+409+253
c^b2-1	like c^b2-2	sequencing with primer b2f9				all 662
lax-3	xf10 lxr1	sequencing with primer lxr2				all 268
uf^1	lxr9 SlLax1341F	length polymorphism	no possibility	249	274	274 + 249 + ~300 from heteroduplex
transgenes						
pJaZP-vector	pGPTV-FOR pGPTV-REV CD61-28 CD61-87	band present or absent internal PCR control (4 Primer PCR) (primers on ls gene)		**wt** 925 bp + no band	**transgenic** 925 bp + **391 bp**	hemi- and homo-zygot are not distinguishable
pJaZP-bli1:	(digest genomic DNA with SmlI to break inverted repeat structures of the RNAi construct)					
	(PCR on digested DNA)			**wt**	**transgenic**	
	Bl-l1for2 Bl-l1rev	transgene product lacks intron		720 bp + no band	720 bp + **319 bp**	

For primer sequences see Table 2.B-2.

Table 2.B-2 Oligonucleotide sequences for identification of mutants and transgenic plants

primer	sequences
Sp-1	ACCCCTTGTGATTGGTAGAGTG
Sp-2	AGTGCCTGGAATGTCTGTGAC
2f10	CCCTTAGATTCAAAAGAAAGGAAG
2r11	TGCATGCAGATGAAATATCCA
b2f9	GGTTACAAAGCAAATGAAGCAA
xf10	TCGTCAGCACCAAAGAAAGTT
lxr1	GATCATCATCAACGAGAGGGTAA
lxr9	CTTCTTTCGCGAGCAGCTAT
SlLax1341F	CCACGTACCTAACGCAATG
lxr2	CATCAACGAGAGGGTAATATCCA
pGPTV-FOR	CCGCAACGATTGAAGGAGCC
pGPTV-REV	AATAGCCTCTCCACCCAAGC
CD61-28	TCCCCTTTTTTTCCTTTCTCTC
CD61-87	AATCCTTAACTTTTCGCGGTCT
Bl-l1for2	GGCCAAATATTAAACACGGAGAG
Bl-l1rev	GGGAAAGTTGTGTTGTTGGGA

2.B.3 Bacteria transformation and selection

Transformations of vectors in *E.coli* were carried out by heat-shock treatment of chemical competent cells as described by Hanahan (1983). In cases where heat-shock transformations were inefficient, electro-transformations were performed using electro-competent cells (ElectroMAX DH5alpha-E Cells, Invitrogen) as described by Dower *et al.* (1988). Competent

agrobacteria cells were transformed using approximately 1µg of plasmids. Subsequently, the cells were incubated for 5 minutes each on ice, in liquid nitrogen and at 37°C for heat shock. After the addition of 800µl YEP, the cells were incubated on a shaker at 28°C for 3 hours, and then plated out on solid YEP medium with proper antibiotics. The concentrations of antibiotics used in this study are listed in *Materials.*

2.B.4 Incubation conditions for bacteria

E. coli were incubated in LB medium at 37°C over night (Sambrook and Russell, 2001) and Agrobacteria in YEP medium at 28°C for 2-3 days with proper antibiotics.

2.B.5 Isolation and purification of plasmid DNA

Plasmid DNA from bacteria was isolated using either the *Plasmid Mini kit* or *Plasmid Midi kit* (Qiagen, Hilden). Purification of PCR products and vectors were done using *Qiaquick PCR Purification* kit (Qiagen, Hilden) or ExoSAP-IT enzyme mix (GE Healthcare)

2.B.6 Semi-quantitative RT-PCR

RNeasy Plant Mini Kit (Qiagen, Hilden) was used for isolation of total RNA from plants. Subsequently, RNA was submitted to DNase digestion using DnaseI (Ambion, Cat# 1906) in (final concentration of >100ng/µl).

For first strand cDNA synthesis, *RevertAidTM H Minus First Strand cDNA Synthesis Kit* (GmbH, Fermentas) was used to transcribe the isolated total RNA according to manufacturer's protocol. 1300ng of total RNA was used for this reaction in 20 µl.

For selection of an internal control BLAST searches with *Arabidopsis* actin genes were performed on the SGN (Mueller *et al.*, 2005) unigene database. The unigene SGN-U314753 was chosen because of a high number of EST members evenly distributed from different cDNA libraries (see http://sgn.cornell.edu/search/unigene.pl?unigene_id=SGN-U314753). Primers SlActin2for2 (GCTATCCAGGCTGTGCTTTC) + SlActin2rev2 (TGCTCCTAGC-

GGTTTCAAGT) were utilized to amplify a 295 bp cDNA fragment spanning over the second intron (annotated by BLAST analyses).

Gene specific primers:

Uniflora: 262 bp from SlLax-for10 (TCGTCAGCACCAAAGAAAGTT) + SlLax-rev2 (CATCAACGAGAGGGTAATATCCA)

Primers for all six *Blind* gene family members amplified fragments of the cds spanning over the second intron. The reverse primer was located 3' of the MYB domain encoding sequence and ensured gene specificity.

Blind-like1: 238 bp from Bl-l1for2 (GGCCAAATATTAAACACGGAGAG) + Bli1-rev11 (TTAGTGGGAATTTGGTGGTGA).

Potato Leaf: 247 bp from 2f45 (GATGAAGAAGATAGGGTAATATGCAGT) + b2r9 (ATG-CTTGAGATTGGGATTGAA).

Bli3: 285 bp from b3f8 (CAAGCATGGAGGATTTTCAGA) + Bl-L3rev (TGGTTGTTGCA-TGATGAGAGG).

Bli4: 291 bp from SlBli4-for10 (TGGAGGGTTCACTGAGGAAG) + Bl-l5rev (GGGTCCA-TATGACTTGTGAATGT).

Bli5: 256 bp from Bli5-for7 (TGCGGCAAGAGTTGTAGGTT) + Bli5-rev6 (CAGCAGAGT-CGCTACTTGGA).

Blind: 274 bp from Cos79-59 (AGCATGGTGATTTTTCTGATGA) + Cos79-60 (CCAAGA-TTTTGTTGGGCTTG).

2.B.7 Polymerase Chain Reaction

Generally, PCR reactions were set as following: 5 µl 10xPCR Buffer, 2.0 µl of 50 mM MgCl, 0.5 µl dNTP (25 mM of each nucleotide), 0.2 µl *Taq*-Polymerase and 1 µl of each Primer (10 pmol/µl) in a 50 µl reaction made up with ddH2O. 10-100 ng of DNA was used as starting DNA template. The *Taq* polymerase was synthesized according to the protocol standardized by Pluthero (1993). Unless specified otherwise, reactions were accomplished using the PCR programme in a T3 Thermocycler by Biometra or the Biozym Multicycler PTC 225: 94°C for 2 min -> 28 to 38 cycles of 94°C for 15 sec, 56-60°C for 30 sec, 72°C for 1 min/kb -> 72°C for 6 min. For cloning work, the amplification of DNA fragments was done using KOD hot

start DNA polymerase (Navogen, Japan). KOD hot start DNA polymerase possesses a 5' to 3'-exonuclease activity. Thus, the PCR products do not have 3'-dA-nucleotid overhang.

2.B.8 iPCR and sequencing

Unknown flanking sequences of genomic loci of interest were obtained by *invertedPCR* (iPCR, Sambrook and Russell, 2001). PCR and iPCR products were purified with ExoSAP-IT enzyme mix (GE Healthcare) and DNA sequencings were accomplished by the MPIZ service unit "Automatic DNA Isolation and Sequencing" (ADIS) using Applied Biosystem (Weierstadt) *Abi Prism 377 and 3700 Sequenzer* by means of *BigDye-terminator chemistry*.

2.B.9 RNA *in-situ* hybridisation

2.B.9.1 Description of probes

Potato Leaf: C-terminal 572 bp antisense-probe (from pGS-C2A, AflII linearized), c-terminal 570 bp sense-probe (from pGS-C2BΔ, HincII linearized).

Blind-like3: C-terminal 524 bp antisense probe from pCR-B3Δfor and sense probe from pCR-B3Δrev (Acc65I and SpeI linearized; PCR fragment of primers Bl-L3-6 (TGACC-ATACATCCATCAGAAAGT) and BL-L3-CDS_RV (ACAACAAAAATTTACAATATA-ATAAAATG) cloned into pCR-Blunt-II-TOPO vector).

Blind: full-length cds antisense probe from linearized pGSMyb5a (Gregor Schmitz, personal communication).

Uniflora: Mixed 180 bp N-terminal and 197 bp C-terminal antisense probes, both excluding the bHLH domain. Synthesized from PCR product of primers 1341F (CCACGTACCTAACGCAATG) + 1521-T7 ((T7)-GTAAACTCTCTCTTTCTTTTCTT-TCG) and lxf8 (CCAAACGATGGTTAATTTAGTCG) + 1916-T7 ((T7)-ACATCACCA-GAAATATTAGTTTCTTCA) and 248 bp N-terminal sense probe from PCR product of primers 1341-T7 + lxr9 (CTTCTTTCGCGAGCAGCTAT).

2.B.9.2 Preparation of tissue sections and hybridization

Sample preparations and in situ hybridizations of 8-mm sections were done as described by (Coen et al., 1990) with slight modifications. 0.03% Tween-20 was added to the fixative, and dewatering of the fixed material was done without NaCl. Plant material was embedded in Paraplast+ (Kendall) in the ASP300 tissue processor (Leica). Probes were not hydrolyzed. After the colour reaction, slides were mounted in 30% glycerol and photographed using brightfield microscopy.

2.B.10 Production of RNAi lines for *Bli1* and *Bli3*

Cloning of RNAi constructs:

Utilizing the restriction sites PmeI and RsrII, the T-DNA cassette of pJawohl17 RNAi vector suitable for Gateway® cloning (Bekir Ulker, MPIZ DNA vector database) was cloned into the binary plant transformation vector pPZP212 backbone (GenBank accession U10462, Hajdukiewicz *et al.*, 1994) including plant selection marker gene nptII (Kan^R). The new vector was named pJaZP. Gene specific DNA fragments were cloned in reverse orientation into pDONR201 and successively into pJaZP via the Gateway® cloning system.

Sequences used for *Bli1* and *Bli3* RNAi constructs:

The complete cds of *Blind-like1* was amplified using primers Bl-l1-cds_fw_gw (GWR-TTT-TTTTCAAAAATCTCTTCTCA) and Bl-l1-cds_rv_gw (GWF-ATCAATAGTACATGATG-ACTTTT). The ENTRY clone was named pENTR-Bl-l1 and the binary RNAi plasmid, pJaZP-Bli1.

A fragment of *Blind-like3* from position +56 to +1025 (including 46 bp of 3'utr) using primers Bl-l3-cds_fw_gw (GWR-CTGAAGAAGATGCTAAGTTG) and Bl-l3-cds_rv_gw (GWF-ACAACAAAAATTTACAATATAATAAAATG) was cloned. The ENTRY clone was named pENTR-Bl-l3 and the binary RNAi plasmid, pJaZP-Bli3.

Transgenic plant production:

Agrobacterium mediated transformation of tomato leaf explants of cv. MM was performed as described (Knapp *et al.*, 1994). Primary transgenics were selected on medium containing Kanamycin.

Five independent *Bli3* RNAi lines and seven independent *Bli1* RNAi lines were obtained. At least four lines per construct exhibited similar developmental defects. The others were not analysed further. All *bli3* lines and three of the *bli1* lines were characterised for their number of T-DNA insertions. A Southern experiment (2.B.12) revealed one quadruple insertion line for *bli1* (bli1-K3) and one triple insertion line for *bli3* (*bli3*-K1). The other six tested lines appeared to be single insertion lines. For experiments described in the results section mainly the two single insertion lines *bli1*-K2 and *bli3*-K2 were utilized. Presence and absence of transgene was tested as described in chapter 2.B.2.

2.B.11 Production of RNAi lines for *Uniflora*

RNAi constructs were cloned as described in chapter 2.B.10.

Two different fragments were utilized. A N-terminal 458 bp fragment using primers lxf1 (ATTCATGCCCCACGTACCT) and lxr1 (GATCATCATCAACGAGAGGGTAA) and a 646 bp fragment comprising the complete cds using primers lxf1 and lxr8 (AAAAACTTAGGCAAACA) were cloned into pJaZP. RNAi plasmids transformed were pJaZP-lax3 and -lax6 (independent clones) carrying the shorter fragment and pJaZP-lax2 carrying the longer fragment.

Four independent transgenic lines phenocopied the development *uniflora* mutants (plant numbers 06227 to 06231, and 07019). Two lines were near phenocopies of *uf* (plant numbers 06207 and 07001). All these lines were from the shorter fragment RNAi construct. One additional line with the shorter fragment RNAi construct and both lines obtained from pJaZP-lax2 transformation resulted weak *uf* like defects (plant numbers 06204, 07041 and 07112). The phenotypic differences probably were not only caused due to individual differences of the transgenic lines, but also by differences in growing conditions (see results) due to different growing time points.

2.B.12 Southern hybridisation

Alkali DNA blotting and radiolabelled detection was performed as described (Sambrook and Russell, 2001). Nylon membranes Hybond XL, Amersham Biosciences, Braunscheig were

utilized. For identification of transgene loci, an approx. 1 kb antisense probe targeting the nptII gene was used.

2.B.13 Plant growth

If not stated explicitly, plants were grown under standard glasshouse conditions with additional artificial light (16-h photoperiod) continuously during the winter period and adjusted to natural light conditions and gradually reduced to 2 h morning and 2 h evening light supplement during summer period. In experiment summer08, plants were grown without artificial light.

2.B.14 Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) was performed with assistance from Rolf-Dieter Hirtz on a DSM 940 (Zeiss). For images of "micro-leaves" fresh tissues were first frozen in liquid nitrogen and subsequently coated with a gold layer under vacuum. For imaging of *uniflora* apices, seedlings were fixed in 4 % PFA solution and critical point lyophilized with assistance from Rolf-Dieter Hirtz.

2.B.15 Computational resources and methods

PCR and sequencing primers were designed with the primer3 tool (Rozen and Skaletsky, 2000). For BLAST analyses and EST and unigene retrieval the databases SGN (Mueller *et al.*, 2005) and GenBank (National Center for Biotechnology Information, NCBI) were utilized.

Assembly and analysing of sequencing results, vector and restriction planning, annotation of genomic sequences and sequence alignments were all performed with the *DNASTAR*® software package.

Image editing for photos and *in-situ* pictures (all in jpeg format), and SEM pictures were performed in MSOffice picture manager. Non-linear brightness/contrast edits were applied.

2.B.15.1 Genome matrix scan for the RAX3 binding motif

Genomic sequences related to single loci were obtained from TAIR ftp service (ftp://ftp.-arabidopsis.org/home/tair/Sequences/blast_datasets/) and scanned using the program MotifLocator (Thijs *et al.*, 2002). MotifLocator is an algorithm using an adapted position-weight matrix scoring scheme. Individual sites are scored by the motif model and a higher-order background model. The score is then computed as the normalized ratio of the motif score and the background score. Matrix searches are superior to simple pattern searches (word searches), as they consider the ratio of preferred nucleotides at ambiguous positions in the defined motif (ambiguous positions of the RAX3 motif: nSVnGGTnGGTKn, Romero *et al*., 1998). The background model takes the frequency of nucleotides in the Arabidopsis intergenic regions into account.

Result tables were produced in collaboration with Maren Heese and Heiko Schoof including the AGI code, name and synonyms of the according locus for each detected hit, the position, score, orientation and sequence of the motif, and the description and annotation of each locus. Names, synonyms and descriptions were retrieved from a web-service offered by www.atidb.org and from the flat file "TAIR_sequenced_genes" (ftp://ftp.arabidopsis.org/home/tair/Genes/TAIR_sequenced_genes) using a script written by Maren Heese.

Conserved elements in close proximity of detected RAX3 elements were identified using the tool CREDO (http://mips.gsf.de/proj/regulomips/credo.htm).

2.C Abbreviations

A. th.	...	*Arabidopsis thaliana*
aa	...	amino acids
AM	…	axillary meristem
AMs	…	axillary meristems
ba1	...	the *barren stalk1* gene from maize
BAC	...	Bacterial Artifical Chromosome
bHLH	…	basic helix-loop-helix
Bl	...	*Blind*
bli1	...	*Blind-like1* RNAi plant

bli3	...	*Blind-like3* RNAi plant
bp	…	base pair
cds	…	coding sequence
CI 0.5	…	confidence interval with significance level 0.05
Craig	...	Tomato cultivar Craigella
cv.	...	cultivar
cvs.	...	cultivars
DNase	…	Deoxyribonuclease
E.coli	…	*Escherichia coli*
EST	…	expressed sequence tag
Hz	…	Tomato cultivar Heinz
iPCR	...	inverse PCR
LU	...	Tomato cultivar Lukullus
MM	...	Tomato cultivar Moneymaker
ORF	…	open reading frame
Os	…	*Oryza sativa* (rice)
RAX	…	*REGULATOR OF AXILLARY MERISTEMS*
RT-PCR	…	Reverse transcriptase PCR
SAM	…	shoot apical meristem
SIM	...	sympodial inflorescence meristem, axillary meristem in reproductive
SNP	…	single nucleotide polymorphism
sp	…	*self pruning*
TGRC	…	Tomato Genetics Resource Center, University of California, Davis, USA
TILLING	...	Targeting Induced Local Lesions IN Genomes
Uf	...	*Uniflora*
utr	…	untranslated region of a mRNA
vs.	...	versus
wt	…	Wild-type
Zm	…	*Zea mays* (maize)

3 Results

3.A The *Blind* gene family

3.A.1 Identification of the *Blind* gene family

Blind was the first gene described from a subclass of R2R3 MYB domain transcription factors involved in branching regulation (Schmitz *et al.*, 2002). R2R3 MYB domain transcription factors are one of the largest families of transcription factors in plants. In *A. thaliana,* this family comprises 126 members. Six of these genes are co-orthologs of the tomato *Blind* gene and form a distinct subfamily (Stracke *et al.*, 2001; Müller *et al.*, 2006). For three genes of this family, *RAX1*, *RAX2* and *RAX3*, a function in the initiation of axillary meristems in *Arabidopsis* has been reported (Müller *et al.*, 2006).

Analyses of public tomato and potato cDNA and genomic sequence databases identified five new paralogous genes in the genus *Solanum*; one in tomato BAC sequence database, three in tomato EST- and one in potato EST-databases. Based on these sequences genomic fragments were amplified and sequenced by PCR and inverse PCR. Thereby more than 21 kb new genomic sequences were obtained, revealing complete coding regions and flanking sequences. In total, six members of the tomato *Blind* gene family are currently known and the new genes were named *Blind-like1* (*Bli1*), *Bli2*, *Bli3*, *Bli4* and *Bli5* (Table 3.A-1; for complete sequences including features see appendix 5.B). All six genes share a conserved gene structure identical to their *Arabidopsis* orthologs with the first 354 bp of the open reading frame encoding the MYB domain and carrying two introns (Table 3.A-1).

A unique spacing of the conserved tryptophans within the MYB domain distinguishes the *Blind* family from all other MYB proteins (Stracke *et al.*, 2001). One additional amino acid preceding the second tryptophan is diagnostic for all proteins of this subgroup. Sequence comparisons demonstrated that the DNA binding MYB domains share 80 to 90 % amino acid identity. Conservation behind the first 118 amino acids of the MYB domain is severely reduced. However, conserved elements are also present in the C-terminal domain. Up to three tyrosines are conserved at the very end of the proteins, mostly surrounded by hydrophobic amino acids and preceded by a lysine five to eight residues prior to the tyrosines (Fig. 3.A-1, inset). As orthologous proteins from poplar, *Arabidopsis* and rice also exhibit this feature (Fig. 3.A-1, inset), this hints at a site of functional importance. Furthermore, putative SUMOylation

sites (ΨKxE, Seeler and Dejean, 2003) and SUMO interacting motifs (e.g. VLxI, where valine, leucine and isoleucine are interchangeable, Perry *et al.*, 2008) are present in several family members and are conserved over species borders. In addition, an element of unknown function, EEIKxL, was detected in Bli1, Bli2, and in the *Arabidopsis* proteins RAX1 and RAX2 (Fig. 3.A-1, inset).

Homologies in the C-terminal part of the genes could also be tracked on nucleotide level (please show data). Therefore, phylogenetic relationships were explored utilizing complete coding sequences of the *Blind* family genes. *MYB35*, the closest homolog to the *Blind/RAX* family in *Arabidopsis*, was used as an outgroup relative. Sequence alignments with ClustalW and bootstrapping unveiled that *Blind-like2* is the closest paralog of *Blind* (Fig. 3.A-1). In addition, the phylogenetic tree indicates a common ancestor for *Blind*, *Bli1*, *Bli2*, *RAX1* and *RAX2*. However, no single pair of orthologous genes could be assigned, thus most genes presumably have arisen from duplications after the divergence of *Arabidopsis* and tomato ancestors.

In phylogenetic trees derived from protein sequence alignments (full protein and MYB domain only, data not shown) Bli4 and Bli5 form a pair of outliers within the group. Especially, Bli5 shows several sequence deviations at conserved residues and is significantly shorter than the other proteins. However, one frame shift and one point mutation in tomato evolution could explain the loss of 56 C-terminal amino acids, which would also include a terminal tyrosine (see also in appendix). Nevertheless, *Bli5* may not be a pseudogene, as there is a well-conserved orthologous gene in potato displaying 90 % amino acid conservation over the entire protein.

Table 3.A-1 Genomic structure of the Blind R2R3 MYB transcription factor family.

	chromo some	up-stream	**ATG to STOP**	down-stream	cds of exon1	intron1	exon2	intron2	cds[a] of exon3	**protein [aa]**
Blind	11	35,000	1542	80,000	136	197	130	397	682	**315**
Bl-like1	9	734	1620	627	136	328	130	401	625	**296**
Bl-like2	6	3412	2071	6457	136	417	130	745	643	**302**
Bl-like3	4	182	1740	297	136	362	130	400	712	**325**
Bl-like4	12	1021	1264	339	136	278	130	137	583	**282**
Bl-like5	8	80,000	1454	46,000	136	711	130	101	376	**213**

Values express bp of DNA sequence, apart from the columns chromosome and protein. The columns up- and downstream display the available genomic flanking sequence for each locus. From *Bli1* additional 1.2 kb downstream sequence is available separated by a 1.5 kb gap. In all six genes, the MYB domain extends from the start of the open reading frame until d 85 bp into the third exon. Complete sequence files are listed in the appendix. [a] figures include stop codons.

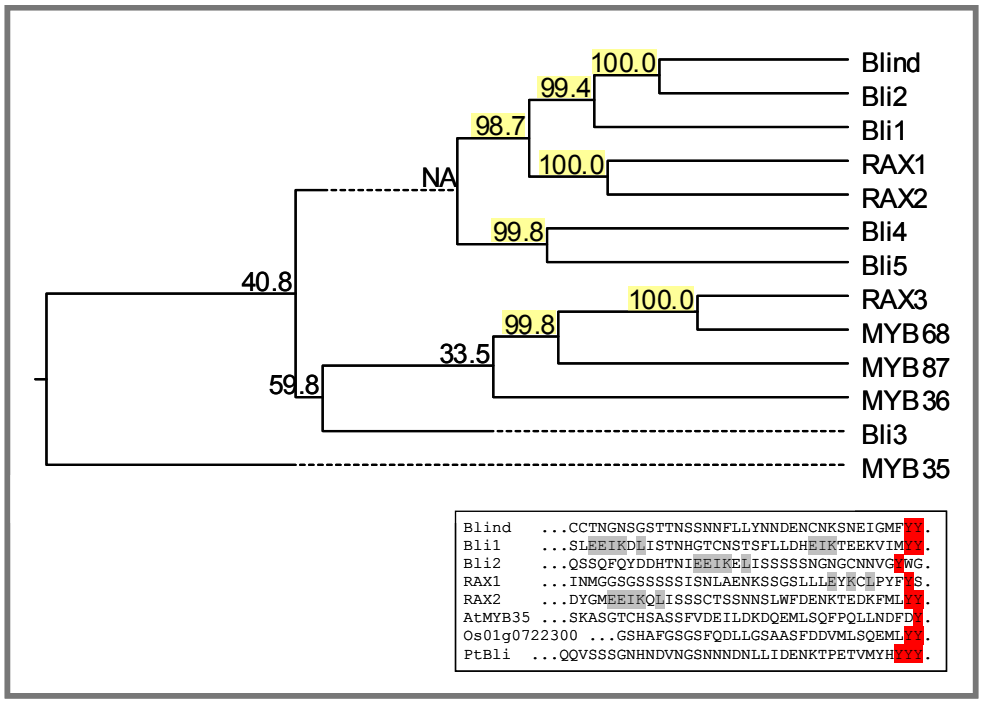


Fig. 3.A-1 Phylogenetic tree of the *Blind* R2R3 MYB transcription factor family from tomato and *A. thaliana*.

Nucleotide sequences of complete open reading frames (plus stop codons) were aligned using ClustalW. *Blind* and *Bli1* to *Bli5* are tomato genes, while *RAX1*, *RAX2*, *RAX3* and *MYB35*, *-36*, *-68* and *-87* are *Arabidopsis thaliana* sequences. *MYB35* was used as an outgroup and is not a member of the *Blind* family. Values represent results of 1000 bootstrap trials. Significant values are highlighted. (Inset) Alignment of the C-terminal end of *Blind* family proteins revealing the conservation of up to three terminal tyrosines (highlighted in red). Additionally, a conserved element (EEIKxL) of unknown function is indicated by grey shading.

3.A.2 Cloning of *Potato Leaf* (*C*)

As described in the previous chapter, the *R2R3 MYB* transcription factor *Blind-like2* (*Bli2*) is the closest paralog of *Blind*. In order to retrieve mutants of *Blind-like2*, a collaboration with Abdelhafid Bendhamane at the Plant Genomics Research Unit (UGRV, Evry France) was initiated. TILLING on the N-terminal sequence of *Bli2* was performed at the UGRV using a mutant population in the variety M82 (Menda *et al.*, 2004). Three families with putative mutations in *Bli2* were identified and bulk family seeds were provided by UGRV. Two alleles affected the coding sequence, while one point mutation located in the first intron and was not analysed further. When testing for the proposed SNPs and screening for homozygous mutants in the populations derived from the provided seeds, a strong leaf development alteration

cosegregated with the mutated alleles. Leaves of homozygous mutant plants of both alleles were simpler than wild-type leaves. The number of leaflets and leaf lobes was severely reduced and serration was nearly abolished (Fig. 3.A-2 A). These defects were reminiscent of the phenotypic defects described for the classical mutant *potato leaf* (Sinha *et al.*, 2001).

The first available scientific reference for *potato leaf* traces back to 1901 (White, 1901). It was one of the earliest mutants in genetic science just after the rediscovery of the Mendelian laws of inheritance (Price and Drinkard, 1908). The origin of the mutant was supposedly classical breeding, as Price and Drinkard described it as a "long known variety" and indeed some old tomato varieties carry "*Potato Leaf*" in their name.

A cross between *potato leaf* (gene symbol *c*, for cut leaf) and *blind-like2* demonstrated that the two mutants are allelic and sequencing of the *Blind-like2* gene in accessions carrying different *potato leaf* alleles proved, that *Blind-like2* is the gene coding for *Potato Leaf* (Fig. 3.A-2 B). $bli2^1$ and $bli2^2$ can now be added to the previously known alleles of *potato leaf*. Furthermore, the mutant *coalita* (*clt*) (Stubbe, 1971) was identified to be allelic to *c* and the provisional alleles c^{prov2} to c^{prov5} could be confirmed and renamed to c^2 to c^5. In addition, the allele c^{prov6} has to be taken from the TGRC list of *c* mutants. No sequence alteration was identified and testing for allelism by crossing was negative. In summary, ten alleles of *potato leaf* are currently known. Nine alleles were analysed molecularly. Seven alleles displayed single nucleotide polymorphisms, one represents a large deletion and one allele probably carries a large insertion (Table 3.A-2 and Fig. 3.A-2 B).

Table 3.A-2 The ten currently confirmed alleles of *potato leaf*

Current symbol	Previous name	Mutation	Effect	Mutagen
$c^{b2\text{-}1}$	*blind-like2*^{1}	G590T	Trp58Leu	EMS
$c^{b2\text{-}2}$	*blind-like2*^{2}	G590A	Trp58Stop	EMS
c^1	*c*	putative large insertion		spontaneous
c^2	c^{prov2}	> 8 kb deletion	gene loss	chemical
c^3	c^{prov3}	A553G	loss of splice site	chemical
c^4	c^{prov4}	G3A	Met1Ile	chemical
c^5	c^{prov5}	A163G	Arg55Gly	chemical
c^{int}	*integerrima*^{1}	G1466A	Asp102Asn	radiation
c^{clt}	*coalita*	T74A	Leu25His	radiation
c^{int2}	*integerrima*^{2}	n.d.		radiation

The abbreviation prov stands for provisional. Nucleotide positions relate to the ATG on the genomic sequence, where A is +1. The mutation in c^4 leads to a loss of the start codon. The next ATG in frame positions at + 352. c^{int2} is an allele found in *Solanum pimpinellifolium* (Stubbe, 1960). The putative insertion of c^1 is located at the C-terminus. EMS … *ethane methyl sulfonate*; n.d. … no data.

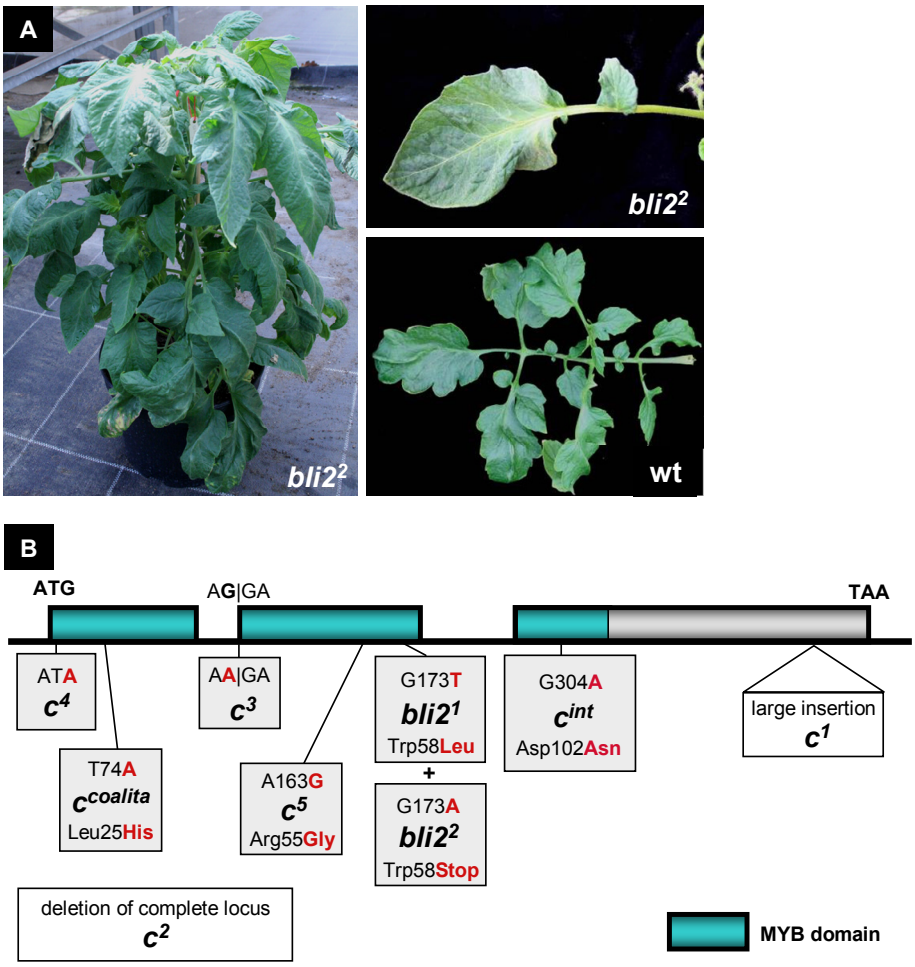


Fig. 3.A-2 Cloning of *Potato Leaf*

(A) The phenotype of *bli2*^{2} mutants resembled the described defects of the mutant *potato leaf.* Leaves had large entire leaflets and the number of leaflets per leaf was strongly reduced compared to the corresponding wild-type (M82). (B) Sequencing of *Blind-like2* in *potato leaf* accessions demonstrated that *Blind-like2* is encoding *Potato Leaf.* Seven alleles exhibit single nucleotide mutations. *c*^{2} shows a deletion of the complete locus (> 8 kb). *c*^{1} exhibits a putative large insertion.

3.A.3 Developmental processes controlled by the *Blind* gene family

In order to elucidate the functions of the three closest paralogs of *Blind*, TILLING was performed for *Bli2* (previous chapter), and silencing by RNAi was used for *Bli1* and *Bli3* (see material and methods 2.B.10). Genotypes labelled *bli1* or *bli3* in this work always correspond to transgenic RNAi loss of function lines (in cv. MM). *blind* and *bli2/c* mutants and *Bli1*, *Bli3* RNAi lines were analysed for phenotypic deviations in the development of aerial plant

architecture. The data presented in this chapter are mainly derived from four large experiments, growing the different loss of function populations in parallel. Additionally, the identified phenotypic defects were confirmed in several individual and smaller populations.

Generally, it is important to mention that most phenotypic defects identified in *bl*, *bli1* and *bli3* plants are strongly dependent on growth conditions. For example, the formation of "king flowers" was described as characteristic for *blind* (Rick and Butler, 1954, Stubbe, 1959 and 1964), but this phenotype was not observed in most experiments performed in this project. Nevertheless, when occurring, it could affect the majority of a population (G. Schmitz, personal communication). Similarly, the phenotype of *Arabidopsis* plants carrying mutations in the *Blind* orthologous genes *RAX1-RAX3* is also strongly dependent on growth conditions (Müller *et al.*, 2006). Unfortunately, the crucial factor in growth conditions affecting the mutant phenotypes in tomato could not be elucidated yet, although there are hints that the daily light dosage plays an important role. The phenotypic variations observed emphasized the importance of wild-type control plants of equal cultivar background grown in a randomized pattern to eliminate background and growth condition effects. These effects clearly influenced leaf complexity, flowering time, inflorescence architecture and other developmental traits.

In summary, the analyses of *bl*, *bli1*, *bli2/c* and *bli3* plants revealed two new functions of the blind gene family, the control of leaf complexity and the regulation of meristem development, and additionally shed a detailed light on the functions and redundancies in axillary meristem initiation and control of organ separation.

3.A.3.1 *C* and *Bli3* control leaf dissection

Wild-type tomato leaves are highly complex consisting of dozens of leaflets, which are variably lobed and bear serrated margins (Fig. 3.A-3 A-D). Several small populations of wild-type plants from cultivar Moneymaker were examined in this study. While the most basal leaf formed only two to three irregularly lobed leaflets, the complexity was strongly increasing during the development of the plant. The sixth leaf displayed highly developed complexity, although it had not reached the full number of leaflets compared to consecutive leaves. It remains elusive, when the increase in complexity stops, but there was repeated evidence that even from leaf seven to leaf ten average leaflet numbers still increase. Generally, the leaflet number was highly variable within each plant, between individuals and cultivars. To analyse wild-type cultivar differences, plants from cvs. Moneymaker and Lukullus, the original

background of the *bl*^{2} mutant, were grown with randomized positions to minimize environmental effects. The leaf below the last leaf of the primary shoot was analysed in order to compare leaves initiated at a similar developmental phase of the plant. Moneymaker leaves produced an average of 30 ± 5 leaflets, while Lukullus leaves harboured in average 23 ± 2 leaflets (significantly different, $p<0.01$, n=12. errors represent standard deviation). For details on first order and second order lateral leaflets, see Fig. 3.A-3 D. This cultivar difference was confirmed independently and affected basal and adult leaves. This result demonstrated the importance of isogenic control plants.

potato leaf plants develop leaves, which are simpler in terms of leaflet number and leaf margin dissection (Kessler *et al.*, 2001). The following general observations in the different mutant lines (see chapter 3.A.2) were made in the present study. The most basal leaf frequently consisted of a single leaflet lacking lobes and serration. The number of leaflets in subsequent leaves increased in a gradient like in wild-type, but never reached wild-type levels. Adult leaves of *potato leaf* almost completely missed small intercalary and second order leaflets (Fig. 3.A-3 A and B). In the experiment described above, *c*^{2} cv. MM generated on average 7 ± 2 leaflets while the wild-type generated 30 ± 5 leaflets (see Fig. 3.A-3 D). Leaflets of *potato leaf* remained nearly unlobed and lacked serration. Furthermore, the leaf blade area of single leaflets exceeded that of wild-type, and leaflets and leaves developed to a size comparable to the control. The terminal leaflet was typically found to be a fusion product of the two distal lateral leaflets and the terminal leaflet (see Fig. 3.A-3 E). Therefore, the resulting terminal leaflet often appeared to be lobed.

Aside from the leaf developmental phenotype, no pleiotropic defects were detected. Thus, *potato leaf* has no function in shoot branching like *Bl, Bli1 and Bli3*, as there was also no expression found in the according domains by RNA *in-situ* hybridisation (see 3.A.4.3). The different alleles of *c* in their different tomato backgrounds did not exhibit obvious deviations from the described defects, with exception of *c*^{int}, which showed an intermediate phenotype (Fig. 3.A-3 A). All other alleles are therefore considered as knock-out mutations. It is remarkable that the function of the gene is conserved in *Solanum pimpinellifolium*, a wild relative of *Solanum lycopersicum*. The mutant *integerrima*^{2} in *Solanum pimpinellifolium* has similar defects in leaf development and was shown to be allelic to *c*^{int} (Stubbe 1960).

Blind-like3 (*Bli3*) plays a similar, but less prominent role in leaf development as *Potato Leaf*. Evidence for control of leaf complexity by *Bli3* was obtained by transgenic plants carrying RNAi constructs targeting *Bli3*. *Bli3* RNAi lines exhibited a reduction in all three levels of leaf complexity (Fig. 3.A-3 C). In the above-described experiment *Bli3* RNAi plants produced only 11 ± 4 leaflets and showed reduced lobing and serration, but no fusions (Fig. 3.A-3 D). This phenotype was observed repeatedly, but was also found to depend largely on growth

conditions. Plants from identical seed batches displayed different levels of defects depending on the experiment, showing also more complex leaves than presented in Fig. 3.A-3 C and D.

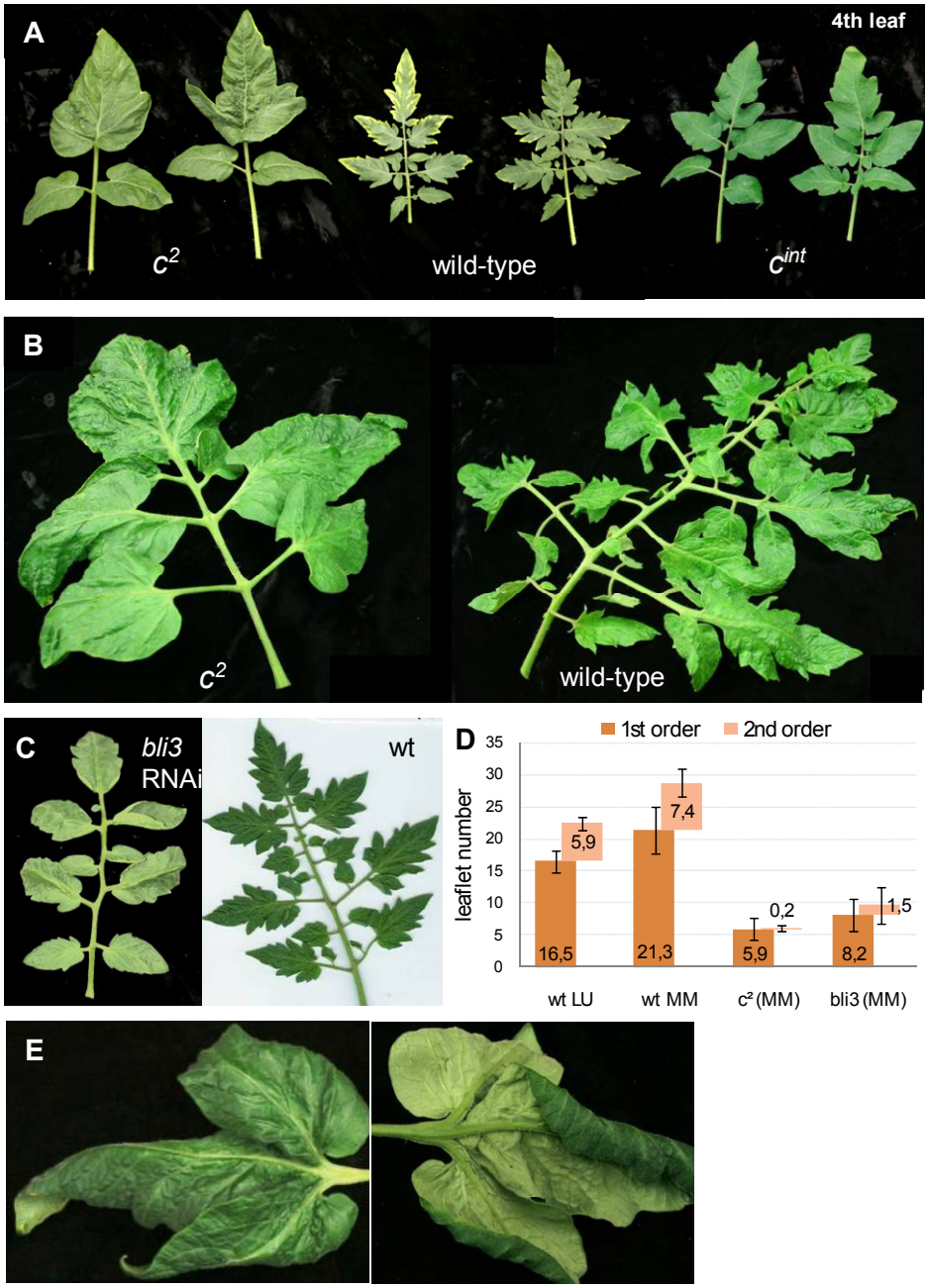


Fig. 3.A-3 *Potato Leaf* and *Blind-like3* control leaf complexity

(A) Fourth leaves from plants of same age 5 weeks after sowing. c^2 shows a high reduction in leaflet number compared to wild-type, but larger leaves; c^{int} has an intermediate phenotype. (B) Adult leaves of c^2 and wild-type (MM) plants; mature leaves of same size and age. Note the enlarged and entire leaflets of *potato leaf* and the missing second order leaflets. (C) *Bli3* RNAi plants displayed intermediate defects in leaf complexity. (D) Mean numbers of lateral leaflets of adult leaves (-1 from sympodial fork) of randomized grown plants (n=12, error bars give the standard deviation). Total leaflet number (see text) equals the sum of primary and secondary lateral plus one terminal leaflet. (E) Fused terminal leaflets in c^2 leading to an excess of leaf blade.

In summary, *Potato Leaf* and *Blind-like3* control leaflet and leaf lobe formation and leaf serration. The size of the mutant leaf blades, give the impression that the lack of lobing and serration is due to missing growth retention at the indentations, rather than due to missing outgrowth of lobe and serration tips (see also chapters 3.A.4.3 and 3.A.5 supporting this model).

Interactions of *c*

Assuming that the regulatory pathway involving *Potato Leaf* is homologous to the molecular pathway of *Bl, Bli1 and Bli3* controlling AM formation, *potato leaf* and leaf development may function as an easy to access model to elucidate these pathways. On the other hand, development of leaf complexity has itself become a research topic of increasing interest. In order to unveil genetic interactions and pathways of *Potato Leaf*, *c* mutants were crossed to more than a dozen of different genotypes with altered leaf compoundness (for an overview see Table 1.B-1 and Table 1.B-2 in introduction). The analyses of double mutants are currently in progress, focusing on mutants for which the underlying gene has been identified. Furthermore, *blind potato leaf* double mutants were established, but revealed no obvious enhancement of either defect in two independent allele combinations, although *Blind* is expressed in similar regions in leaf primordia like *C* (see chapter 3.A.4.2).

***C* in eggplant and potato**

Finally, the potential role of *Potato Leaf* in inter-species variation of leaf shapes was studied. Obviously, *potato leaf* leaves share some similarities with leaves of *Solanum tuberosum*. However, partial protein sequence (obtained by genomic PCRs, missing only the terminal 22 amino acids) of *C*-alleles from the tetraploid potato cultivar *Desiree* did not show major alterations compared to the tomato gene. 95 % of the 280 amino acids are conserved and the divergent 5 % are not affecting any conserved residues. Another putative role in species diversification for *Potato Leaf* was assumed based on a QTL study, mapping a QTL for leaf lobing differences between *eggplant* and its wild relative *S. linnaeanum* (Doganlar *et al.*, 2002). The identified QTL on chromosome six spans a large region putatively including the *Potato Leaf* locus. *C* was PCR-amplified and genomic sequence was obtained from both species. However, no evidence for a functional difference could be obtained when comparing the full-length protein sequences from the two species showing only three amino acid exchanges at non-conserved positions. Nevertheless, in both species, potato and eggplant, differences in the regulatory sequences of *C* may still be responsible for the simpler leaf phenotype.

3.A.3.2 Prevention of concaulescent fusions by *Bl, Bli1 and Bli3*

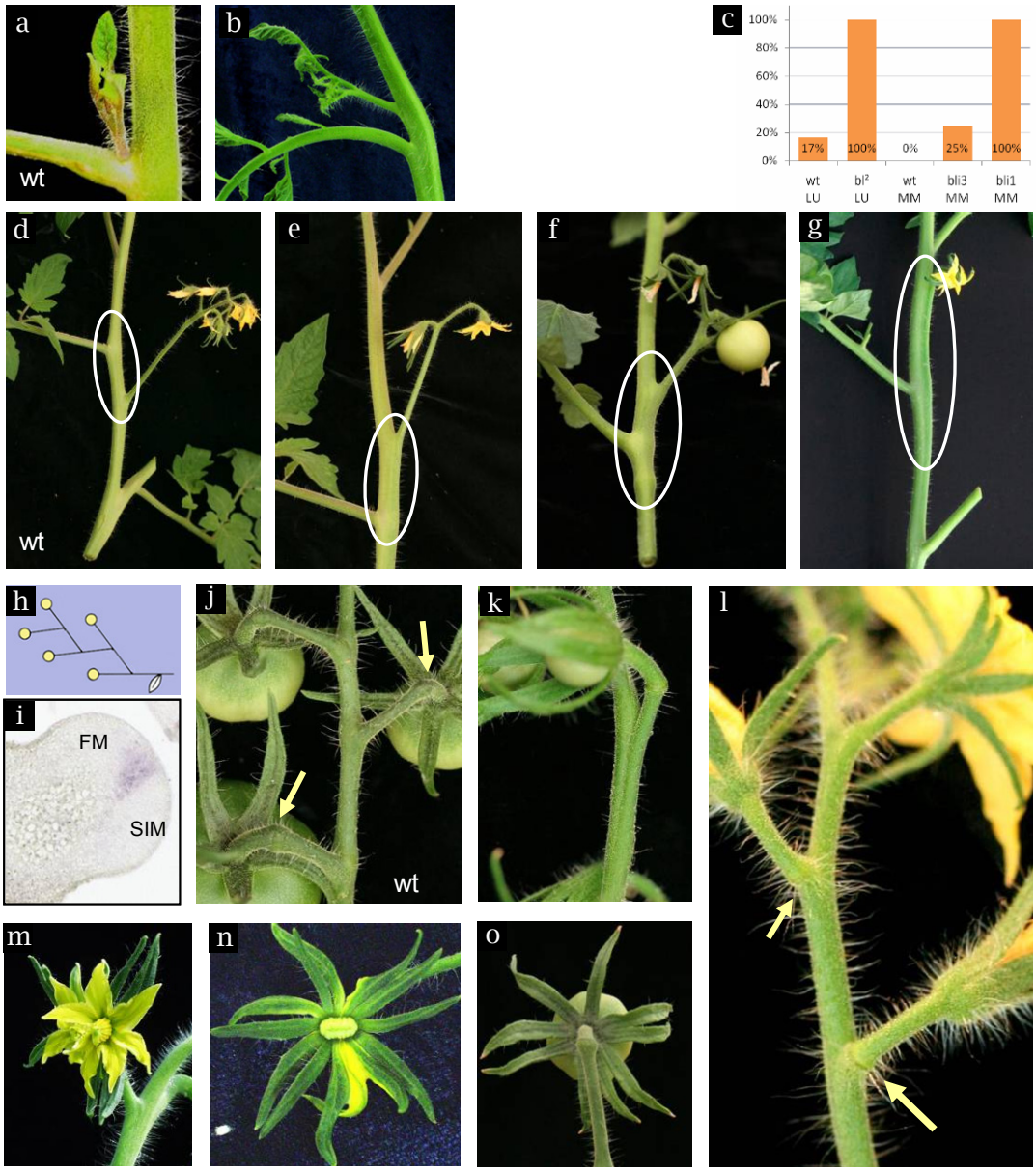


Fig. 3.A-4 Concaulescent fusions of vegetative and reproductive side-shoots

(a) wild-type vegetative side-shoot. (b) Concaulescent side-shoot of *blind* in the leaf axil below the sympodial fork. (c) Frequencies of inflorescences that displayed "reduced pedicels" (see text; n=24 wt MM, 11 bl^2, 12 for others) (d) Recaulescence of the sympodial shoot in wild-type. (e-g) Concaulescence of the sympodial shoot in *blind* mutants. Ovals encircle the two axils of the sympodial shoot. (h) scheme of a cyme, the tomato inflorescence type (i) RNA *in-situ* hybridisation showing the *Blind* expression domain separating the apical flower meristem (FM) and the sympodial inflorescence meristem (SIM) (see chapter 3.A.4.2) (j) close up of a wild-type inflorescence. Arrows point at the abscission zones (joints) within the pedicels of the tomato fruits. (k-o) Continuum of concaulescent fusions of apical flower meristems and SIMs (details in the text). (k) *bli1 bli3* (l) *bli1* (m-o) *blind*.

Normally, axillary shoots grow out of the axil between the subtending leaf and the shoot (Fig. 3.A-4 a). A side-shoot fused to its parental shoot is called concaulescent, in contrast to recaulescent fusions, where the side-shoot unites with the petiole of the leaf. Solanaceae are known to exhibit different kinds of fusions of shoots and organs, thereby influencing shoot architecture. Under constant conditions, the pattern of fusions is controlled genetically and in this chapter *Bl, Bli1 and Bli3* are shown to be involved in this process.

bl, *bli1* and *bli3* plants exhibited fusions of shoots and flowers and length differences of internodes, peduncles and pedicels. Characterisation of these defects unveiled that one principal function of *Bl, Bli1 and Bli3* is the separation of axillary meristems from their parental shoot during the vegetative and reproductive phases of development.

During vegetative development of *blind,* two side-shoots were affected. First, with moderate penetrance, the fast developing side-shoot below the sympodial fork was fused to the stem (Fig. 3.A-4 b) and secondly, this was frequently the case for the sympodial shoot (Fig. 3.A-4 e-g, for sympodial shoot development see introduction Fig. 1.C-1). In wild-type, the sympodial shoot normally unites with its subtending leaf (recaulescent fusion) which leads to a final positioning of this leaf up to 20 cm above the inflorescence peduncle. The peduncle comprises the internode formed by the main shoot after initiation of this last leaf (Fig. 3.A-4 d). In the *blind* mutant, concaulescent fusions of the sympodial shoot even exceeded the recaulescence and therefore the point of separation between the inflorescence peduncle (primary shoot) and the sympodial shoot (axillary shoot) was found above the axil of the sympodial shoot and its subtending leaf (Fig. 3.A-4 e-g). In four independent *blind* populations penetrance was 75 % to 90 % (n = 10, 10, 12 and 29). In *Bli1* and *Bli3* RNAi lines these concaulescent fusions of the sympodial shoot were also observed, but with lower penetrance (the observed maximum was 14 % in *bli1 bli3* double transgenic plants, n = 22). In seven wild-type control populations only 0 to 3 % of sympodial shoots deviated from the normal recaulescent pattern of development (total n = 210, three observations of concaulescence exceeding the recaulescent fusion).

In tomato inflorescences, the apical flower meristem forms the flower pedicel including the joint, while the sympodial inflorescence meristem (SIM) forms the side-shoot and therefore the inflorescence internode. (shown in introduction 1.D and Fig. 3.A-4 h and i). Hence, fusions between the flower pedicel and inflorescence internodes are concaulescent fusions, homologous to the concaulescence in vegetative development. Such fusions between the flower pedicel and inflorescence internodes were observed in *bl*, *bli1* and *bli3* plants (Fig. 3.A-4 k).

In *bli1* and *blind* (cv. Craigella), concaulescence in reproductive development led predominantly to the loss of the proximal part of the flower pedicels. In wild-type, a flower pedicel is divided by the formation of an abscission zone, the so-called joint (see Fig. 3.A-4 j, arrows.) In *bl* (cv. Craigella) and *bli1* inflorescences the first (proximal) part of the pedicels was often fused with the internode of the next sympodial inflorescence unit (Fig. 3.A-4 k, l). Although this fusion was often so strong that the two parts were completely merged, the fusion was mostly resolved at the joint (Fig. 3.A-4 l). The graph in Fig. 3.A-4c shows an exemplary experiment analysing frequencies of inflorescences harbouring this kind of fusion. Generally, *Blind* and *Bli1* appeared to play a dominant role in prevention of concaulescent fusions in inflorescences compared to *Bli3*.

In *blind* mutants of cv. Lukullus concaulescence frequently exceeded the point of joint formation, causing fused flowers (Fig. 3.A-4 m). A continuous degree of fusions was observed, in strong cases resulting in seemingly single flowers, displaying increased organ numbers (Fig. 3.A-4 m-o). The presence of such fusions was strongly dependent on the cultivar background. In two populations (n=12 and 21) of bl^2 in cv. LU, 75 % and 100 %, respectively, of the inflorescences developed fusions exceeding the joint. In contrast, two populations of bl^1 in cv. Craigella grown in parallel displayed this defect only in 25 % of their inflorescences (n=12, both). This resulted in a different appearance of the inflorescences of *blind* plants in the two cultivars. Besides, the genetic background of cultivar Lukullus influenced also other traits of *blind* inflorescences (less micro-leaves and leafiness, see chapters 3.A.3.3 and 3.A.3.4). Alternatively, the different alleles might cause the different phenotypes, but analyses of a cross of bl^2 cv. LU with cv. MM and the assumption that both both alleles are knock-out alleles, favour the cultivar as a cause.

It is noteworthy, that these fusions did not prevent the formation of the subsequent sympodial inflorescence meristem, and therefore are not the cause for the precocious termination of *bl*, *bli1* and *bli3* inflorescences. Finally, *bli1 bli3* and wild-type tomato inflorescences almost never exhibited fusions exceeding the joint.

In summary, the defects described here for *Bl*, *Bli1* and *Bli3* loss of function plants demonstrated a function for the three genes in the separation of shoot apical and axillary meristems in vegetative and reproductive development of tomato.

3.A.3.3 *Bl*, *Bli1* and *Bli3* control the initiation of vegetative and reproductive AMs

The initiation of vegetative AMs is controlled in a "zonal" fashion

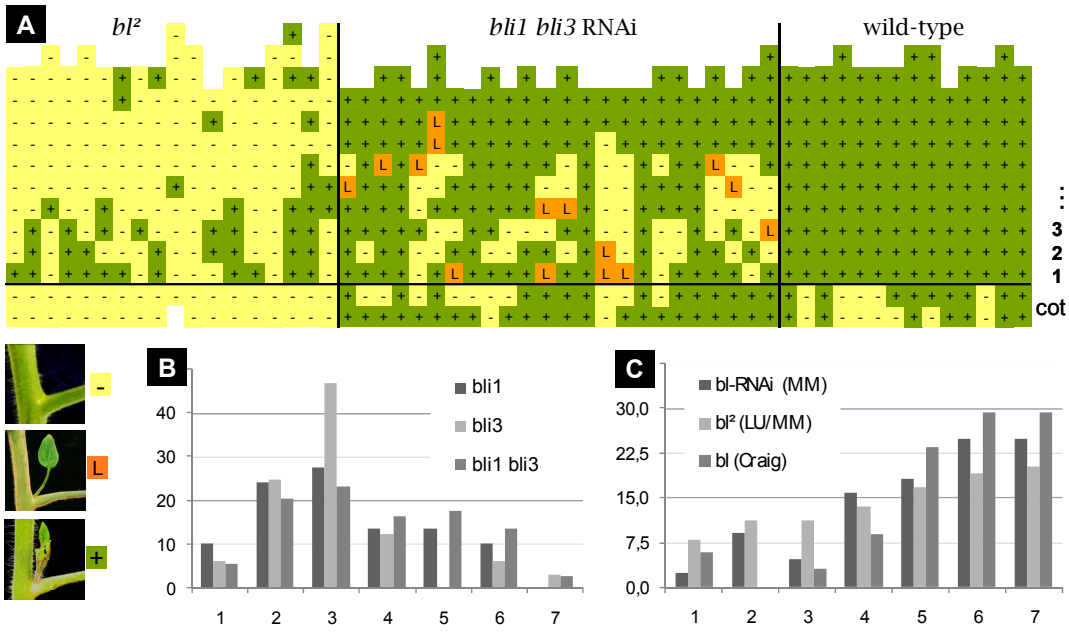


Fig. 3.A-5 Zonal branching defects of *bl*, *bli1* and *bli3* plants.

(A) Side-shoot formation in bl^2, *bli1 bli3* and wild-type plants. Cotyledons (cot) and all leaf axils of the primary shoot were judged to be barren (-), harbour a terminating structure (L) (see also chapter 3.A.3.4) or to carry an axillary bud or shoot (+). Each column represents a single plant from cotyledons up to the last leaf of the primary shoot. bl^2 and wild-type are from a segregating population (cvs. LUxMM) and *bli1 bli3* double transgenic plants are in cv. MM. (B, C) Relative distribution of barren axils in the first seven leaves ("1" indicates the most basal leaf). (B) Summed up results of three independent experiments for each genotype ($n_{(barren\ axils)}$ = 29, 32 and 73 for *Bli1*-, *Bli3*- and double RNAi lines, respectively). (C) Single experiments for three different *Bl* loss of function genotypes ($n_{(barren\ axils)}$ = 44, 89 and 34 for *Bl* RNAi, bl^2 and bl^1 respectively).

Bli1 and *Bli3* single and double transgenic RNAi plants were analysed for their branching pattern during vegetative development in parallel with *Blind* loss of function plants in three independent experiments. All three experiments demonstrated that *Bli1* and *Bli3* exert a function in AM formation. Experiment "summer08", where plants were grown without artificial light, displayed the strongest branching defect for double transgenic plants. Plants silenced for *bli1* and *bli3* exhibited a defect in side-shoot formation in 40 % of their vegetative leaf axils (leaves one to seven, Fig. 3.A-5 A). The majority of affected axils

remained barren, while some produced terminating axillary structures (Fig. 3.A-5 A, for detailed explanation see chapter 3.A.3.4).

Similar patterns, although less pronounced, were obtained in two repetitions with artificial light supplements. The three experiments revealed that *bli1* and *bli3* formed empty axils mainly in the first six leaves after the cotyledons, with a peak at the second and third leaf axil. *bli3* displayed an obvious maximum of barren axils at the third leaf, while the defect of *bli1* was broader (Fig. 3.A-5 B). The phenotypic penetrance varied from experiment to experiment, a fact that is also described for the branching defect of *blind* (Schmitz *et al.*, 2002). The average number of axils lacking side-shoots within the first seven leaves of each population was 2-5 % for *bli3* and 2-10 % for *Bli1* RNAi plants (n=12 to 80). Double RNAi lines demonstrated that *Bli1* and *Bli3* act redundantly, as *bli1 bli3* plants exhibited an enhancement of the mutant phenotype. 5-40 % of the first seven leaf axils lacked side-shoots in the double transgenics. The distribution of barren leaf axils was similar to that of *Bli1* single RNAi lines (Fig. 3.A-5 B).

In the three experiments, *Blind* loss of function populations grown in parallel lacked side-shoots in 40 % to 70 % of the first seven leaf axils (n=12, 13 and 18). Except for the most basal one, the relative distribution of these barren leaf axils in *blind* was complementary to that observed in *bli1* and *bli3* (Fig. 3.A-5 C). *blind* plants often produced side-shoots in the axils of leaves number two and three and the adjacent leaves, the "zone" where *bli1* and *bli3* plants exhibited the strongest defects (Fig. 3.A-5 C, see also Mapelli and Kinet, 1992).

Furthermore, although *bli1 bli3* plants never lacked the sympodial shoot, the percentage of accessory bud formation in the axil of the leaf subtending the sympodial shoot was reduced by about 50 percentage points compared to wild-type (analysed in primary transgenic plants three times, n=12 to 36). Occasionally wild-type controls also harboured empty axils, but summarizing the three experiments these were less than one percent of leaf axils that remained barren (0.6 %, sum of three experiments, n=50) and no terminating axillary structures were ever observed.

In summary, it could be shown that in tomato members of the *Blind* gene family control the initiation of vegetative AMs in a zonal and partial overlapping fashion, as it was also described for *Arabidopsis* (Müller *et al.*, 2006). Since tomato and *Arabidopsis* are two distinctly related dicots, it seems to be of evolutionary benefit to regulate side-shoot formation in different phases of vegetative development by independent genes, possibly enabling the plant to react more flexible to environmental influences.

Reduced flower numbers in *Bl*, *Bli1* and *Bli3* loss of function plants

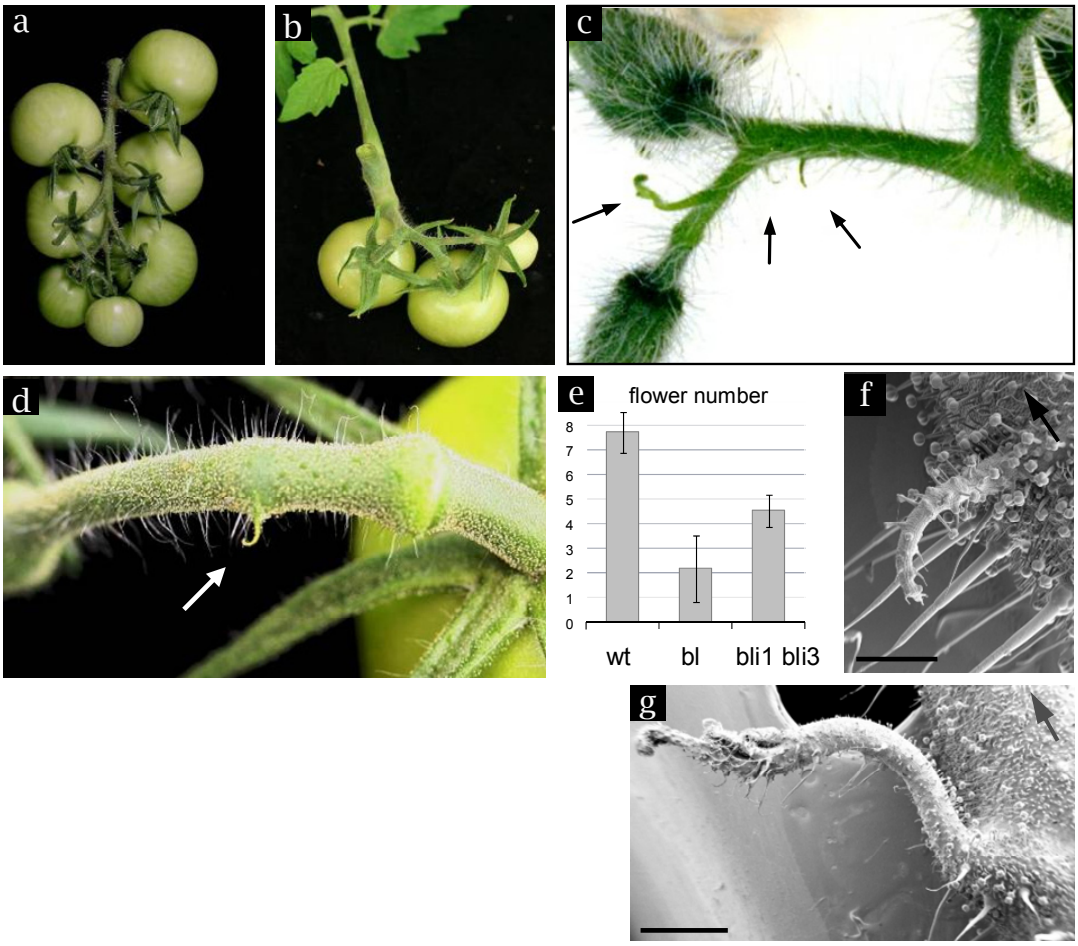


Fig. 3.A-6 *bl*, *bli1* and *bli3* initiate less inflorescence meristems

(a) Wild-type (cv. MM) tomato truss. (b) Truss of *Bli1* RNAi plant, consisting of only three fruits. (c, d) Exemplary images of "micro-leafs" (arrows) with barren axils in inflorescences of *bl*, *bli1* and *bli3* plants. (c) bl^1 in AC, (d) *Bl* RNAi in MM. (e) Average flower numbers of the first inflorescence of wild-type, bl^2 and *bli1 bli3* plants (wild-type and bl^2 from a segregating population (LUxMM), *bli1 bli3* double transgenics in MM; n=14, 17 and 13 respectively; error bars give standard deviation). (f, g) SEM pictures of "micro-leaves" (scale bars 0.5 mm (f) and 1 mm (g); arrows indicate growth direction).

Apart from the primary flower, all flowers of tomato inflorescences depend on the initiation of an axillary inflorescence meristem, called sympodial inflorescence meristem (SIM) (introduction, 1.D) and therefore the number of flowers is determined by the number of meristems initiated in an inflorescence.

In the experiment "summer08", the number of flowers in *bli1 bli3* double transgenic plants was reduced to an intermediate level compared to wild-type whereas it was strongly reduced in bl^2 inflorescences (Fig. 3.A-6 b, e). The failure to initiate SIMs in *bl*, *bli1* and *bli3* was often accompanied by the development of rudimentary leaf-like or pin structures, which were

named micro-leaves (Fig. 3.A-6 c, d, f, and g). Strikingly, equal rudimentary leaf-like structures were also found in *uniflora* reproductive development (see chapter 3.B.3). These micro-leaves in inflorescences are considered to represent the leaves of reproductive phytomers that are fully suppressed during wild-type development (see introduction 1.D and discussion 4.B).

Besides, the presence of micro-leaves at non-terminal positions in the *bl*, *bli1* and *bli3* inflorescences (Fig. 3.A-6 c) demonstrated that the failure to initiate a SIM did not always lead to precocious termination of the *bl*, *bli1* and *bli3* inflorescences, but frequently the inflorescence meristem produced another phytomer prior to its transformation into a flower meristem.

Statistical analysis displayed that 71 % of bl^1 inflorescences harboured micro-leaves, while only 5 % of the inflorescences of the control developed micro-leaves (n=17 and 43, cv. Craigella). A similar experiment with *Blind RNAi* plants resulted a 70 % vs. 6 % frequency of inflorescences bearing micro-leaves (n=30 and 47). In bl^2 in cv. Lukullus, grown in parallel to the above mentioned bl^1 population, only 24 % of 21 inflorescences showed micro-leaf formation. This is probably due to background effects on inflorescence architecture of *blind* (for modifying effects of cv. LU see also 3.A.3.2 and 3.A.3.4).

3.A.3.4 *Bl*, *Bli1* and *Bli3* regulate the development of meristems

An adult tomato plant simultaneously possesses dozens of shoot apical meristems, all fulfilling specific developmental programs. Differences in these programs become obvious as early as during the initiation of the meristems. A meristem in the axil of a basal leaf appears five to six plastochrons after the initiation of its subtending leaf (Gregor Schmitz, personal communication). It will then form several leaves and one sympodial inflorescence meristem (SIM), before terminating into a flower meristem. In contrast, a sympodial shoot meristem forms already about one plastochron after the initiation of its subtending leaf, develops with high velocity and normally initiates only three leaves and one SIM prior to termination. However, the fastest developing tomato AMs are formed in inflorescences. A SIM develops to the size of its parental meristem within one plastochron. It will then form only one phytomer, harbouring the next SIM, before again terminating into a flower meristem (Helm, 1951; Danert, 1958 and analysed in this project).

Finally, the primary shoot apical meristem of the next plant generation needs to be initiated and programmed alike other meristems. The primary shoot apical meristem is established

during embryogenesis at an axial position between the cotyledons and this process is known to involve at least one gene that is also involved in AM formation, namely *Goblet*, the recently identified tomato ortholog of the *Arabidopsis CUC* genes (Blein *et al.*, 2008; Berger *et al.*, 2009).

Under constant environmental conditions, timing and velocity of meristem formation and the size and identity of the newly formed meristems are genetically controlled, determining what organs will be formed when and where (see also introduction). Data in the two following subchapters demonstrate that *Bl, Bli1 and Bli3* are such genetic factors influencing the development of all types of apical meristems.

Terminating growth and underdeveloped meristems in *bl*, *bli1* and *bli3* plants

i. Terminating axillary structures replacing vegetative side-shoots

Bl, *Bli1* and *Bli3* loss of function did not merely lead to the presence or absence of AMs, but to a continuum of defects in the formation of AMs. Instead of functional wild-type AMs, *bl*, *bli1* and *bli3* plants frequently displayed side-shoots that terminated with the formation of two leaves. In more severe cases leaves or single leaflets replaced the side-shoots. Even more pronounced reduction resulted in the formation of small pins with a rudimentary, often cup-shaped, leaf blade in the axils of *bl*, *bli1* and *bli3* leaves (Fig. 3.A-7 a-e). In three experiments the population-wide penetrance of such terminating axillary structures varied from null to more than a third of the amount of barren axils independent of whether *bl*, *bli1* or *bli3* function was lost ($n_{(plants)}$ =12 to 80). These structures were formed predominantly at the border of the zones of barren axils and axils with normal side-shoot development (compare with Fig. 3.A-5).

ii. Terminating shoot apical meristems in *Bli3* RNAi seedlings

Besides frequent problems or failures of germination, termination of the shoot apical meristem (SAM) after the formation of two normal leaves was noticed repeatedly in *Bli3* RNAi populations. This termination mostly occurred as a "consumption" of the SAM leading to the formation of a terminal small pin, leaflet or irregular leaf (f). Growth continued from axillary buds, if they had been initiated (Fig. 3.A-7 f_1). In addition, *Bli3* RNAi plants repeatedly formed a 2-3 mm sized pin on the stem after the formation of two leaves in plants that did not show obvious termination. These structures appeared equivalent to the pins often formed upon termination and might represent primary termination and consecutive development of an AM as a sympodial shoot.

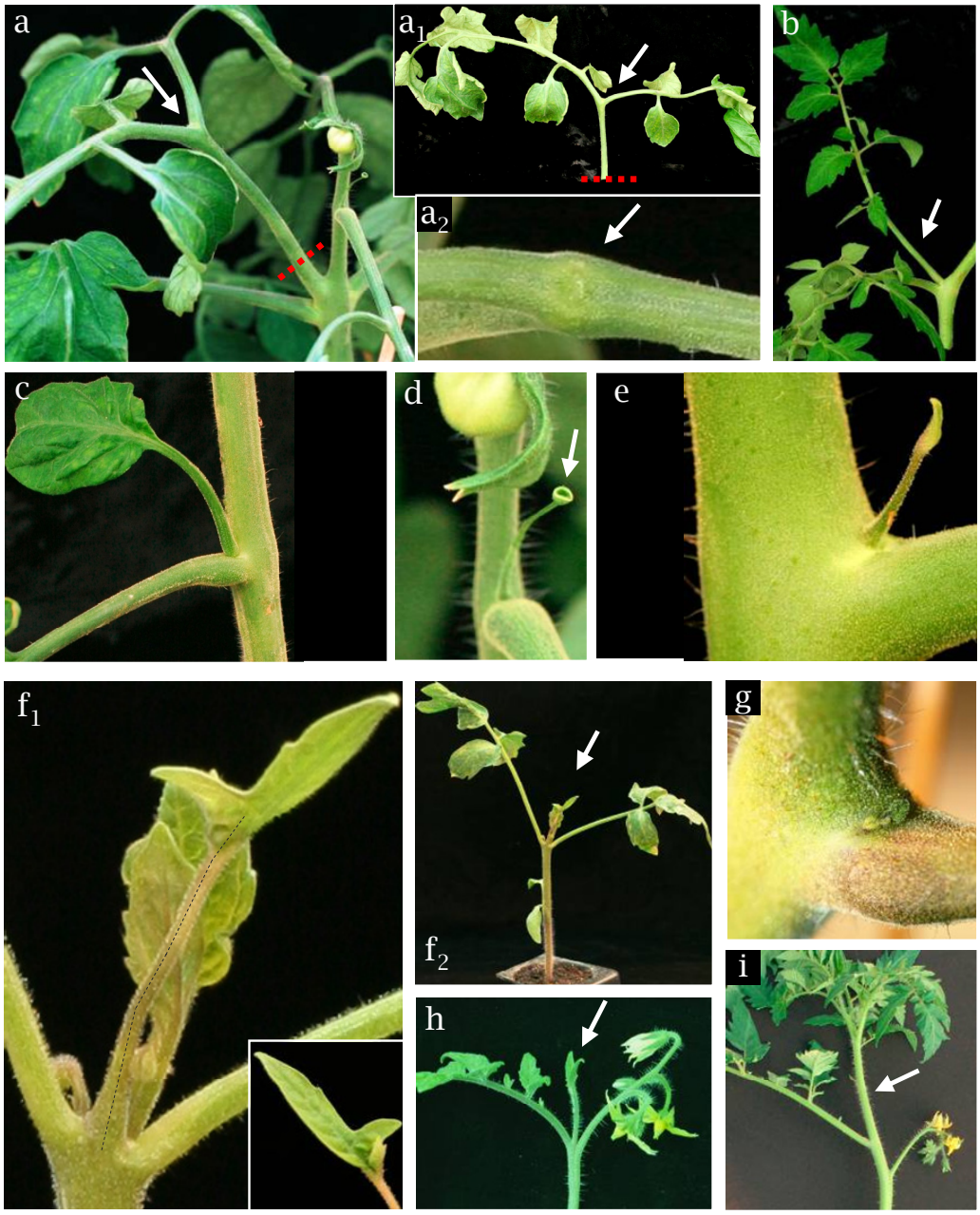


Fig. 3.A-7 Terminating and underdeveloped meristems in *bl*, *bli1* and *bli3* plants.

(a-e) Images representing the continuous levels of terminating axillary growth replacing side-shoots in *bl*, *bli1* and *bli3* plants. (a) A side-shoot terminating after two leaves (arrows marks the termination point) (a_1) explant of (a) showing only the terminating axillary shoot. (a_2) close up of termination site. (b) A compound leaf or (c) a simple leaflet replacing a normal side-shoot. Axillary pins with terminal (d) cup shaped or (e) rudimentary leaf blade replacing side-shoots. (f_1) close up of terminating *Bli3* RNAi seedling. The shoot apical meristem is terminating into a rudimentary leaflet (broken line and inset) after two normal leaves were formed (f_2). (g, h) Examples of underdeveloped axillary shoots, (g) vegetative bud, and (h) sympodial shoot. Compare to wild-type sympodial shoot (i) at similar stage of inflorescence development.

In two independent *Bli3* RNAi populations, also segregating for a *Bli1* RNAi construct, the penetrance of these events was recorded as 15 % termination and 4 % pins (n=45) in experiment "summer 08", and 4 % termination and 6 % pins in a repetition experiment (n=52). Remarkably, all clear termination events were found only in single *Bli3* RNAi plants in both segregating populations. In wild-type this kind of termination or pin formation was never observed.

iii. Underdeveloped vegetative and sympodial side-shoots in *bl*, *bli1* and *bli3*

Fig. 3.A-7 g shows a vegetative *bli1* leaf axil harbouring a rudimentary bud. Such buds were extremely delayed in their development compared to wild-type buds, but they still could grow out days after elimination of all other shoot tips. However, even this outgrowth was slow in comparison to wild-type buds. Furthermore, in *bl*, *bli1* and *bli3* plants a delayed development of the sympodial shoot was repeatedly noticed (Fig. 3.A-7 h, i). Generally, most axillary buds that were formed in *bl*, *bli1* and *bli3* plants appeared to develop later or slower compared to wild-type buds, although no detailed analysis on this was performed.

Altered inflorescence architecture and flowering time in *bl*, *bli1* and *bli3* plants

i. Evidence for altered sympodial flowering time

In three experiments, flowering time of the primary shoot was not altered in *blind* mutants. However, flowering time of the sympodial shoots was altered in two experiments (the majority of plants in the third experiment (summer08) lacked sympodial shoot initiation). In both experiments the average number of leaves in the first sympodial shoot was increased in bl^2 cv. LU plants, while decreased in bl^1 cv. Craigella plants (data experiment one see Fig. 3.A-8 b; data experiment two: cv. Craigella, bl^1 2.40 ± 0.43 vs. 3.50 ± 0.38 control; and cv. LU, bl^2 3.42 ± 0.38 vs. 3.25 ± 0.35 control; $n_{mutants\ and\ controls}$ =12, error gives CI 0.05). Statistically significant ($p<0.01$) were the sympodial late flowering of bl^2 cv. LU in the first experiment and the sympodial early flowering of bl^1 cv. Craigella in the second experiment (the mutants were grown with their corresponding wild-types in randomized positions).

ii. Reduced leaves preceding the first flower

bl mutants repeatedly formed reduced leaves prior to floral termination of the primary apex (Fig. 3.A-8 c). This might represent a gradual transition to reproductive growth or could be the result of lacking SIM initiation (see discussion 4.C). In any case, this defect is highly reminiscent of the pseudoshoot formation in *uniflora* mutants (see chapter 3.B.3).

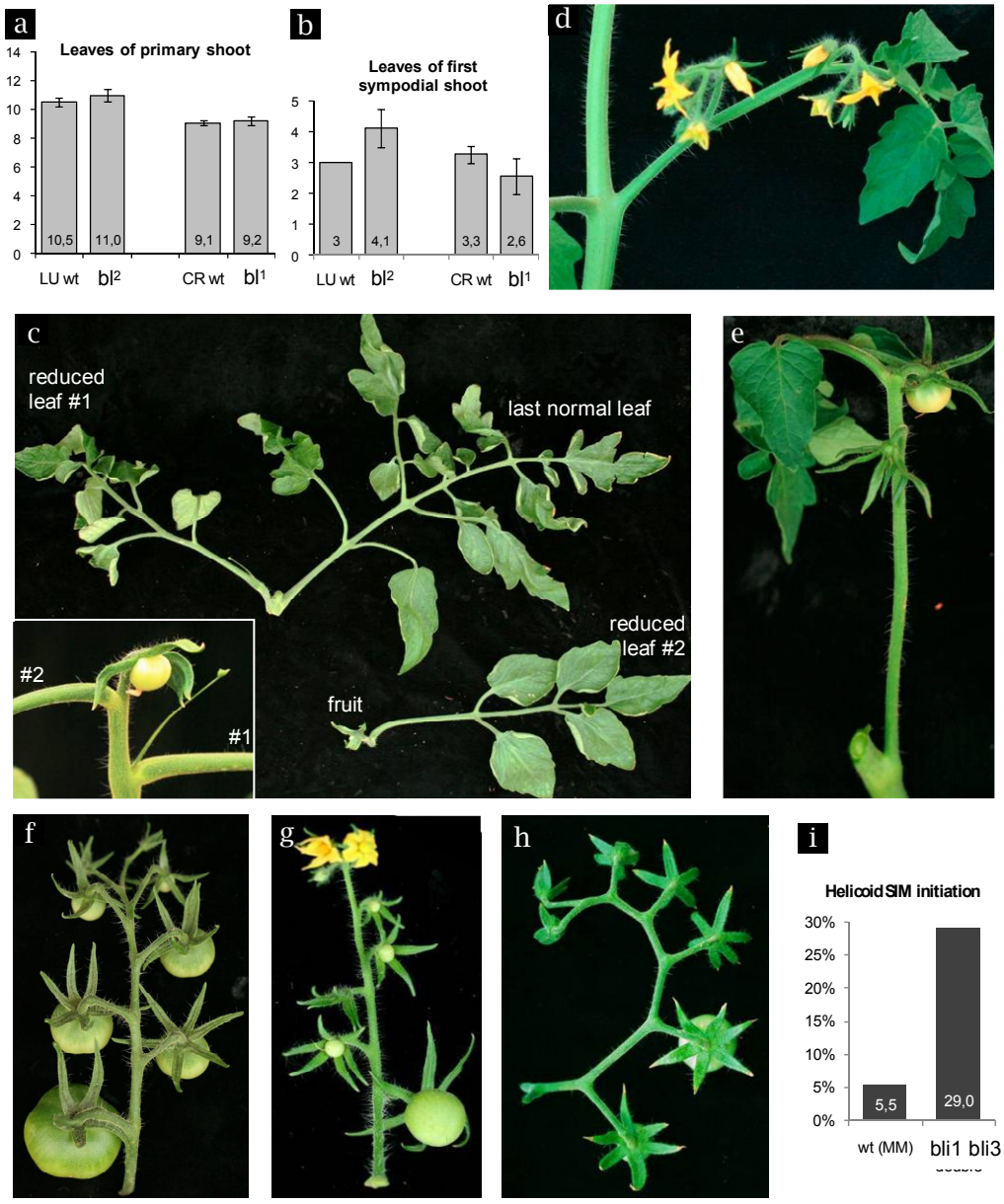


Fig. 3.A-8 Inflorescence architecture and flowering time in *bl*, *bli1* and *bli3*

(a, b) Flowering time experiment with bl^2 in cv. LU and bl^1 in cv. Craigella. (a) Leaf number of the primary shoot of mutants did not deviate from controls. (b) Number of leaves in the first sympodial unit was significantly increased in bl^2 (n = 9 and 12 for bl^2 and control, n=7 and 11 for bl^1 and control; error bars show CI 0.05). (c) Reduced leaves (#1 and #2) preceding flower formation of a bl^2 *Bli1 RNAi* plant (in LUxMM); #1 is the first reduced leave formed, #2 the second; the inset shows the plant prior to dissection. (d) Example of a leafy (at second node) as well as branched (at first node) inflorescence with one elongated internode (*bli1*). (e) Example of leafy inflorescence (terminal) with upright and elongated peduncle (bl^2 in LUxMM). (f-i) *bli1* and *bli3* plants show random positioning of SIM initiation. (f) Wild-type scorpioid cyme (LUxMM). (g) irregular SIM initiation (*bli1*) and (h) complete helicoid cyme formation (*bli3*). (i) Percentage of SIMs initiated at the helicoid position in the first inflorescence of *bli1 bli3* double transgenics compared to wt (n = 69 and 110).

iii. Leafy inflorescences and elongated peduncles and internodes

In two out of three experiments, *bl* mutant plants displayed an increase of inflorescences harbouring leaves compared to wild-type. In the first experiment 24 % of bl^2 inflorescences were leafy (n=22), while the segregating wild-type plants only generated 4 % leafy inflorescences (n=28, cv. MMxLU). An independent experiment analysing bl^1 in cv. Craigella resulted in 52 % leafy inflorescences compared 11 % in the wild-type (n=17 and 46). However, bl^2 in cv. Lukullus did not show this phenotype in three experiments. This represents the third case of specific differences of the inflorescence phenotype of bl^2 in the cultivar background Lukullus compared to the other *bl* mutant lines (see chapters 3.A.3.2 and 3.A.3.3 strongly increased fusions of flowers and decreased micro-leaf formation).

Single and double *Bli1* and *Bli3* transgenic RNAi plants did not show a significant increase of leafy inflorescences compared to control plants. However, qualitative differences (number, size and position of leaves in inflorescences) appeared, but were not statistically analysed.

Furthermore, often upright and elongated peduncles and elongated inflorescence internodes (Fig. 3.A-8 d, e) contributed to a unique mutant appearance of *bl*, *bli1* and *bli3* inflorescences.

iv. Formation of branched inflorescences

Bl and *Bli1* loss of function plants frequently displayed branched inflorescences (Fig. 3.A-8 d). A branched inflorescence results from the formation of more than one SIM by a single apical inflorescence meristem (introduction, 1.D). One example of intermediate penetrance showed 11 % of all bl^1 inflorescence meristems producing two SIMs prior termination vs. only 2 % of the control group (cv. Craigella, n=55 and 506, respectively). Although not all *blind* and *bli1* populations analysed displayed increased frequencies of branched inflorescences, similar penetrances as described above were repeatedly observed.

v. Distorted inflorescence phyllotaxy in *bli1* and *bli3* plants

One of the most prominent alterations in inflorescence architecture of *bli1* and *bli3* plants is the deviation from the wild-type inflorescence phyllotaxy. The positioning of the third and all consecutive wild-type SIMs is alternating to the preceding SIM initiation side and thus resulting in the typical zig-zag pattern of the tomato inflorescence (or so called scorpioid cyme, introduction, 1.D). In *bl*, *bli1* and *bli3* inflorescences this positioning of SIMs failed and consecutive SIMs were initiated nearly randomly (Fig. 3.A-8 g), which in some cases, when all SIMs were initiated on the same side, led to the formation of a helicoid cyme, a morphological novelty for tomato (Fig. 3.A-8 h). Distorted inflorescence phyllotaxy in *bli1* and *bli3* lines was found in all three experiments and *Bli1 Bli3* double RNAi populations

exhibited a slightly increased frequency compared to single transgenic plants (Fig. 3.A-8 i). In *bl* mutants this could not be analysed, because the number of SIMs per inflorescence was too low and the fusions (see chapter 3.A.3.2) were often too strong impeding analysis.

Analysing vegetative wild-type AMs on the primary shoot and in primary leaf axils of shoots up to the fifth order (in cv. MM), revealed that the first leaf of a vegetative AM was randomly initiated on either side, in relation to its parental phytomer and the preceding parental phytomers. Thus, random phyllotaxy of the first phytomer of an AM is a normal character in vegetative development.

Summarizing the last two chapters, *Bl, Bli1 and Bli3* control not only the decision whether to initiate an AM or not, but are also needed to establish the well-defined pattern of development of shoot apical meristems in vegetative and reproductive development.

Whether there is a common underlying mechanism responsible for these regulatory functions remains a subject of speculation. It might be that most of the defects described in the last two chapters are direct and indirect consequences of improper meristem initiation. However, in order to explain all of the phenotypic aberrations, *Bl, Bli1 and Bli3* might also act on already established meristems, influencing their development, independently of their obvious function in meristem initiation (see discussion 4.C).

3.A.4 Expression analysis of the *Blind* gene family

3.A.4.1 Semi-quantitative RT-PCR analysis

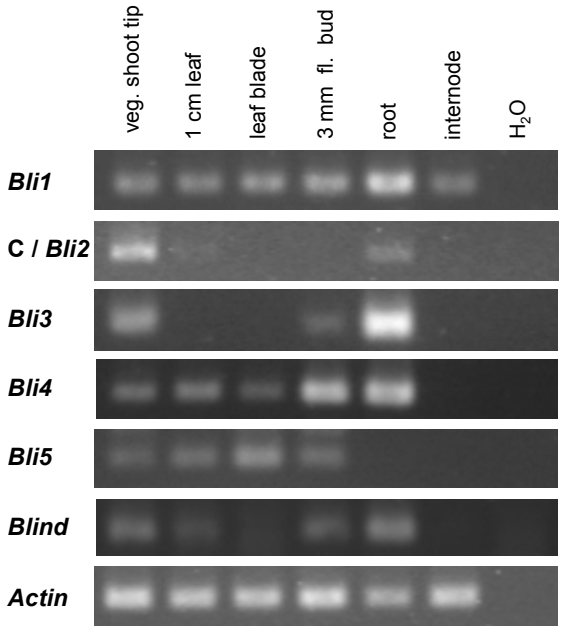


Fig. 3.A-9 Semi-quantitative RT-PCR of the *Blind* gene family

Total RNA was isolated from wild-type tomato cv. MM. Expression of six tomato MYB genes of the *Blind* subfamily was analysed by RT-PCR, performing 34 PCR cycles with primer pairs spanning the second intron. *Actin* cDNA was amplified to control similar concentrations of total cDNA (28cycles).

To analyse the expression pattern of the *Blind* gene family, total RNA was isolated from tissues harvested from wild-type tomato plants at late afternoon grown under artificial light supplement. Vegetative shoot tips (< 5 mm), harvested under the binocular using razorblades, included the four to five youngest leaf primordia. Complete young leaves of 1 cm size were harvested to investigate expression in already compound, but not mature leaves. Leaf blade tissue was harvested from mature, non-senescent leaves of about 40 cm size excluding midvein and leave margin tissue. Roots were harvested from soil-grown plants and vegetative internodes of about 3 mm diameter were harvested from mature plants.

All six genes analysed were expressed in vegetative shoot tips, including tissues like the SAM and leaf primordia. Except for *Bli5,* all genes were also expressed in roots. Generally, all genes exhibited differently regulated expression (Fig. 3.A-9), with *Bli2* / *C* and *Bli3* showing the most restricted expression. *Bli1* was expressed in all tissues, slightly upregulated in roots

and it was the only gene active in internode tissue. Compared to the *Arabidopsis Blind* orthologous gene family, *Bli1* shows similarity to the expression of *RAX2* (Müller *et al.*, 2006). Interestingly, *C* and *Bli3* were not or only very weakly expressed in leaf tissues. To analyse the expression domains of *Bl*, *C* and *Bli3* in more detail RNA *in-situ* hybridisation was performed (see next section).

3.A.4.2 Pattern of *Blind* mRNA accumulation

RNA *in-situ* hybridisation is a valuable tool to analyse the expression pattern of a gene at cellular level. Cells harbouring mRNA of a gene of interest can be identified using a specific antisense probe. However, the mRNA pattern detected by *in-situ* hybridisation does not always fully reflect the areas of protein activity, due to potential control of mRNA translation, protein modification, stability or transport.

RNA *in-situ* hybridisation with *Blind* antisense probe revealed two major expression domains, namely in the axils of leaf primordia and on the adaxial side of axillary meristems. Sections from thirteen tomato apices of three independently harvested populations revealed the following detailed mRNA patterns. Expression of *Blind* was found at the position of incipient leaf primordia (P0) where it usually comprised two to six cells in all three dimensions (Fig.3.A-10 a16, b, c; non-filled arrows). As soon as leaf primordia became morphologically distinguishable, strong hybridisation signals were detected adaxially of the primordia (Fig.3.A-10 a14). In transverse sections, this expression domain was oval- to band-shaped, covering at least half of the boundary between the SAM and the leaf primordium, and two to six cells broad. In the longitudinal axis, the hybridisation signal started at the L1, reached through all cell layers of the SAM and faded out in the region where cells start to become vacuolated (Fig.3.A-10 a, b, c; filled arrows). Sometimes, especially at P0, the L1 and the L2 did not express *Blind* (Fig.3.A-10 c). The axillary expression of *Blind* was detected until the oldest leaf axils analysed here (P6) (Fig.3.A-10 a32-34).

Blind expression was also detected on the adaxial side of young vegetative and reproductive axillary meristems, separating the axillary meristem from the parental shoot (Fig.3.A-10 d, e). This expression correlates with the function of *Blind* in preventing concaulescent fusions in vegetative and reproductive development (see chapter 3.A.3.2). Furthermore, *Blind* transcript was detected at the adaxial flanks of leaf primordia in most specimens (arrowheads Fig.3.A-10 a, b). This expression pattern was often weak and inconsistent, but showed close similarity to the pattern of *Potato Leaf* transcript (see chapter 3.A.4.3 for details).

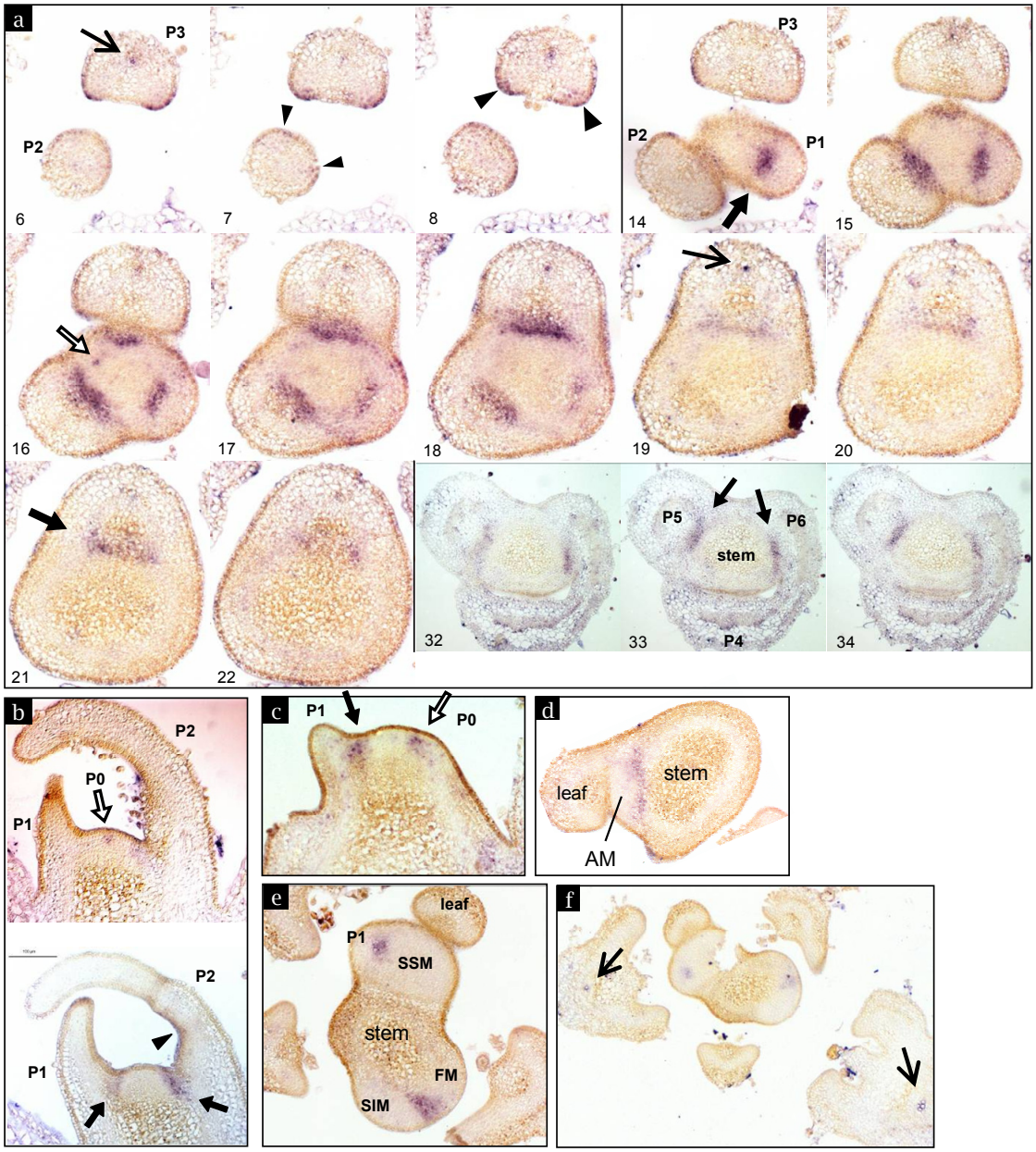


Fig.3.A-10 RNA *in-situ* hybridisation of *Blind*

Sections of tomato seedlings were hybridised with an anti-*Blind* probe. (a, b, c) Transverse and longitudinal sections from apices at early (a, b) and late (c) vegetative stage. Numbers in (a) give the number of sections relative to the tip of P2 (=section 0). (a16, b, c) Non-filled arrows point to expression at P0. (a7, a8, b) Arrowheads indicate expression in leaf primordia. (a14, a21, a33, b, c) Arrows point at expression domains in the axils of leaf primordia respectively young leaves. (d, e, f) Transverse sections of seedlings at reproductive stage. (d) *Blind* signal between the stem and a new axillary meristem (AM), prior to leaf primordium initiation of the AM. (e) Transverse section of reproductive shoot tip basally of the first flower meristem. *Blind* transcripts accumulate between the second flower meristem (FM) and the latest sympodial inflorescence meristem (SIM). Furthermore, *Blind* is expressed in the axil of P1 of the sympodial shoot meristem (SSM). (f) Same specimen as in (e). (a6, a19, f) Evidence for expression in putative provascular cells (arrows with open heads).

All *Blind* expression domains described here sometimes appeared cloudy, i.e. not well defined, as cells adjacent to the described areas often showed faint expression signals. Finally, there was some evidence for *Blind* RNA in putative provascular and vascular cells. However, this result was inconsistent and needs further analyses (Fig.3.A-10 a6, a19, f arrows with open heads).

3.A.4.3 Transcript accumulation of *Potato Leaf* (*C*) in shoot tips

Tomato seedlings at early and at late vegetative stages were fixed, sectioned and hybridised with a *Potato Leaf* antisense probe made from the C-terminal region of the gene. Seven shoot tips from three independently harvested populations bearing dozens of leaf primordia were successfully analysed by RNA *in-situ* hybridisation. Generally, expression signals were detected within leaf primordia, being strongest in P2 to P4, whereas no significant hybridisation signals were observed in the shoot apical meristem or in axils of leaf primordia.

Discrete expression domains were present at the adaxial flanks of leaf primordia at different positions in relation to the development of the compound tomato leaf. The earliest expression was found at positions prior to any visible formation of lateral leaflet primordia (arrowheads in Fig. 3.A-11 a, b; Fig. 3.A-12 and Appendix Fig. 5.A-1). It remains unknown, whether these cells give rise to the leaflet primordia, or are marking the boundary of incipient leaflet primordia. At a later developmental stage, starting with P3 or P4, when lateral leaflet primordia are morphologically distinguishable, *Potato Leaf* mRNA was detected at their proximal and the distal axils (Fig. 3.A-11 a, b, c; Fig. 3.A-12 and Appendix Fig. 5.A-1; unfilled arrows mark expression in the proximal axils of leaflet primordia, filled arrows point at distal expression). Finally, *Potato Leaf* was detected at the emargination between developing leaf lobes (Fig. 3.A-11 c and Fig. 3.A-12; arrows with open heads). The different expression domains were mostly ball shaped with one to six cells of strong expression in diameter, focused just below the outermost cell layer, often also including this layer. Sometimes the central domain was accompanied by very faint expression in some adjacent cells.

In summary, *Potato Leaf* mRNA was detected in leaf primordia a) prior to leaflet formation, b) at axils of leaflet primordia and c) between developing leaf lobes. Taken together *Potato leaf* expression seems to mark incipient or actual areas of inhibited growth within leaf primordia during the compound leaf development. This is reminiscent of the expression of

Blind in the shoot apex. The expression domains of *Potato Leaf* fit to its function in the formation of leaflets and leaf lobes (see chapter 3.A.3.1).

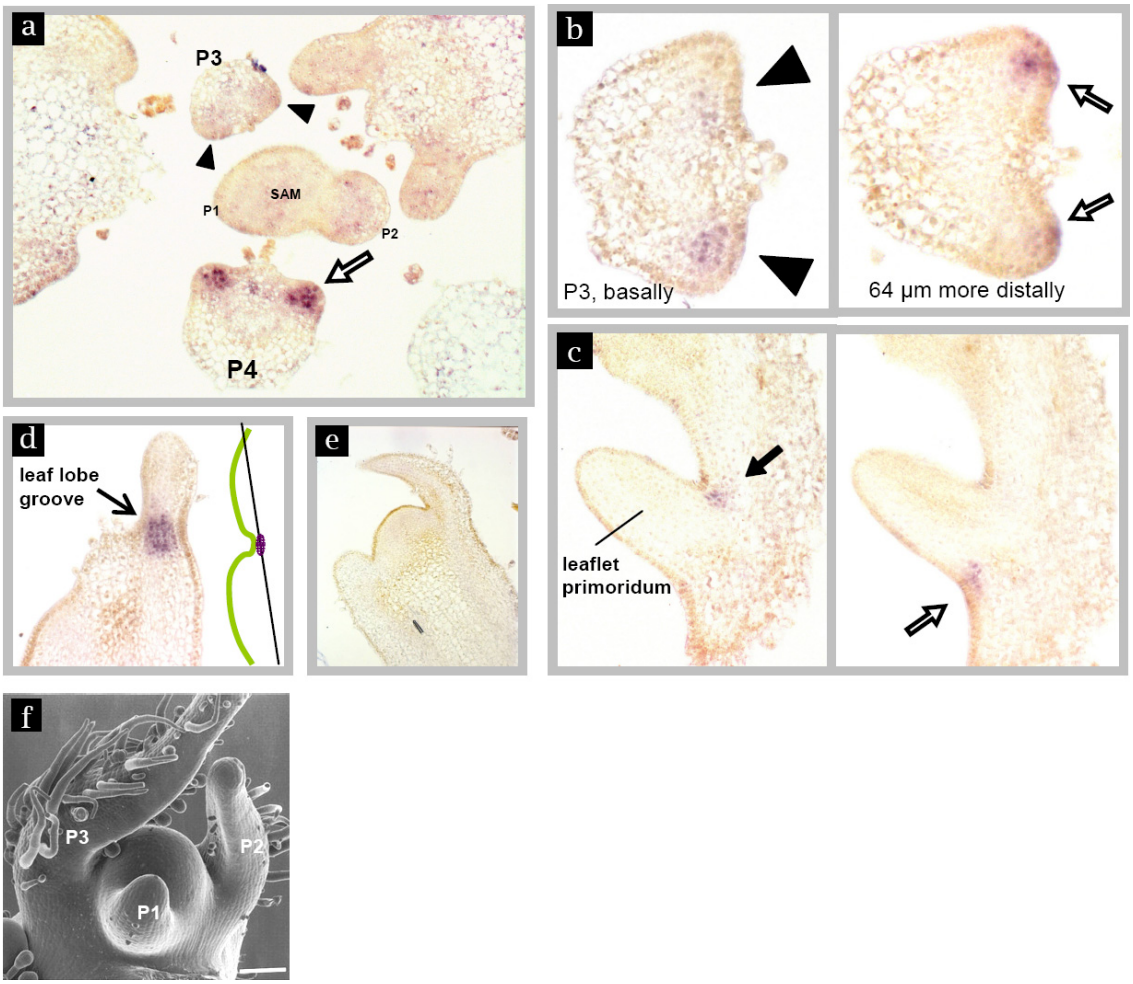


Fig. 3.A-11 Transcription profile of *Potato Leaf*

Tomato seedlings were fixed at vegetative stage and 8 µm sections were hybridised with an anti-*C* probe. (a) Transverse section basally of the summit of the SAM. For serial pictures of this specimen, see Appendix Fig. 5.A-1. (b) Transverse sections through a late P3, close to the axil of the primordium (left) and more distal, just basally of the first leaflet primordia pair (right). (c, d) Partial longitudinal sections of large leaf primordia.

Potato Leaf expression was detectable at the adaxial flanks of young leaf primordia prior to leaflet formation (arrowheads in a, b), in the proximal axils of leaflet primordia (unfilled arrows in a, b, c), at the distal axil of leaflet primordia (filled arrow in c) and putatively at the emargination between two forming leaf lobes (arrow with open head in d). In (e) a sense *Potato Leaf* probe was used as a negative control. (f) SEM of wt shoot tip (Szymkowiak *et al.*, 1999).

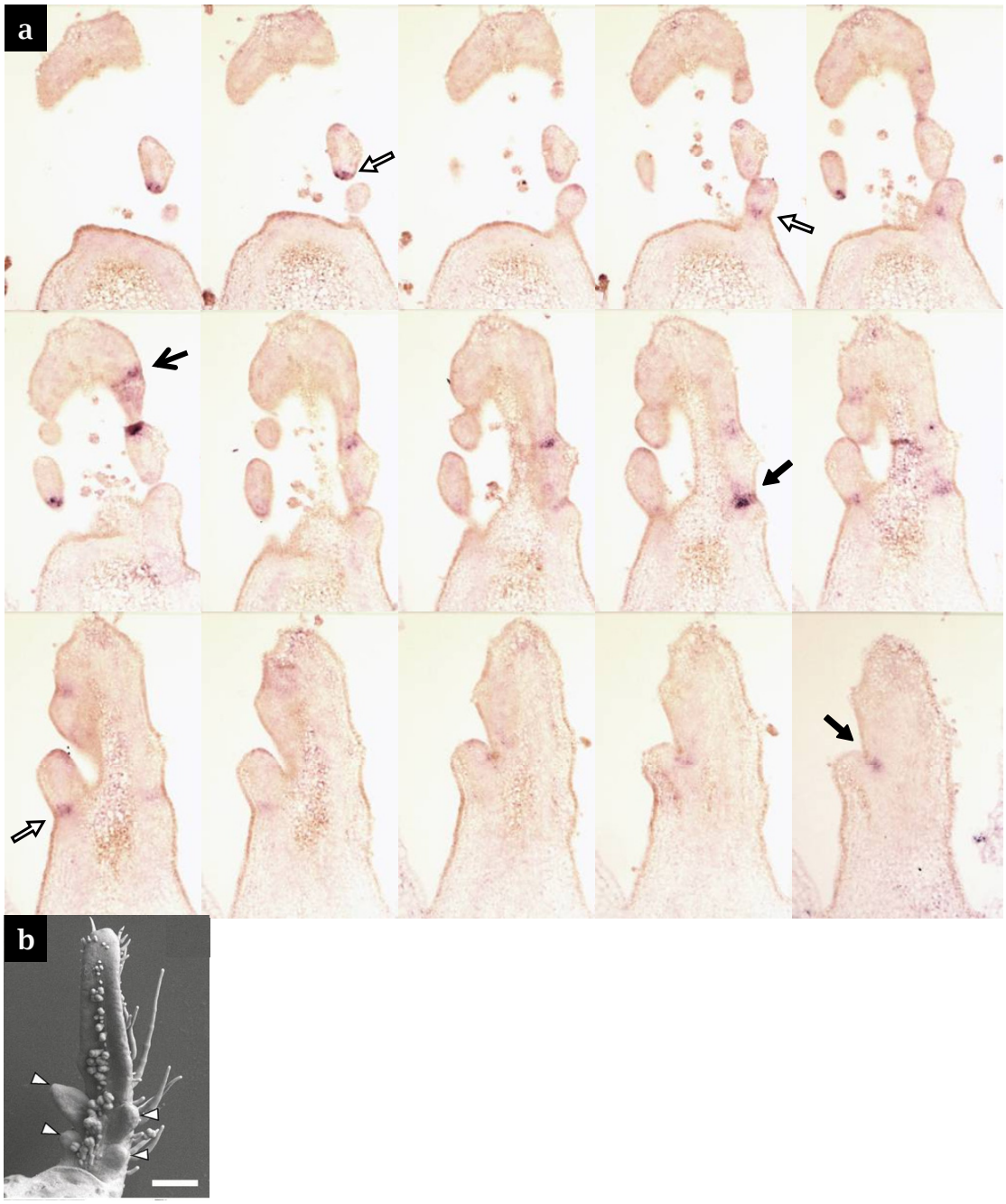


Fig. 3.A-12 *Potato Leaf* expression analyses in serial sections of a young leaf

(a) Fifteen serial sections of 8 µm through a juvenile leaf at the developmental stage P3 are shown. Tissues of distinct identities show transcript accumulation of *Potato Leaf*. Non-filled arrows point to expression at the proximal axil of leaflet primordia. Filled arrows mark hybridisation signal at distal axils of leaflet primordia. The arrow with an open head demonstrates expression in a developing leaf lobe sinus. (b) SEM picture of a leaf at approximately similar age and orientation as shown in (a) (from Reinhardt *et al.*, 2005).

Furthermore, initial results could be obtained for expression of *Blind-like3*. Preliminary evidence was gained from a single sectioned seedling. *Bli3*-mRNA accumulated in P3 prior to lateral leaflet primordium formation and in domains congruent with the expression of *Potato*

Leaf (Fig. 3.A-13 a). In addition, the proximal axils of the lateral leaflet primordia in P4 showed *Bli3* expression. Furthermore, *Bli3* was present in a newly formed axillary meristem at positions of the first two incipient leaf primordia (Fig. 3.A-13 b, c). This expression data correlates well with the function of *Bli3* in controlling leaf complexity and axillary meristem formation in basal leaf axils of the primary and secondary shoots.

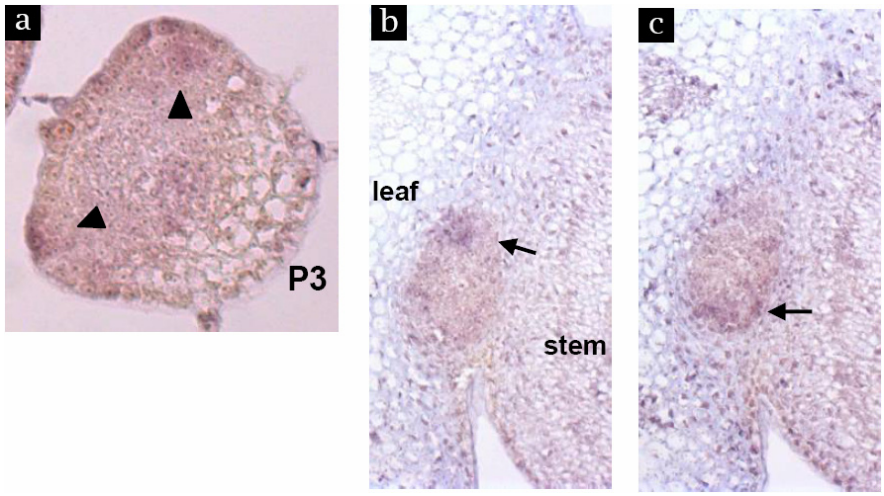


Fig. 3.A-13 *Blind-like3* RNA *in-situ* hybridisation

Transverse sections of a single tomato seedling hybridised with a *Bli3* antisense probe. *Bli3* mRNA accumulated at similar areas as *Potato Leaf* transcript. Exemplarily, (a) shows expression at the adaxial flanks of P3 prior to leaflet primordia formation (arrowheads). Additionally, *Bli3* transcripts accumulate in a newly formed axillary meristem at positions of the incipient leaf primordia (b, c; arrows).

3.A.5 Ectopic expression of *Blind* suppresses growth and partially complements *potato leaf*

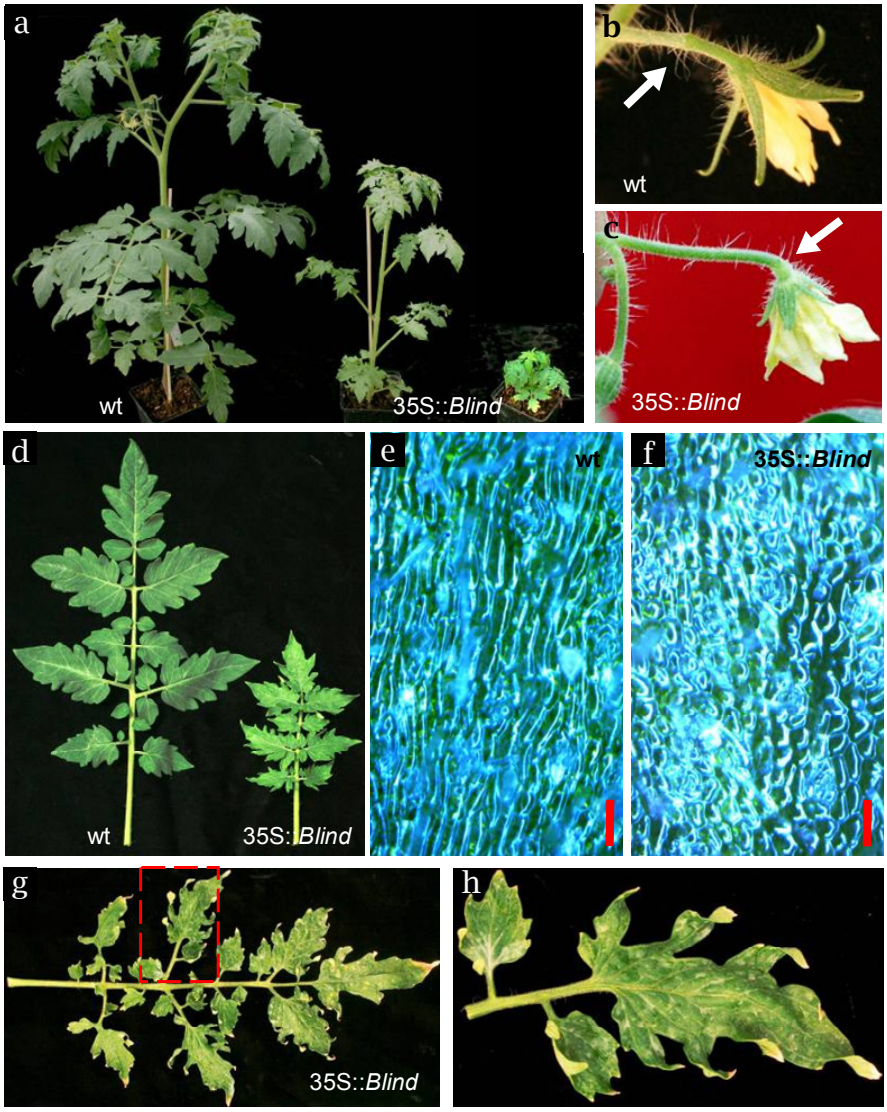


Fig. 3.A-14 Ectopic expression of *Blind* suppresses growth

(a) Two 35S::*Blind* transgenic plants and a wild-type tomato plant and of equal age (all cv. MM). (b) Wild-type flower with pedicel and joint (arrow). (c) Pedicel and flower of a 35S::*Blind* plant exhibiting the joint next to the flower base. Note also the reduced sepal size relative to other organs. (d) Young leaves of similar age from wild-type and 35S::*Blind* plants. (e, f) Cells at the abaxial surface of the rachis of leaves like in (d). (e) wild-type cells show cell elongation in direction of leaf growth, while cells of 35S::*Blind* leaves largely lacked cell elongation (f). (g) Mature leaf of 35S::*Blind* plant displaying deeply lobed and serrated leaf margins and twisted lobe tips. (h) Close-up of leaflet from (g) showing the abnormally deep and broad lobe sinuses.

Transgenic plants expressing *Blind* under the control of the cauliflower mosaic virus promoter were produced and kindly provided by Gregor Schmitz (personal communication). Fig.

3.A-14 displays two plants of independent transgenic lines showing retarded growth of all aerial plant parts. These plants represent intermediate levels of phenotypic deviations, because several transgenic lines frequently displayed such severe growth retardations that they failed to develop further than a few centimetres of height and finally died (Gregor Schmitz, personal communication). However, at least four independent lines showing intermediate defects could be analysed in several generations in the course of this project. The plants exhibited very slow growth and strongly delayed development. Flowering, fruit set and fruit ripening were much slower than in wild-type. Generally, all visible parts of the plant were retarded in growth equally, however the retardation of growth at leaf lobe and serration sinuses and the retardation of sepals were specifically pronounced (Fig. 3.A-14 b, c, g and h).

Leaves remained smaller than in the controls throughout their life span (Fig. 3.A-14 d). Abaxial epidermis cells of the rachis of 35S::*Blind* leaves largely lacked cell elongation compared to wild-type (Fig. 3.A-14 e, f). This indicates a function for *Blind* in the suppression of cell elongation. However, epidermal cells of sepals of 35S::*Blind* flowers were of equal size compared to wild-type (data not shown), indicating that another mechanism causes this specific reduction of sepals.

Another unique feature of 35S::*Blind* inflorescences was the appearance of the abscission zone (joint) of flower pedicels, which in the wild-type divides a flower pedicel into a proximal and distal fragment, of about two third and one third length respectively, while in 35S::*Blind* inflorescences the joint was located in close proximity to the flower base and the distal part of the pedicel failed to develop normally (Fig. 3.A-14 b, c).

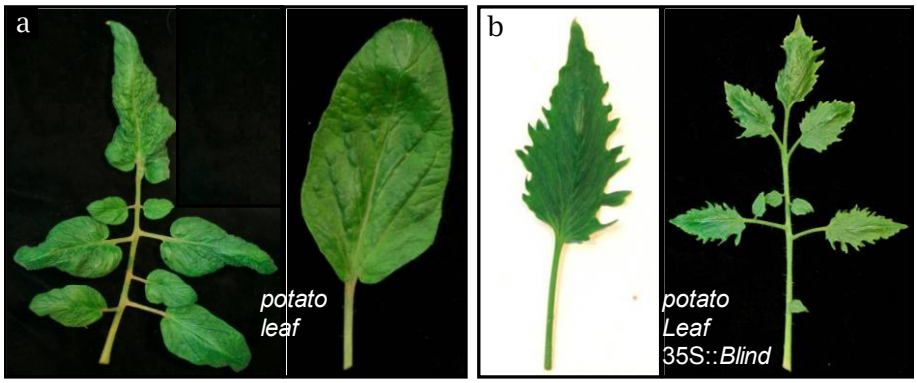


Fig. 3.A-15 Ectopic *Blind* expression induces serration of leaf margins in *potato leaf*

(a) Mature leaf from *c* mutant plant and close up of lateral leaflet displaying entire margins. (b) Leaf and close-up of lateral leaflet of a *potato leaf* plant expressing Blind under the control of the 35S promoter.

The phenotype of 35S::*Blind* plants displaying deeply lobed and serrated leaf margins (Fig. 3.A-14 g, h) is opposing the phenotype *potato leaf* displaying a lack of sinus formation (Fig. 3.A-15 a). When considering the expression pattern of *Potato Leaf* (chapter 3.A.4.3), it seems

likely that 35S::*Blind* enhances the lobing and serration pre-patterned by *Potato Leaf*. To test this hypothesis 35S::*Blind* plants were crossed to *potato leaf* mutants.

potato leaf plants expressing *Blind* under control of the ubiquitously active cauliflower mosaic virus promoter, indeed restored a strong serration of leaf margins (Fig. 3.A-15 b). However, leaflet and leaf lobe formation defects of *potato leaf* were not complemented. This indicates that *Blind* can fulfil a similar function as *Potato Leaf*, but the development of the complex wild-type tomato leaves requires a defined pattern and timing of this function.

Due to these results and due to the fact that *Potato Leaf* and *Blind* share a highly conserved DNA binding domain, it is likely that the two genes can regulate the same downstream targets and that their different function in wild-type is determined by their expression pattern only. To test this hypothesis transgenic plants expressing *Blind* under the control of a *Potato Leaf* promoter are currently generated (Gregor Schmitz, personal communication).

Furthermore, as no *Potato Leaf* gain of function plants are available yet, 35S::*Blind* plants were used as a putative mimic of *Potato Leaf* gain of function and were crossed with several mutants developing simple leaves or entire leaf margins. Analyses of the resulting plants in parallel to double mutants generated with *potato leaf* shall help to place the function of *C* in a context to other described players in leaf complexity regulation.

3.A.6 Candidate target genes of the Blind protein family

R2R3 MYB proteins are known to act as DNA binding transcriptional regulators (for references see Stracke *et al.*, 2001), leading to the obvious question what genes might be regulated by the Blind gene family. Microarray transcriptome experiments comparing young axillary tissues of *blind* and wild-type tomato plants yielded limited information due to technical difficulties with the small amount of tissue and due to the limited number of genes represented on the tomato microarray chips (G. Schmitz, personal communication).

A complementary technique used to elucidate target genes of transcription factors is the identification of a binding motif by random target site selection. Random oligonucleotides are affinity purified and subsequently sequenced, ideally revealing a short sequence element specifically recognised by the DNA binding protein of interest. This element can then be searched in promoters of genes of interest.

In 1998, Romero *et al.* investigated DNA binding capabilities of the *Arabidopsis* MYB transcription factor super family. Fortunately, one of the proteins analysed in that study was

RAX3, a co-ortholog of Blind. The consensus of the oligonucleotides retrieved in that experiment was nSVnGGTnGGTKn, notably including the core binding motif GGTnGGT, recognised by different subclasses of MYB proteins. However, base frequencies in the sequenced affinity purified oligonucleotides yielded additional information. 77 % of all sequences fitted to the consensus: GGKGGTAGGTGS. The frequencies of the four nucleotides in each position of the sequenced oligonucleotides are published as a small matrix (Romero *et al*., 1998, Table 3.A-3) Due to the high amino acid sequence identity and the conserved functions of the *Blind* gene family across species a conserved binding behaviour of all family members can be assumed. The RAX3 binding element was therefore used to search for downstream targets of *Blind* and its homologs.

Table 3.A-3 *Arabidopsis* RAX3 DNA binding specificity matrix (Romero *et al.*, 1998)

Matrix:	A|	22	0	18	14	0	0	0	78	0	0	0	0	4
	C|	18	14	4	9	0	0	0	4	0	0	0	0	32
	G|	42	86	78	55	100	100	0	4	100	100	0	96	60
	T|	18	0	0	22	0	0	100	14	0	0	100	4	4
consensus:		n	S	V	n	**G**	**G**	**T**	n	**G**	**G**	**T**	K	n

Figures give base frequencies in the affinity purified, sequenced and aligned oligonucleotides from a target site selection experiment with *Arabidopsis* RAX3 (former AtMYB84) protein (modified from Romero *et al.*, 1998).

Matrix scans of the *Arabidopsis* genome

The high complexity of the motif made it feasible to filter out candidate target genes in a genome wide search. The *Arabidopsis* genome annotation release TAIR6 listed 31407 genes and provided sequence sets comprising genomic up- and downstream sequences, untranslated regions (utr) and introns of each of the genes. These sequence sets were screened for elements matching the RAX3 binding matrix (Table 3.A-3) using the position-weight matrix scan algorithm MotifLocator (Thijs *et al.*, 2002). Every hit, i.e. every sequence element, in the output list of the scan had a score indicating its relatedness to the matrix (see in materials and methods, 2.B.15.1). A threshold score of 0.9097, yielded only sequence elements that had no mismatch to the core motif GGTnGGT. 317 hits scored 0.9097 or higher in a scan of the sequence sets, 1000 bp upstream region, 5' utr, first intron, 3' utr and 500 bp downstream region (data not shown). All genes connected to these 317 hits were screened for a potential relation to meristem development based on descriptions retrieved from public databases (see chapter 2.B.15.1). The outcome of this screen were seven putative target genes of the *RAX* family, namely *ACC1, ULT1, RDR2, PUP8, LRP1, CYCB1;1* and *APC1* (Table 3.A-4).

Using position-weight matrix scans, the number of hits is dependent on the threshold used. The stringent threshold score of 0.9097 resulted in the mentioned 317 hits. However, potential

RAX3 binding elements with a lower degree of similarity might be lost that way. A table comprising 3920 candidate genes was retrieved by setting a lower threshold (0.85) and by broadening the selection of the scanned potential regulatory regions to 3000 bp upstream, 5' and 3' utrs, all intron and 1000 bp downstream sequences of the *Arabidopsis* gene loci. This larger dataset, including detailed information, can be compared to future tomato microarray results. Examples retrieved from the extended list of 3920 genes by keyword searches are *ESR1 (Dornröschen), ETR1, TCP10, TCP19, TCP21, Phabulosa, CUC1, AGO6 (pinhead protein), and APC2*. However, based on current knowledge, neither any threshold setting nor any subset of potential regulatory regions can be proven biologically most meaningful.

Table 3.A-4 Candidate target genes of the RAX/Blind family

AGI-code ***Name*****,** Synonyms	Descriptions
AT1G36160 ***ACC1*****,** acetyl-CoA carboxylase1, Gurke, Pasticcino3	Encodes an acetyl-CoA carboxylase. Mutant displays uncoordinated cell divisions, which are enhanced by cytokinins. Mutant also has aberrant organization of the apical region in the embryo and abnormal root and shoot development.
AT4G28190 ***ULT1*** Ultrapetala1	Encodes a novel Cys-rich protein with a B-box like domain that acts as a negative regulator of meristem cell accumulation in inflorescence and floral meristems as loss-of-function ult1 mutations cause inflorescence meristem enlargement, the production of extra flowers and floral organs, and a decrease in floral meristem determinacy.
AT4G11130 ***RDR2*****,** RNA-dependent RNA polymerase2	Encodes RNA-dependent RNA polymerase that is required for endogenous siRNA (but not miRNA) formation.
AT4G18195 ***PUP8*** Purine permease8	Member of a family of proteins related to PUP1, a purine transporter. May be involved in the transport of purine and purine derivatives such as cytokinins, across the plasma membrane.
AT5G05560 ***APC1***	Arabidopsis thaliana E3 ubiquitin ligase, putative subunit of anaphase promoting complex
AT4G37490 ***CYCB1;1*** CyclinB1;1	Cell cycle regulator, cyclin-dependent protein kinase CYCB1;1. Functions as an effector of growth control at G2/M. Regulated by TCP20.
AT5G12330 ***LRP1*****,** Lateral root primordium1	LRP1: Protein of unknown function expressed in lateral root primordia and induced by auxin

317 hits from a matrix scan for the RAX3 binding element in regulatory regions of the *Arabidopsis* genome were filtered for genes with published information on their function and potential relation to meristem development, yielding seven putative candidate target genes of the RAX transcription factor family.

CycB1;1 and *LRP1* were chosen for further investigation

CycB1;1 is one of the best studied cell cycle regulators and the RAX3 binding element in the promoter of *CycB1;1* displays one of the best fitting sequences compared to the published RAX3 binding matrix. Moreover, this sequence element occurs in a tandem duplication. Therefore, *cycB1;1* mutants, kindly provided by Arp Schnittger, were grown under short day conditions and analysed for axillary bud formation as described in Müller 2006. However, they revealed no deviation in branching compared to wild-type.

LRP1 is a protein of the small SHI family of transcriptional regulators (Kuusk *et al.*, 2006) thought to positively regulate auxin biosynthesis. The *LRP1* promoter is active in the process of lateral root initiation (Smith and Fedoroff, 1995) and in addition, *LRP1* is highly expressed in the shoot apex (AtGenExpress, Schmid *et al.*, 2005). The motif found in the promoter region of *AtLRP1* is not present in other members of the *Arabidopsis SHI* family, but is highly conserved in rice, maize and Lotus japonicus *LRP1* genes and accompanied by additional conserved motifs in all four species. The unknown motifs GGARVVA and AGMAWA(a)HA occur eleven, respectively eight times in close proximity (< 50 bp) to the RAX binding elements in the four species, partly even in conserved distances to it (Fig. 3.A-16). In conclusion, this strongly suggests that a member of the RAX family or another MYB protein with similar binding specificity plays a regulatory role for LRP1 and its orthologs, probably in combination with other factors, that bind to the associated elements.

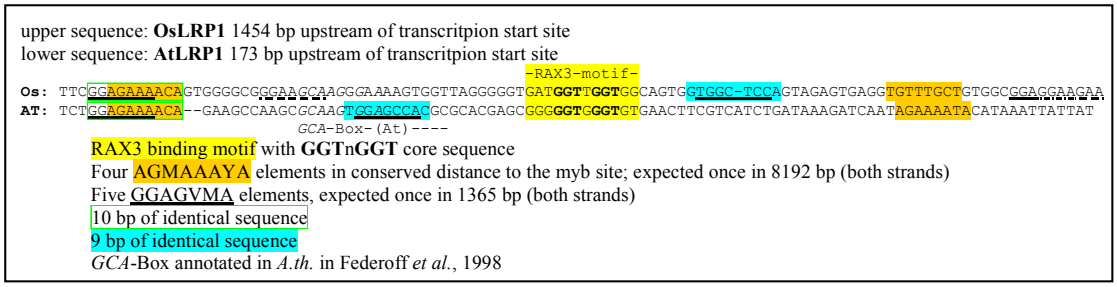


Fig. 3.A-16 Alignment of conserved cis-regulatory blocks with central RAX3 binding site in *Arabidopsis* and rice *LRP1* promoters.

The figure exemplarily illustrates conserved elements associated with RAX3 binding motifs in *LRP1* promoters of distinctly related species. Genomic sequences of putative orthologs of *LRP1* were analysed in four species (*O.s., Z.m., A.th. and L.j.*) and revealed in all four species the presence of RAX3 motifs accompanied by two elements conserved to the elements indicated in the figure by the orange shading and thick underlining.

Additionally, a preliminary result of the tomato microarray experiment indicated that the tomato *LRP1* mRNA is down regulated in the *bl* mutant (G. Schmitz, personal communication). To test for potential function of LRP1 in axillary meristem initiation,

axillary bud formation was analysed in *lrp1* plants, kindly provided by Eva Sundberg. However, no deviations from the control plants could be detected.

3.B *Uniflora* is the tomato ortholog of the rice branching regulator *LAX PANICLE*

3.B.1 Tomato contains an ortholog of *OsLAX*

The rice mutant *lax panicle* (*lax*) fails to initiate axillary meristems (AMs) during reproductive development (Komatsu *et al.*, 2001, Fig. 3.B-1 A). In addition, vegetative AMs are no longer formed in the double mutant *lax spa*, although *spa* single mutants produce similar numbers of AMs developing into tillers as the wild-type (Fig. 3.B-1 C). In 2003, Komatsu *et al.*, identified a bHLH transcription factor encoding *OsLAX*. Noteworthy, the orthologous maize gene, *barren stalk1* (*ba1*), was identified to exert a conserved function in shoot and inflorescence branching, as *ba1* mutant plants lack almost all axillary meristems (Ritter *et al.*, 2002; Gallavotti *et al.*, 2004). Wild-type plants of both species can form rudimentary bracts during reproductive growth. They resemble reduced leaves subtending meristems that will develop into inflorescence branches, spikelet pairs or spikelets. In *lax* and *ba1* mutants, these bracts are enlarged and exhibit barren axils (Komatsu *et al.*, 2001, Fig. 3.B-1 B).

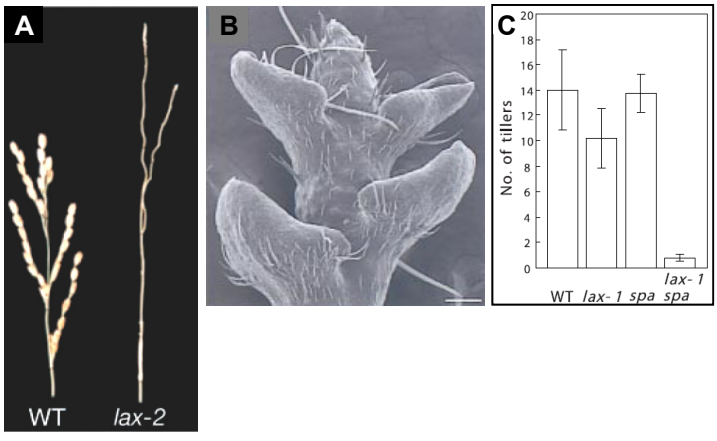


Fig. 3.B-1 *OsLAX* controls initiation of axillary meristems in the vegetative and reproductive development of rice (Komatsu *et al.*, 2001 & 2003)
(A) *lax* mutant plants lack panicle branches and spikelets due to the inability to initiate axillary meristems. (B) SEM picture of mutant reproductive shoot tip displaying enlarged panicle bracts lacking AMs. (C) In the *spa* mutant background *LAX* is essential for the formation of tillers.

Given this crucial role of this two orthologous grass genes in branching regulation, it was of interest to search for related proteins in dicot species. Recently an ortholog could be characterised in *Arabidopsis* revealing indeed a conserved function. AtLAX, formerly named AtbHLH140, is a newly identified member of the subclass VIII of the bHLH transcription

factor family in *Arabidopsis thaliana* (Yang, 2008). Loss of *AtLAX* function strongly enhances the inability of *rax1* and *rax1 las* double mutants to initiate AMs in the vegetative and reproductive phase of *Arabidopsis* development (Yang, 2008).

BAC-end sequence databases revealed the presence of a *LAX*-orthologous sequence in tomato and genomic iPCR resulted in amplification of the complete open reading frame (597 bp, no intron) of *SlLax* and flanking sequences (>1.2 kb up- and downstream, see appendix for complete sequence including features). The bHLH protein domains of SlLax, ZmBA1, all *Arabidopsis* bHLH genes from subclass VIII and the orthologous gene family from rice were aligned using ClustalW. The resulting phylogenetic tree shows a distinct clade containing the four LAX orthologous sequences (Fig. 3.B-2). This indicates that probably one ancestral *LAX* gene diverged from other genes in the subclass prior to the separation of monocots and dicots. Additionally, sequences from poplar and *Vitis vinifera* confirmed this relationship, as in both species, unambiguously orthologous genes exist (data not shown). This is in contrast to the *MYB* gene family of *Blind*, were no single genes could be assigned to an orthologous partner when comparing tomato and *Arabidopsis* sequences (see chapter 3.A.1).

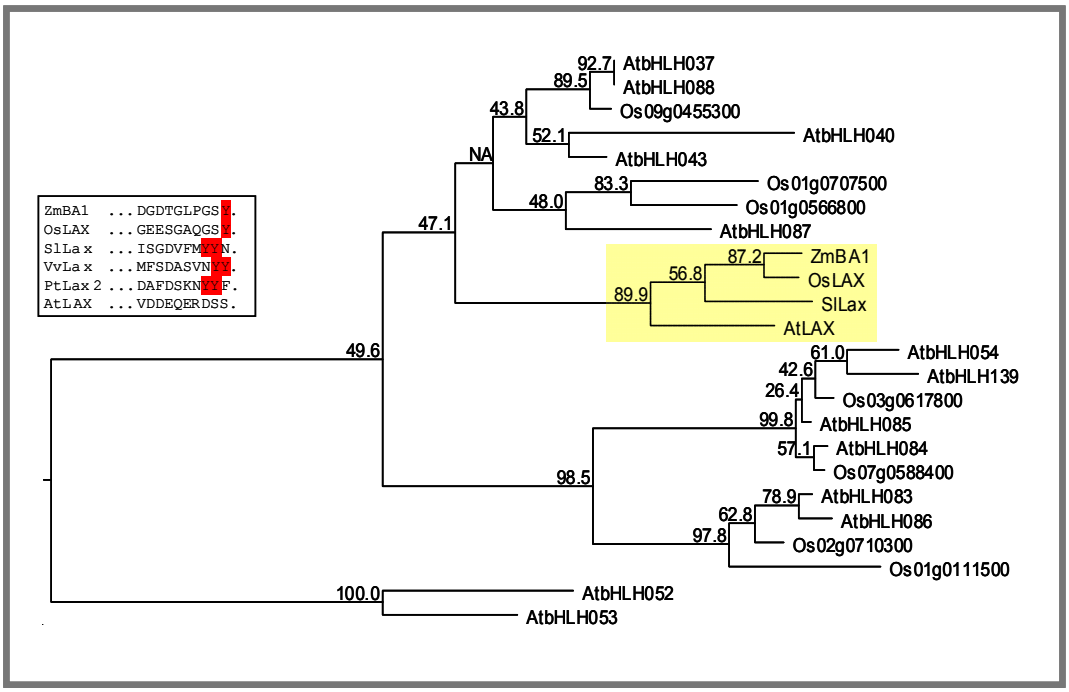


Fig. 3.B-2 Protein sequence comparisons of bHLH transcription factors related to LAX

Protein sequences of bHLH domains were aligned using ClustalW. Members of bHLH subclass VIII (Yang, 2008) from *Arabidopsis* and rice are represented together with ZmBA1 and SlLax. Values represent percentages of 1000 bootstrap trials. The subclade of *LAX* orthologs is highlighted. The inset shows an alignment of the ten terminal residues of different LAX proteins highlighting the conservation of one or two terminal tyrosines.

Sequence comparisons of the orthologous LAX proteins demonstrate high conservation of the DNA binding bHLH domains. ZmBA1 and SlLax share 88 % identity in the bHLH domain, which is gradually decreasing in the flanking regions. Strikingly, the presence of terminal tyrosines in the LAX proteins is conserved throughout many species, with the exception of *A. thaliana* (Fig. 3.B-2, inset), which is reminiscent of the *Blind* gene family (see chapter 3.A.1). Furthermore, SUMO interaction motifs (e.g. VLxI, where valine, leucine and isoleucine are interchangeable, Perry *et al.*, 2008) were found in the tomato, *Arabidopsis*, maize and poplar LAX-orthologs. Besides, an element of unknown function (QMMQQ) was detected in many of the orthologs (data not shown).

3.B.2 *SlLax* encodes *Uniflora*

The aim of this project was to answer the question whether the conserved function of the grass genes, *LAX* and *ba1*, is shared by the orthologous *SlLax* gene in tomato. To this end, RNAi constructs targeting *SlLax* were cloned and tomato plants cv. MM were transformed (see Materials and Methods). *SlLax* RNAi revealed normal vegetative growth, but severely impaired reproductive development producing predominantly solitary flowers. (and next chapters). Flowers had a wild-type appearance and were fertile. Surprisingly, the *SlLax* RNAi plants phenocopied the classical tomato mutant *uniflora* (*uf*, Fehleisen, 1967) (Fig. 3.B-3 b). The gene underlying the defects in the mutant *uniflora* remained unknown up to now. Sequencing *SlLax* in the uf^{1} mutant revealed that *SlLax* indeed carries a mutation in uf^{1}. An insertion mutation consisting of a 25 bp duplication leads to a frame shift before the bHLH domain (Fig. 3.B-3 e). A *uniflora* line, where the mutant locus was introgressed into cv. AC was obtained from IPK-Genebank, Gatersleben. DNA sequence analysis uncovered the same mutation in *SlLax* as found in uf^{1} cv. PTN, thus giving evidence that this mutation is causal for *uniflora*.

Furthermore, new mutant lines were identified to be allelic to *uniflora* by Y. Eshed (personal communication). The mutants, e1316 and e1383 originated from an EMS mutagenised population in cv. M82 (Menda *et al.*, 2004). Seeds were kindly provided by Y. Eshed and plants developed equivalent defects as uf^{1} and *SlLax* RNAi (Fig. 3.B-3 c and chapter 3.B.4). Sequencing of *SlLax*/*Uniflora* in e1316 and e1383 revealed a non-sense mutation right after the bHLH encoding sequence. Both mutants carried the same mutation, indicating that the original mutant may have been duplicated due to technical reasons during the mutagenesis

project. The new allele of *uf*, named *uf*^{Y}, completes the line of evidence that *SlLax* encodes *Uniflora*.

Besides, TILLING of *Uniflora*/*SlLax* was initiated. In cooperation with A. Bendhamane (UGRV, Evry, France) the above-mentioned EMS mutagenised population was screened. Interestingly the mutant allele *uf*^{Y}, which originated from the same population, was not found by this screen, indicating that TILLING is probably not an exhaustive screening method. Nevertheless, three new alleles, *lax*^{1}, *lax*^{2} and *lax*^{3}, could be identified (see appendix). However, all three mutations did not affect conserved amino acid residues and did not cause obvious phenotypic deviations.

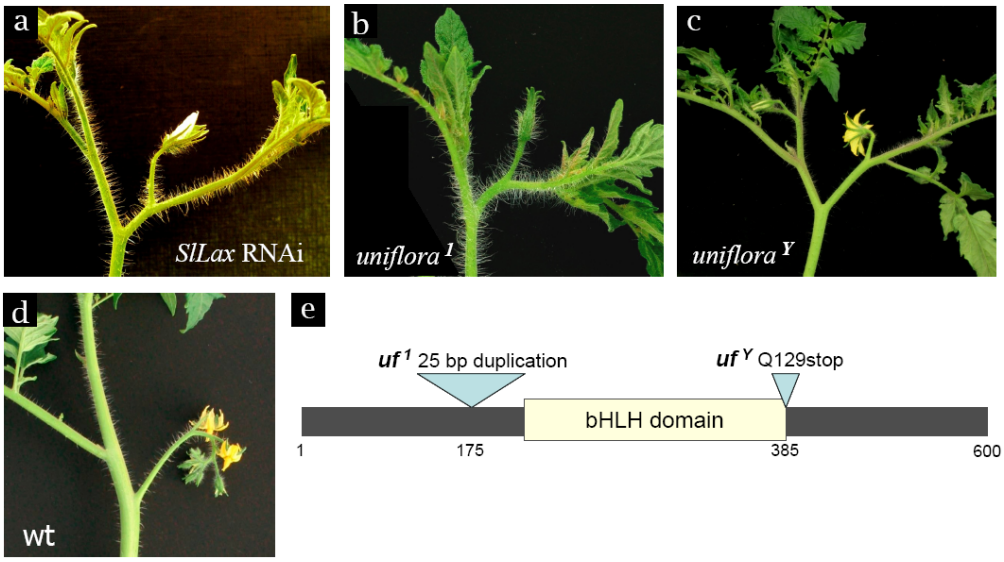


Fig. 3.B-3 Identification of the *Uniflora* gene

(a) Aberrant inflorescence of *SlLax* RNAi plant producing only solitary flowers, besides other defects. (b, c) *uf*^{1} and *uf*^{Y} plants phenocopied *SlLax* RNAi (for description of development see consecutive chapters). (d) Wild-type tomato inflorescence. (e) Schematic representation of the different *uf* alleles. Numbers below the bar indicate bp. *uf*^{1} carries a 25 bp duplication leading to a frameshift and stop of the open reading frame before the bHLH domain. *uf*^{Y} harbours a single nucleotide substitution (C385T) that leads to a stop after the bHLH domain.

3.B.3 *Uniflora* controls SIM initiation and reproductive development

3.B.3.1 *uniflora* in the literature

The mutant *uniflora* was first described in 1967 (Fehleisen) and was subject of several scientific studies since then. In 1967, it was reported that *uniflora* causes "one important modification: side branches of the inflorescence are suppressed and there persists only one axis that ends in only one flower."

The original mutant was isolated in the Argentinean fresh market tomato cultivar *Platense* (Accession LA1200, TGRC). Later, this allele, *uniflora*^{1}, was introgressed into the European cultivar Ailsa Craig. Analysing this line, *uniflora* was described as a late flowering mutant forming inflorescences of always only a single flower (Dielen *et al.*, 1998 and 2004). No suppressed or aberrant branching of *uf* inflorescences was observed in these studies. In fact, it was stated that, after reproductive transition of *uf* plants, "the vegetative meristem of *uf* transformed directly into a unique flower that consumed totally the apical meristem". *uf* plants were described as always late flowering, although the degree of flowering delay compared to the wild-type was dependent on growing conditions. Late flowering was extremely enhanced in conditions with low daily light energy integral. Furthermore, frequent release of side shoots from apical dominance was noted, most frequently in the leaf-axils number 8 to 13, the region where wild-type plants undergo reproductive transition. This was interpreted as a partial evocation of *uf* plants (Dielen *et al.*, 1998 and 2004).

In contrast to the single flower inflorescence description, another study described, that *uniflora* "inflorescences are indeterminate and mostly leafy with rare replacements of a leaf by a solitary flower". This structure was named vegetative inflorescence or pseudoshoot (Lifschitz *et al.*, 2006; the background of the analysed *uf* plants was not reported).

3.B.3.2 *uniflora* in the cultivar *Platense*

Trying to resolve these discrepancies in literature and in order to obtain a better understanding of the developmental role of *Uniflora*, different approaches at the macroscopic and microscopic level were used to analyse the phenotypic deviations of *uniflora* in a detailed manner. *uf* ^{1}, cv. *Platense* (LA1200), exhibited severe germination and fertility constraints.

Only a limited number of plants could be generated. However, all plants displayed inflorescences that developed obviously only one axis and only one flower (Fig. 3.B-4). Preceding this terminal flower, one or more nodes were visible macroscopically, but no functional sympodial inflorescence meristem (SIM) was formed. Often the SIM was completely absent and only a small pin or reduced leaf was found, which was reminiscent of the branching defect described for *bl*, *bli1* & *bli3* inflorescences (see chapter 3.A.3.3). Alternatively *uf* [1] cv. Platense plants formed inflorescence phytomers displaying different kinds of reduced leaves with terminating or shoot-like axillary structures.

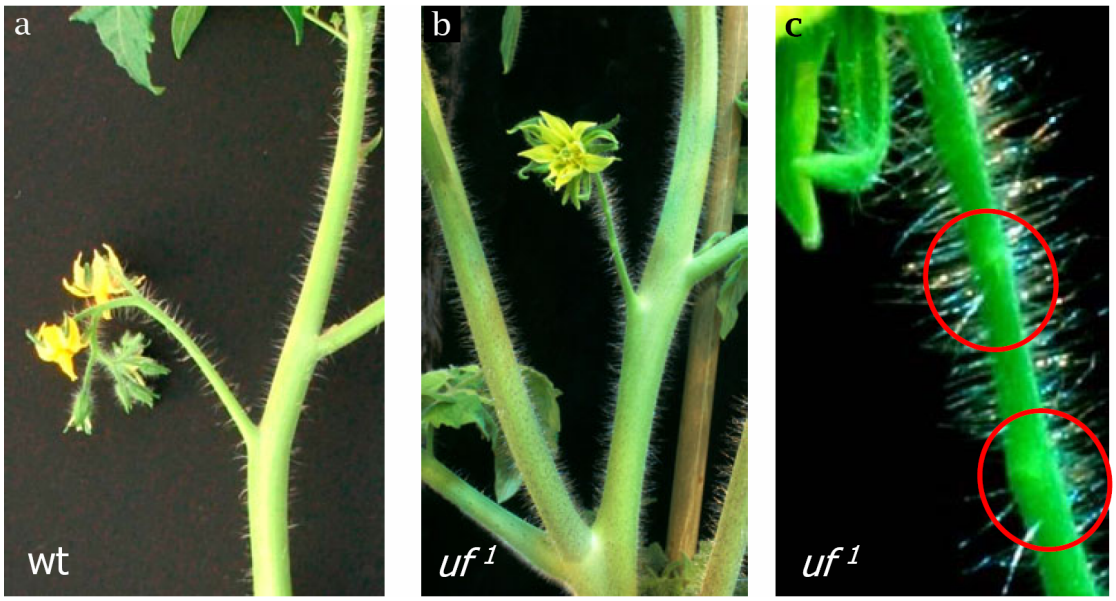


Fig. 3.B-4 Inflorescence development in *uf* [1] cv. Platense

(a) Wild-type inflorescence. (b) *uf* [1] in cultivar *Platense* (accession LA1200). (c) Close up of peduncle in (b) showing two nodes prior to floral termination lacking any inflorescence branches.

3.B.3.3 Abnormal phytomers in *uniflora*

The phenotypic defects of *uf* [1] were analysed in a bigger number of plants using the Ailsa Craig near isogenic line MLE567 (IPK-Genebank, Gatersleben). Germination and seed production was good in this line. Several hundred plants were grown at 10 independent time points within a period of more than one year. Apex development was studied from the seedling stage to reproductive development using a binocular with 50 times magnification and scanning electron microscopy (SEM) as well as the development of the mutants was studied macroscopically.

During vegetative development, no deviations from the wild-type were observed in *uniflora* plants. However, when Ailsa Craig control plants had switched to reproductive growth, *uf*

plants typically started to produce aberrant phytomers instead of normal reproductive ones (for wild-type development see introduction, 1.D). All three elements, the AM, the leaf and the internode, in these *uf* phytomers deviated from wild-type reproductive phytomer elements and this deviation was highly variable and exhibited a continuum of severities.

Defects in axillary meristem development displayed a gradient, from complete absence to the formation of near wild-type reproductive branches. Regularly axillary meristems failed to initiate and axils remained barren during the lifetime of the plant (Fig. 3.B-5 a, b). If axillary structures initiated, they often terminated instead of establishing a functional side-shoot. Their form ranged from arrested protrusions of a few cells over radially symmetric pins to rudimentary leaflets or even small compound leaves (Fig. 3.B-5 c). If axillary meristems initiated, timing, size and velocity of initiation and growth was delayed or reduced, with a continuum of severities observable. In some cases, small axillary buds became only visible late in development (observed on macroscopic level). In other cases, small bulges were visible in nodes of only a few plastochrons of age. The fastest axillary meristems initiated nearly with the timing and pace of a wild-type SIM, reaching the size of the apical meristem within one or two plastochrons (Fig. 3.B-5 d-g). These axillary meristems either displayed vegetative growth or continued the aberrant reproductive growth of *uf*, before eventually terminating again into a flower.

Leaves in aberrant *uf* phytomers were either fully suppressed, as in wild-type, or were partially released. The latter resulted in the presence of abnormal structures at a position subtending an axillary meristem. The size of these structures spanned a continuum from protrusions of a few single cells over pin-like structures and highly reduced leaves to leaves that were only slightly reduced when compared to wild-type adult leaves (Fig. 3.B-6). Primordia of aberrant leaves formed trichomes in basipetal sequence, bearing the largest trichomes on their distal tip in contrast to normal vegetative leaf primordia, which form trichomes in an acropetal pattern. Additionally, the diameter at the base of such reduced leaf primordia was smaller. Both characteristics were expressed in varying degrees, most likely correlating with the final size of these leaves or leaf-like organs (Fig. 3.B-6).

Furthermore, it was observed in all experiments, that there was no obvious correlation between the gradual release of leaves and the gradual defects in AM formation. This means that large branch meristems and barren axils were both subtended by nearly normal vegetative leaves or by fully suppressed leaves. Finally, internodes were reduced or elongated without any obvious pattern. However, the peduncle was often morphologically identical to the wild-type, displaying a reduced diameter and an increased stiffness compared to vegetative shoot internodes (data not shown).

Noteworthy, the primary shoot apical meristem of *uf* normally developed more than one aberrant phytomer prior to floral termination, in contrast to the (mostly) single inflorescence phytomer formed by wild-type SAMs after the switch to reproductive growth. Additionally, the number of such aberrant nodes prior to termination was random and no obvious correlation between position and grade of defect of a single phytomer was observed. In mature Ailsa Craig *uniflora* [1] plants, the sum of this abnormal phytomers led to the formation of a shooty, respectively leafy, inflorescence-like structure (Fig. 3.B-9 b), which shall be called pseudoshoot using the term of Lifschitz *et al.*, 2006.

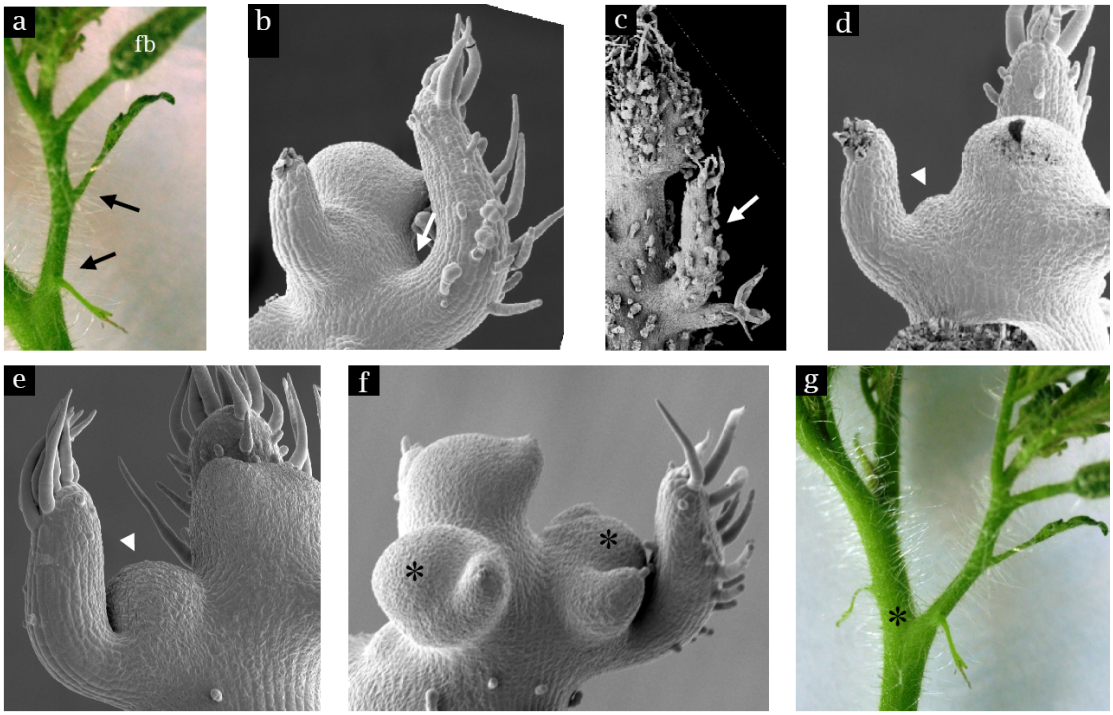


Fig. 3.B-5 Gradual impairment of axillary meristem formation in *uf* [1]

uf [1] plants (cultivar Ailsa Craig) were imaged after transition to pseudo-reproductive growth. (a, b) Nodes of *uf* [1] pseudoshoots lacking the development of AMs (arrows; fb flower bud). (c) A leaf primordium replacing an axillary meristem (arrow). (d, e) AMs in *uf* [1] pseudoshoots (arrowheads) developing much slower than wild-type reproductive AMs but faster than vegetative AMs. (f, g) AMs developing with similar velocity as in wild-type inflorescences, forming strong pseudoshoot branches (asterisks).

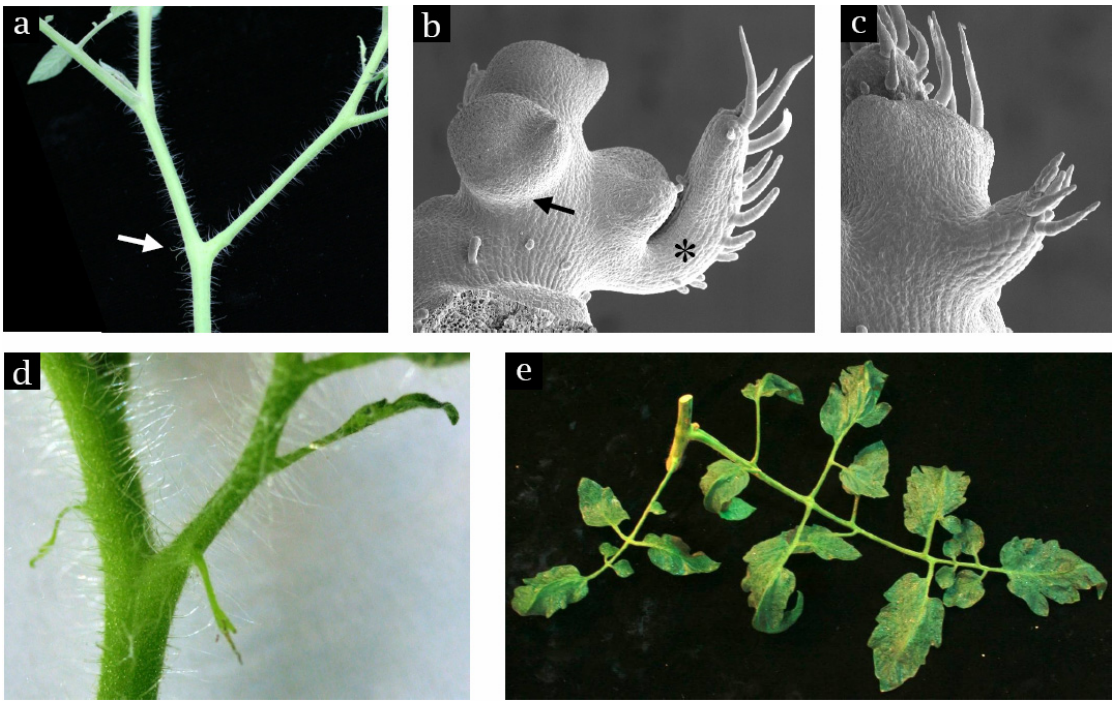


Fig. 3.B-6 Leaves in *uf*[1] pseudoshoots are gradually released

Complete suppression of leaf development in pseudoshoot phytomers like in wild-type inflorescence phytomer (a, b, arrows). Partially released leaves from rudimentary (c, d, b asterisk) to small compound or close to normal vegetative leaves (e with 6 resp. 15 leaflets). All images are from *uf*[1]- plants from Ailsa Craig cultivar.

3.B.3.4 Flowering time of *uniflora*

Flowering time, namely the number of normal leaves formed prior to any reproductive or pseudo-reproductive structure, was not obviously altered in experiments described in previous chapters. To unveil minor differences a careful flowering time experiment was conducted. The number of normal leaves and the days from opened cotyledons to the first opening flowers were counted in *uf*[1] (Ailsa Craig) and control plants. An excess of seeds was sown in parallel. Seedlings with open cotyledons within a time window of two days were selected, transplanted and grown to maturity in a computer randomised positioning. *uniflora* plants did not form more normal leaves (9.6 ± 0.3, n=12; Fig. 3.B-7 a) than Ailsa Craig wild-type plants (9.7 ± 0.5, n=11; error values give confidence intervals with α = .05). Nevertheless, *uf* plants needed in average 5,8 days longer from open cotyledons to opening of the first flower (43.9 ± 1.5 vs. 38.1 ± 1.7; Fig. 3.B-7 b). This can be explained by the increased number of phytomers initiated by the *uf* pseudoshoot preceding floral termination.

The axillary shoot in the last leaf axil of the primary shoot (sympodial shoot) continues vegetative growth of wild-type tomato plants (see Fig. 1.C-1). All wild-type sympodial shoots formed three leaves before terminating into the second inflorescence. In contrast, in *uniflora* the side shoot originating from the axil of the last normal leaf, initiated 5.9 ± 0.2 leaves before switching to reproductive or pseudo-reproductive growth (Fig. 3.B-7 c). In addition to the delayed flowering, the axillary shoot of the last normal vegetative leaf of *uniflora* is often slower than the pseudoshoot or than a wild-type sympodial shoot and is not acquiring the leading position in growth (not shown). The results of this flowering time experiment are in line with observations in several smaller experiments counting the number of leaves prior to the first and second pseudo-reproductive termination in *uf* plants.

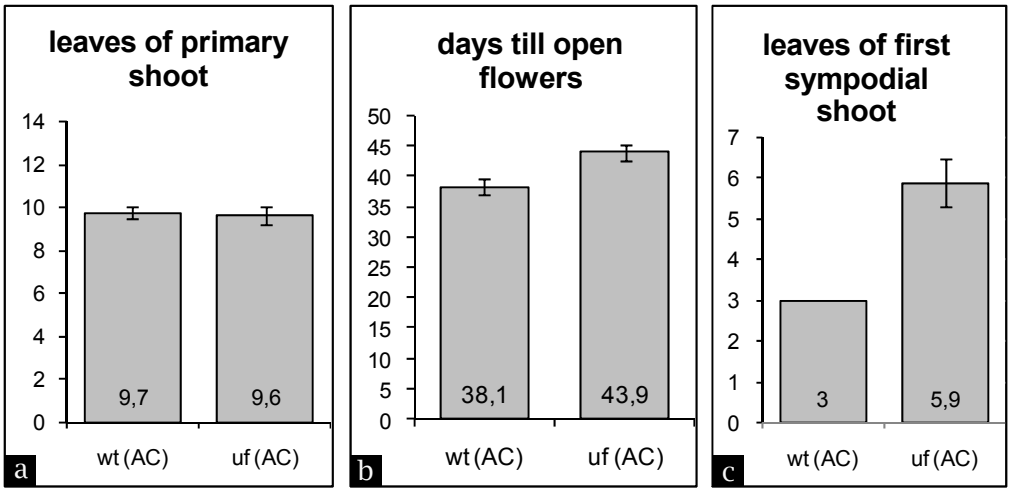


Fig. 3.B-7 Flowering time of *uf*^{1} in Ailsa Craig

(a) *uniflora* plants formed 9.6 ± 0.3 normal leaves in the primary shoot (n=12). This did not deviate from Ailsa Craig wild-type plants (n=11; 9.7 ± 0.5). (b) Time from open cotyledons to open petals was increased in *uf* by 15 % (43.9 ± 1.5 days vs. 38.1 ± 1.7 days) (c) The axillary shoot of the last normal leaf of the primary shoot flowered always after three leaves in wild-type but only after 5.9 ± 0.2 in *uniflora*. (error bars: confidence interval, significance level 0.05)

3.B.3.5 Summary of *uniflora* development

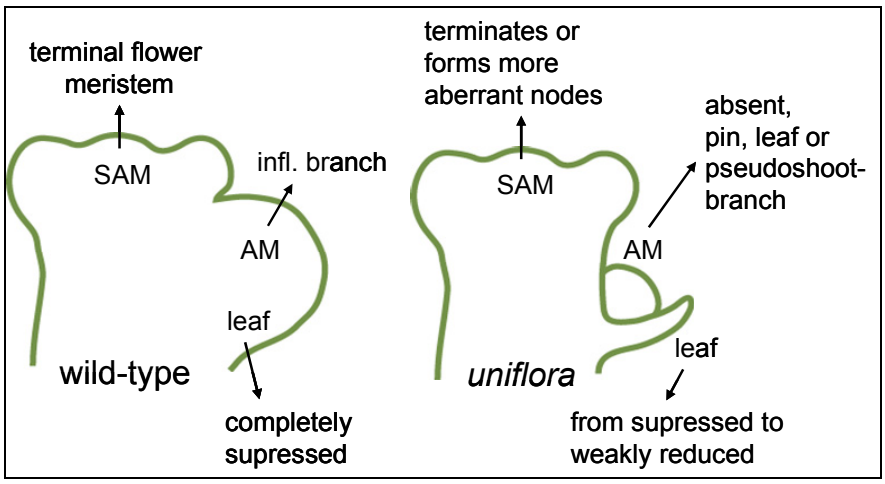


Fig. 3.B-8 *uniflora* shows gradual alterations in the formation of reproductive phytomers

Schematic drawing of wild-type and *uniflora* shoot apices after transition to reproductive respectively pseudo-reproductive growth. The tips of both drawings show the primary SAM converting to a flower meristem bearing sepal primordia.

Taken together, *uf* plants failed to form proper reproductive phytomers. When wild-type plants switch to reproductive growth, *uf* plants showed gradual defects in the formation of axillary meristems and in the suppression of leaves. Fig. 3.B-8 gives a schematic illustration and summary of the organogenesis in *uniflora* and wild-type shoot apical meristems after reproductive transition.

Remarkably, where the wild-type initiated SIMs, *uf* either produced retarded axillary meristems, terminating axillary structures or totally lacked axillary meristem initiation. This lack of AMs indicates, that the function of *uniflora* is conserved compared to the orthologous grass genes *LAX* and *ba1*, and the orthologous gene *ROB* in *Arabidopsis,* which are all regulators of axillary meristem initiation (see chapter 3.B.1). Moreover, the gradual impairment in AM formation is reminiscent of loss of function phenotypes of the branching regulators from the *Blind* gene family (see chapter 3.A.3.4). Therefore, *Uniflora* represents a new branching regulator in tomato. Additionally, the analysis of *uniflora* displayed that *Uniflora* controls proper reproductive development of apical and axillary meristems

Finally, the observations of both, Fehleisen, 1967 and Lifschitz *et al.*, 2006 could be confirmed and now understood at the level of organogenesis. The discrepancy between the

description as inflorescence with suppressed branching and the description as pseudoshoot can be now explained to be due to different expression of the gradual defects of *uf* (Fig. 3.B-9). The observations that the vegetative meristem directly converts to a flower meristem and that *uf* is always late flowering (Dielen *et al.*, 1998), could not be confirmed by the performed analyses.

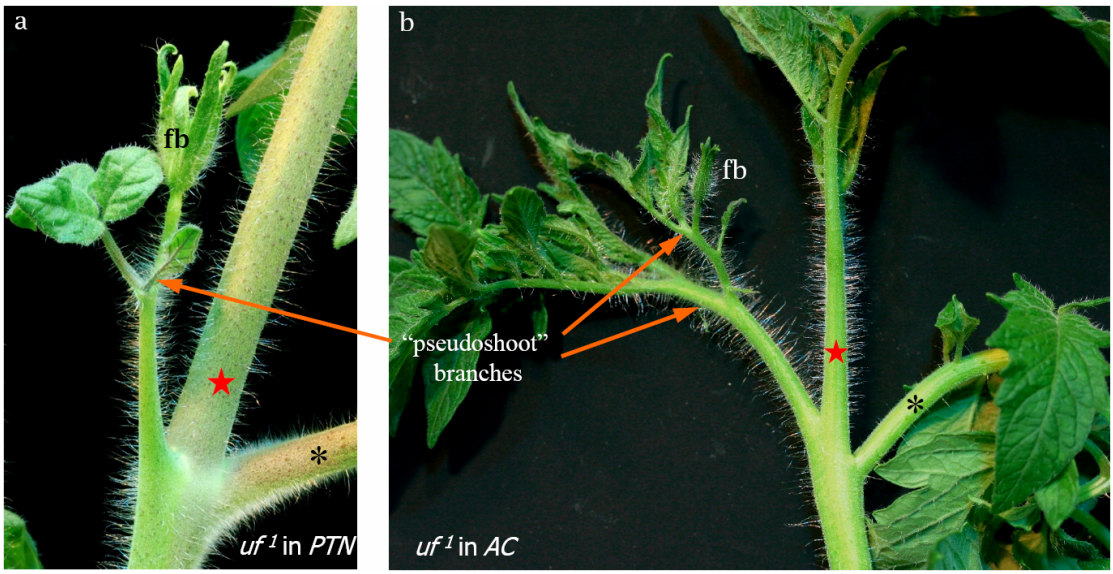


Fig. 3.B-9 Single flower and pseudoshoot formation in *uniflora*

(a) Inflorescence of *uf* ^{1}in cultivar *Platense*. Formation of a single abnormal phytomer prior to floral termination, harbouring a weak axillary shoot in the axils of a rudimentary pin. (b) Pseudoshoot of *uf* ^{1}in cultivar Ailsa Craig. Formation of four abnormal phytomers preceding termination. These phytomers display two pseudoshoot branches and two rudimentary leaves with barren axils. asterisks: petiole of the last normal leaf formed; stars: sympodial shoot equivalent; fb: flower bud.

3.B.4 Suppression and weak expression of *uf* developmental defects.

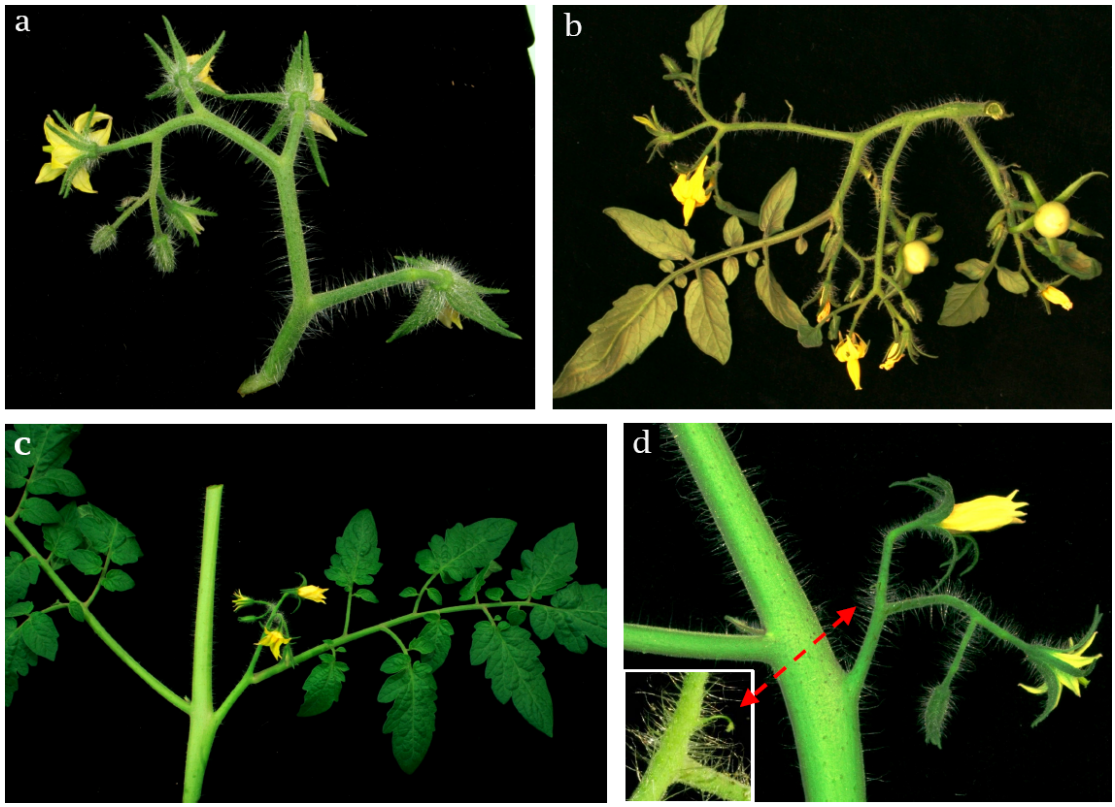


Fig. 3.B-10 Mild phenotypic defects of *uniflora*

(a) Inflorescences of *Uf* RNAi in cv. MM (line 09055). (b) uf^{1} in cv. AC x M82. (c, d) uf^{1} *sp* in cv. AC x Hz.

To further analyse the function of *Uniflora*, different populations of uf^{1}, uf^{Y} and *Uf* RNAi were examined. These were populations of uf^{1} in cv. AC, uf^{Y} in cv. M82 segregating for the gene *self pruning* (Pnueli *et al.*, 1998); uf^{1} (cv. AC) in a F2 population of a cross to cv. M82 segregating for *sp*; uf^{1} *sp* double mutant in cv. ACxHz and T0 and T1 generations of weak RNAi lines (see Material and Methods, 2.B.11). Five to twelve mutant plants of each population were evaluated macroscopically.

Plants of the line uf^{1} in cv. AC, grown in parallel to the other mentioned lines, developed as described in the previous section. The typical solitary flowers and pseudoshoots of *uniflora* were also observed in some inflorescences of each of the other mutant lines. However, many if not most inflorescences and pseudoshoots in these other mutant lines produced an increased number of flowers, (Fig. 3.B-10), indicating that the number of phytomers generated by the reproductive or pseudo-reproductive meristems prior to their floral termination was markedly decreased in these lines. Nevertheless, inflorescences of these lines developed several deviations in comparison to the wild-type, like reduced leaves prior to the first flower (Fig.

3.B-10 c), leafy inflorescences (Fig. 3.B-10 b), micro-leaves (Fig. 3.B-10 d inset), branched inflorescences (Fig. 3.B-10 c) and termination after formation of a few flowers (Fig. 3.B-10 d). Interestingly, these *uf* plants even displayed consecutive flower formation, however the wild-type scorpioid cyme phyllotaxy was distorted (Fig. 3.B-10 a, d).

Genotyping the *sp* locus in the populations segregating for *sp* indicated that *sp* might have a mild suppressing effect on the developmental aberrations of *uniflora*, yet this needs to be analysed in more detail. However, the major suppression of *uniflora* was independent of *sp* and thus probably due to unknown modifiers of the field tomato cultivars M82 and Heinz (Hz).

Strikingly, besides the already noted parallels in phenotypes of *bl*, *bli1* and *bli3* and *uniflora*, the described mild *uniflora* defects revealed even stronger reminiscence on *bl*, *bli1* and *bli3* inflorescence development (compare to chapters 3.A.3.3 and 3.A.3.4, Fig. 3.A-6, Fig. 3.A-7 and Fig. 3.A-8).

3.B.5 Pattern of *Uniflora* mRNA accumulation

Due to the defect in reproductive development of *uniflora*, reproductive shoot tips of wild-type tomato seedlings were analysed for the expression of *Uf* by RNA *in-situ* hybridisation. Preliminary results of two successfully hybridized reproductive tomato apices indicate that *Uf* mRNA accumulates at the border between the last and the penultimate meristem formed (Fig. 3.B-11). The last meristem formed is the youngest sympodial inflorescence meristem (SIM, introduction Fig. 1.D-1). This expression domain of *Uf*, adaxial of the reproductive axillary meristem, is highly similar to the expression domain of *Blind* (chapter 3.A.4.2) and the expression domains described for the *Uf* orthologous genes *LAX*, *ba1* and *ROB*, from rice, maize and *Arabidopsis,* respectively, which all show expression adaxially of newly initiated reproductive AMs (Komatsu *et al.*, 2003; Gallavotti *et al.*, 2004 and Yang, 2008).

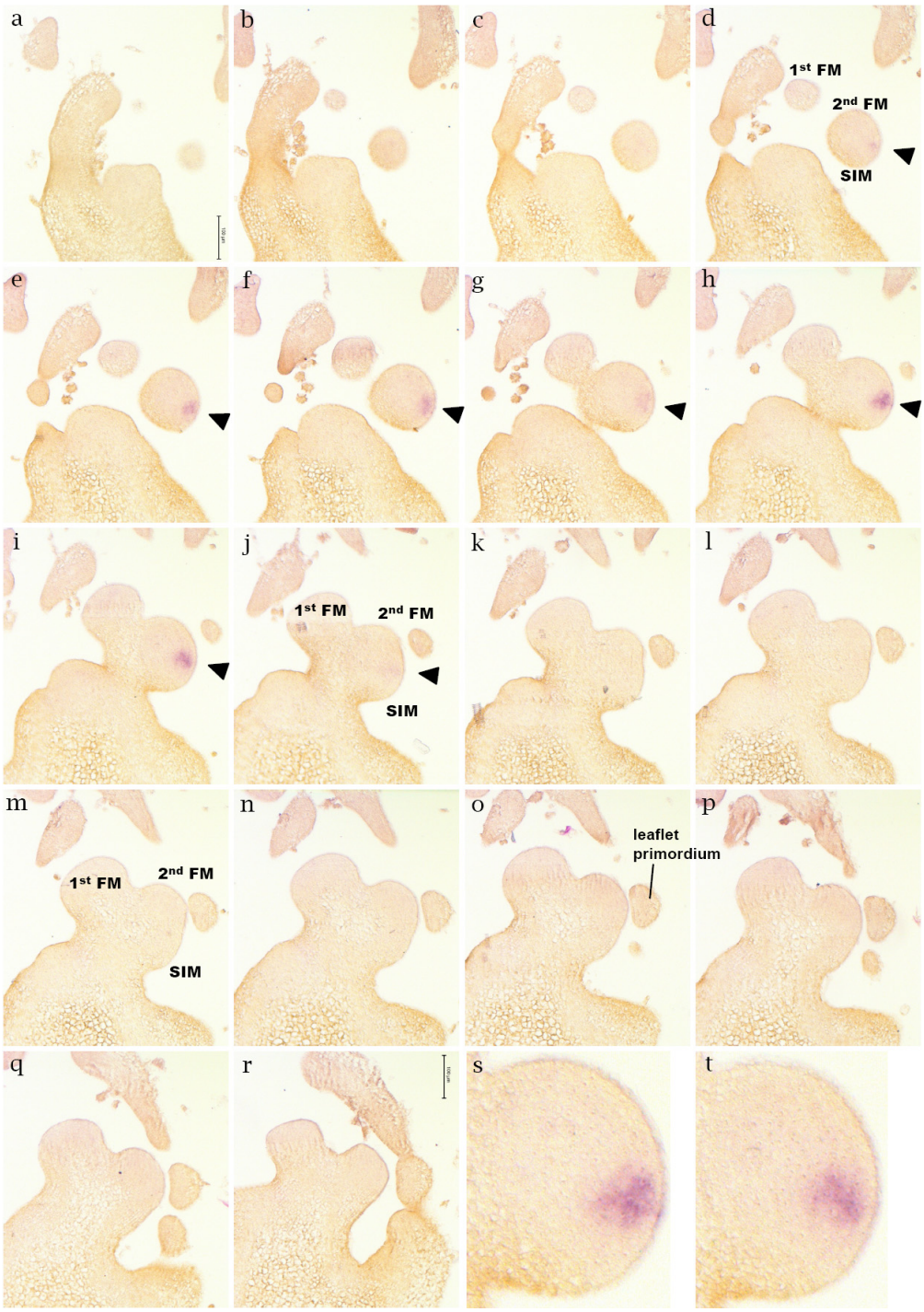


Fig. 3.B-11 RNA *in-situ* hybridisation of *Uf*

Reproductive shoot tips of wild-type tomato seedlings (cv. MM) were fixed and 8 µm sections were hybridised with *uf* antisense RNA probes. (a-q) Serial sections of a developing inflorescence harbouring two floral meristems (FM) and one sympodial inflorescence meristem (SIM). Expression of *Uniflora* was detected in sections d – j (arrowheads), presumably at the border between the youngest apical meristem (2nd FM) and the newly initiated SIM. (s and t) Close-ups of the expression domains from sections h and i. Section (r) displays the continuity of the leaf primordium on the right of the inflorescence (three sections between sections q and r are not presented; scale bar (a) to (r) 100 µm).

4 Discussion

4.A RNA interference - value and limitation

RNA interference is utilized to obtain loss of function plants of genes of interest. In the present study, this technology proved to be of great value. *SlLax* was identified as an orthologous gene of the rice *LAX* and maize *ba1* genes (3.B.1), two important branching regulators of grass species (Komatsu *et al.*, 2003; Gallavotti *et al.*, 2004). Silencing of *SlLax* resulted in the identification of the classical tomato mutant *uniflora*. *SlLax* RNAi lines phenocopied the development of *uniflora* mutant plants, which led to the assumption that *SlLax* is the gene mutated in *uniflora*. Sequencing of *SlLax* in two alleles of *uniflora* and in an introgression line of uf^{l} in Ailsa Craig revealed that all *uniflora* mutant lines carry sequence alterations in the *SlLax* gene disrupting the production of normal protein (3.B.2). Strikingly, the majority of the primary transgenic RNAi plants obtained by transformation with the *SlLax* RNAi plasmids, pJaZP-lax3 and pJaZP-lax6 (see 2.B.11), were indistinguishable from uf^{l} mutant plants in cv. AC. This again demonstrated the principal capability to knock out the function of a target gene of interest and not interfere with other developmental processes.

Two more genes were targeted by RNAi in this project, the *Blind* paralogous genes, *Bli1* and *Bli3*. Silencing of each of these genes led to subtle defects in vegetative AM formation and to changes in inflorescence architecture besides other developmental defects (3.A.3). Most of these phenotypic alterations were found to be enhanced when double transgenic lines, silencing both genes, where analysed. This indicates that the two genes might act redundantly. Independently, *Bli3* RNAi unveiled a function for this gene in the formation of leaf complexity (3.A.3.1).

In summary, RNAi silencing of *Bli1* and *Bli3* revealed two new regulators of tomato aerial plant architecture that act to large extent in the same developmental processes as controlled by *Blind* and *Potato Leaf* (Schmitz *et al.*, 2002 and 3.A.3). Especially for the processes controlled by the *Blind* gene, the RNAi lines enabled a more detailed insight, demonstrating that the three genes, *Bl, Bli1 and Bli3*, prevent concaulescent fusions, control AM initiation and influence meristem development. All three functions are exerted during the vegetative and reproductive phase of development (3.A.3). Furthermore, the transgenic lines indicated that *Bli1, Bli3* and *Blind* redundantly control the initiation of axillary meristems in complementary zones along the vegetative shoot. Consequently they might be causal for the remaining ability of *blind* knock out mutants to initiate axillary meristems (3.A.3.3). Triple loss of function plants are currently produced to test this hypothesis.

Nevertheless, RNAi is only an indirect method and does not always lead to a phenocopy of a knock out mutant. Therefore, the question remains how reliable the data for *bli1* and *bli3* are. The low efficiency and the time consuming nature of tomato transformation did not allow the generation of several dozens of independent transgenic lines. Nevertheless, multiple lines could be obtained for both genes (see chapter 2.B.10), which displayed the same general defects, as described in the results section. However, knockout mutants of *Bli1 and Bli3* still might show stronger defects than those observed in the RNAi lines. To test this hypothesis, mutants can be obtained by TILLING approaches. Currently, TILLING alleles of *bli3* are analysed (for allele information see appendix).

Another potentially weak point of RNAi lines might be an unspecific silencing of close paralogs. Only mutants can fully answer this question, but the specificity of the defects of *Bli1* and *Bli3* RNAi lines compared to *blind* and *potato leaf* mutants strongly suggest a specific silencing of the targeted gene. Silencing of *blind* can be excluded regarding the complementary branching defects observed (Fig. 3.A-5) and the similarity of *Blind* RNAi to *blind* mutants (Schmitz *et al.*, 2002 and Fig. 3.A-5). Indeed *Blind* RNAi lines are another good example for the capability of RNAi to phenocopy the mutant phenotype. *Bli3* RNAi defects were specific in branching, yet a weak cross silencing of *Potato Leaf* cannot be excluded. This would be expected rather in *Bli1* or *Blind* RNAi plants, as these two are closer related to *Potato Leaf* than *Bli3* is (3.A.1). However, both, *Blind* and *Bli1* silencing, did not result simpler leaves.

Testing for a potential cross silencing of *Blind* or *Potato Leaf* in *Bli1* and *Bli3* RNAi plants by qRT-PCR was considered useless due to four reasons. First, the defined expression domains of *Blind* and *Potato Leaf* (see 3.A) preclude harvesting plant samples with exact equal proportions of expressing cells of the total cells harvested. Second, the altered architecture e.g. less leaflets or less SIMs in the RNAi plants, reduces the presence of tissues where *Blind* or *Potato Leaf* are expressed and, therefore, indirectly leads to a relative reduction of their expression. Third, the *Blind* gene family might be auto-regulatoring, therefore a knock down of one gene would lead to expression changes of others. Fourth, the specific defects observed when comparing loss of function plants of all four genes with each other rule out that *Bli1* and *Bli3* RNAi completely silenced *Blind* or *Potato Leaf* and strongly indicate that the silencing of *Bli1* and *Bli3* was specific.

In summary, the present study is another example demonstrating the value of RNA interference technology.

4.B *Bl*, *Bli1* and *Bli3* act together with *Uniflora*

Plant transcription factors of the bHLH and MYB protein classes are known to physically interact in several processes (for review see Ramsay and Glover, 2005). Therefore, interaction of the MYB domain protein Blind and the bHLH domain protein Uniflora and their co-orthologs in *Arabidopsis*, RAX1 and ROB, are currently under investigation and could already be demonstrated for *Arabidopsis* (Yang and Wang, personal communication).

This thesis provides strong support that the two gene groups indeed work together. The phenotype of *uniflora* (*uf*) was highly reminiscent of the reproductive phenotype of *blind*, especially when comparing *uf* in cv. PTN to *blind* in cv. LU. Moreover, *uniflora* lines with weak or supressed phenotypic deviations resembled the reproductive phenotypes of *bli1* and *bli3* plants. Four aspects of these phenotypic similarities shall be briefly summarized here:

First, the *MYB* genes and the *bHLH* gene are both needed for axillary meristem initiation during reproductive growth. Loss of function of either led to the lack of sympodial inflorescence meristems (SIMs), i.e. many phytomers in reproductive development displayed no sign of AM initiation (3.A.3.3 and 3.B.3). In both genotypes, this block in AM formation is frequently accompanied by the occurrence of "micro-leaves".

Normally, tomato suppresses the development of morphologically distinguishable leaves (bracts) in inflorescence phytomers. However, in *Arabidopsis* the suppression of bracts is known to be dependent on the presence and the correct reproductive identity of the axillary meristems (Long and Barton, 2000 and references therein). Therefore, micro-leaves in *bl*, *bli1* and *bli3* inflorescences displaying barren axils (see 3.A.3.3) are most likely the consequence of lacking SIM initiation and are considered as outgrowing tomato bracts, which are otherwise probably subsumed by the inflorescence internodes formed from the SIMs. Strikingly, equal structures are also formed in *uniflora* (see 3.B.3), where they either bear barren axils or subtend partially vegetative axillary shoots, which are also rarely detected in the *bl*, *bli1* and *bli3* plants. Moreover, very similar structures also subtend SIMs in the tomato mutants *falsiflora* (data not shown) and *compound inflorescence* (Lippman *et al.*, 2008), in which SIMs develop with defective reproductive identity. Similarly, enhanced development of bracts was described for *lax* and *ba1*, the *uniflora* orthologous mutants of rice and maize (Komatsu *et al.*, 2001; Ritter *et al.*, 2002). Thus, like in *Arabidopsis*, suppression of leaf development (bracts) in tomato reproductive phytomers is dependent on the presence and correct reproductive identity of axillary meristems. On the other hand, in some cases "micro-leaves" or pin structures in *uf*, *bl*, *bli1* and *bli3* inflorescences may also represent a termination event of an improperly initiated SIM.

Secondly, if SIMs are formed in *bl*, *bli1*, *bli3* and *uf*, they frequently exhibit an abberant development or identity. They show vegetative characters, like indeterminacy, random phyllotaxy, leafiness or shooty development (3.A.3.4 and 3.B.3). The similarity becomes even more obvious when analysing weak or suppressed *uf* phenotypes (3.B.4). Third, the formation of reduced leaves prior to the first reproductive phytomer indicates a direct or indirect function in the reproductive transition of the shoot apical meristem (Fig. 3.A-8 c and Fig. 3.B-6). Fourth, both, *uniflora* and *blind*, generate sympodial shoots displaying altered flowering time. Moreover initial RNA in-situ hybridisation experiments revealed expression of *Uniflora* in the same tissue of the developing inflorescence as detected for *Blind* (3.A.4.2).

This line of evidence supports the view that these transcription factors act as heterodimers, although they are probably not fully dependent on their binding partner to exert their function. Similar findings have been described for other MYB/bHLH pairs (Hartmann *et al.*, 2005). Another important fact is that the *Blind* gene family consists of at least six members and no complete knockout of *Blind* related functions could be analysed yet, while *Uniflora* probably does not possess redundant paralogs. However, some developmental deviations described in this work indicate that *Uf* and the *Blind* gene family also function independently, e.g. concaulescent fusion or vegetative branching defects were not detected in *uf*.

In summary, the present and previous studies in tomato, *Arabidopsis*, rice and maize (Fehleisen, 1968; Schmitz *et al.*, 2002; Yang, 2008; Wang, personal communication; Müller *et al.*, 2006; Komatsu *et al.,* 2003 and Gallavotti *et al.*, 2004) have demonstrated that both, the *LAX*-bHLH and the *Blind*-MYB gene groups, are crucial players in the concert of genes needed for the initiation of all kinds of axillary meristems unveiling a conserved mechanism functioning in grasses and dicot species.

In addition, data in this work disclosed the *Blind* gene family and *Uniflora* as regulators of apical meristem development and identity, as briefly summarized above. Possible cause-effect relations of this function will be discussed in the next section.

4.C *Bl, Bli1*, *Bli3* and *Uniflora* regulate development and identity of apical meristems

Beside the described defects in organ separation and in early steps of AM initiation, several defects were observed in *bl*, *bli1*, *bli3* and *uniflora* that affect development and identity of apical meristems (3.A.3.4 and 3.B.3.3). These defects are summerized in the following lists. Phenotypic aberrations affecting axillary meristem and side-shoot development found in both loss of function groups:

- late or slow formation of SIMs and vegetative sympodial AMs (detailed analysis is described for *uniflora* 3.B.3.3) and slowly developing side-shoots or sympodial shoots
- terminating axillary organs
- SIMs developing vegetative characters, like indeterminacy, random phyllotaxy, elongated internodes, leafiness or shoot formation
- altered flowering time of the sympodial shoot

Furthermore the following defects affected development of the primary SAM:

- termination of the primary SAM after the formation of two leaves in *Bli3* RNAi lines.
- late flowering of the primary shoot in *uniflora* (as described in literature (3.B.3.1) and very recently also detected in the present work (data not shown) (also note the altered flowering time in plants carrying mutations in the *Blind* co-orthologous *Arabidopsis* gene *RAX1* (Müller *et al.*, 2006))
- generation of reduced leaves by the primary apical meristem prior to reproductive phytomer formation in *uniflora* and *blind*
- development of elongated, shoot-like peduncles and
- indeterminacy of the primary SAM after reproductive transition, leading to branched inflorescences in *bl*, *bli1*, *bli3* and *uniflora*.

Many of the listed defects, like the slow or late formation of AMs or the nature of terminating axillary structures were observed in continuous increments of severity in MYB and bHLH loss of function plants. This leads to the assumption that probably all aberrations, from the formation of barren axils over terminating axillary structures to the formation of axillary shoot apical meristems showing aberrant development, represent only a continuum of severities of

the same principle defect. Nevertheless, it remains questionable if aberrant meristem fate is indeed the mild penetrance of the same principle function leading also to a complete lack of AMs. Even if this is the case, how can other defects affecting the primary SAM be explained, since the primary SAM and axillary meristems are not considered to be equivalent? Alternatively, more than one function might be fulfilled independently by *Uniflora* and the *MYB* genes.

Two hypothetical scenarios shall be described here, trying to explain the developmental defects of in *bl*, *bli1*, *bli3* and *uniflora* apical meristems.

Scenario 1. The *MYB* and *bHLH* genes act only prior to the formation of AMs.

The *MYB* and *bHLH* genes set the right time point and velocity of AM initiation. Consequently, the extreme case of too slow or too late initiation is the failure. Furthermore, it needs to be postulated that the right timing and pace of axillary meristem initiation is a prerequisite for the new meristem to fulfil the right developmental program, in other words timing and pace of AM initiation are crucial for the correct meristem identity. A too late, small or slow initiation of sympodial inflorescence AMs (SIMs) in *bl*, *bli1*, *bli3* and *uniflora* then could lead to defective reproductive identity and to the establishment of the described vegetative characters within the inflorescence. The same effect could influence development of the vegetative sympodial meristem and thereby explain the variation of the sympodial flowering time. Furthermore, the defects related to the transition of the primary SAM could be explained as indirect effects that might be due to a missing signal which confirms proper sympodial identity of the last AM formed and which influences the timing of floral termination of the apical meristem (first from the vegetative, then the inflorescence sympodial AM). Such a feedback mechanism, confirming the presence of a functional SIM, could also explain the compound inflorescences of tomato mutants like *sft*, *s*, *an* or *falsiflora* (for review of inflorescence mutants see Samach and Lotan, 2007). Finally, the termination of *bli3* could be caused by improper initiation of the primary SAM during embryogenesis, explaining why always only two leaves are formed just like terminating axillary shoots in *blind* plants do. However, this hypothetical scenario has problems to explain the late flowering of *uniflora* (or the early flowering of *rax1*).

Scenario 2. *Bl, Bli1*, *Bli3* and *Uniflora* not only act in the initiation of AMs, but also act on already established apical meristems.

Experiments decapitating blind plants (Mapelli and Lombardi, 1982) and histological analysis (Mapelli and Kinet, 1992) showed that AMs in barren leaf axils of blind are completely lacking. In Arabidopsis, focused STM expression is missing in barren leaf axils of mutants of the Blind orthologous genes RAX1-RAX3 (Müller et al., 2006). Therefore, it is suggested that

Bl, Bli1, Bli3 and Uniflora act prior to AM initiation. Nevertheless, it is likely that they exert a second, independent function controlling the reproductive transition and reproductive identity of apical meristems. This interpretation implies a non-cell autonomous signal promoting reproductive transition and/or identity, originating from the *Blind* and *Uniflora* expression domains and directly or indirectly reaching apical meristems.

The two interpretations are not completely different, but deal with the question of an indirect secondary effect or a direct function in reproductive transition and identity control.

In addition, one can speculate whether the MYB and bHLH proteins regulate transcription only in cells prior to the formation of axillary meristems according to their mRNA expression patterns, that precede AM outgrowth in many of the analysed systems (3.A.4.2; Müller *et al.*, 2006; Yang, 2008; Komatsu *et al.,* 2003 and Gallavotti *et al.*, 2004) or whether the proteins are transported into establishing or already fully established meristems and then directly influence their development (this was very recently described for the LAX protein from rice, Oikawa and Kyozuka, 2009). Alternatively, several other scenarios could be postulated. For example the separation of the AM and its parental meristem could be suggested as the primary function that is needed to maintain or establish correct identities of both meristems. However, no directional correlations could be identified, substantiating this hypothesis. Nevertheless, the question has to be asked, what the relation of organ separation and axillary meristem development could be (see also chapter 4.D).

4.D Organ separation and axillary meristem initiation

Detailed analysis of *Bl*, *Bli1* and *Bli3* loss of function plants revealed that these genes are involved in the separation of AMs from their parental shoots. Loss of function plants developed fusions of side-shoots with the stem and fusions of inflorescence internodes with flower pedicels of varying severities. Because the tomato inflorescence is a cyme, inflorescence internodes represent side-shoots, while the preceding flower pedicels represent the corresponding parental shoots (see introduction 1.D). Therefore, the vegetative and the reproductive fusions produced in *bl*, *bli1* and *bli3* plants are specific concaulescent fusions. Interestingly, fusions of vegetative side-shoots were only observed in the last two side-shoots formed in vegetative development, the sympodial shoot and the one below. In contrast to other axillary meristems, the AMs giving rise to these two side-shoots initiate faster, i.e. less plastochrons elapse between formation of a leaf primordium to bulging of its AM. A similar effect can be detected in many species; AMs toward the reproductive switch of the apical meristem develop faster then truly vegetative ones. Even faster AM formation takes place in

the inflorescence, consequently in can be summarized that *Bl, Bli1 and Bli3* are needed to prevent concaulescent fusion of fast evolving axillary meristems.

The same three genes acting in the separation of organs proved to be involved in the initiation of axillary meristems. This is not the first example of a coincidence of separation defects and a defect in axillary meristem initiation in developmental mutants. The recently described tomato mutant *goblet* exhibits fusions of multiple organs (Berger et al., 2009; Blein et al., 2008). *goblet* plants are characterised by leaf petioles fused to the stem and barren leaf axils (unpublished data). Similarly, *Arabidopsis* mutants of the orthologous *cuc* family produce organ fusions and lack axillary meristems (Aida *et al.*, 1997 and Raman *et al.*, 2008). Another pair of orthologous genes involved in organ separation and in axillary meristem initiation are the *Ls* and *LAS* genes of tomato and *Arabidopsis*, respectively (Schumacher *et al.*, 1998 and Greb *et al.*, 2003). Moreover, recently more mutants were identified that develop barren leaf axils as well as organ fusions (*scarface*, *filomena1* and e3221, unpublished). Remarkably, the mRNA expression patterns of *Blind*, *Potato Leaf*, *Ls* and *GOB* all mark tissues of presumptive organ formation: at P0 prior to leaf primordia formation, at leaf primordia flanks prior to leaflet primordia formation and at leaf axils prior to AM formation. Consecutively the mRNA accumulates at the boundaries of these organs (see chapters 3.A.4.2, 3.A.4.3, Greb *et al.*, 2003; Blein et al., 2008; Berger et al., 2009)

The question has to be asked why *Bl, Bli1 and Bli3* and more generally, why some regulators of AM initiation, are also needed to prevent organ fusions. Are these independent consequences of one the same principal function, or do these genes possess two functions independently controlling the two processes, or is improper organ separation even causal for missing AMs or incorrect development of AMs? However, the lack of a directional correlation between the degree of fusions on the one hand and the lack or malformation of axillary meristems on the other hand, does not allow an easy explanation, correlating the two processes.

4.E Development of leaf complexity and axillary meristem initiation employ homologous mechanisms

The reverse genetics approach, elucidating the function of *Blind* orthologous genes, revealed redundant functions of *Bl, Bli1 and Bli3* in the control of shoot branching and organ separation. In addition, TILLING of *Blind-like2*, the closest paralog of *Blind*, unveiled an unexpected and on the first glimpse unrelated new function in the *Blind* gene family. TILLING of *Blind-like2* led to the identification of the classical mutant *potato leaf.* Allelism

tests and detection of sequence alterations in currently nine alleles demonstrated that *Blind-like2* is *Potato Leaf*. *potato leaf* / *blind-like2* mutants develop leaves with highly reduced complexity, almost completely lacking second order and intercalary leaflets, leaf lobing and serration. *Potato Leaf* is a key regulator of leaf complexity (Hareven *et al.*, 1996; Kessler *et al.*, 2001), that was described as a Mendelian gene already in 1908 (Price and Drinkard). Strikingly, ectopic expression of *Blind* by the cauliflower mosaic virus 35S promoter could complement the serration defect in *potato leaf* plants. Currently, complementation tests, utilizing *Potato Leaf* promoter sequences driving *Potato Leaf* expression and alternatively the same promoter driving *Blind*, are in progress to answer the question, if the *Blind* protein can fully replace the function of *Potato Leaf.*

Furthermore, RNAi induced silencing of *Bli3* resulted in plants exhibiting a branching defect and a loss of leaf complexity. The observations, that *Blind* and *Potato Leaf* share the highest protein similarity within the *Blind* gene family, that ectopic expression of *Blind* can complement the serration defect of *potato leaf* and that *Bli3* RNAi plants develop a branching and leaf complexity defect, provide evidence that one and the same function of the *Blind* MYB gene family is needed for both, AM initiation and compound leave development .

Indeed, the link between the two processes became more evident in the recent past, due to the discovery of several mutants displaying both, a failure in axillary meristem initiation and a defect in development of leaflets, leaf lobes or leaf serration.

Highly similar defects as found in loss of function plants of the *Blind* gene family are present in the *goblet* (*gob*) mutant. *gob* mutants produce leaves that lack second order and intercalary leaflets, leaf lobing and leaf serration, just as described for *potato leaf* (Blein et al., 2008; Berger et al., 2009). Additionally, theses plants lack almost all AMs in vegetative and reproductive development (unpublished). A similar situation was described for the *Goblet* orthologous genes in *Arabidopsis*, regulating AM initiation and leaf serration (Raman *et al.*, 2008; Nikovics *et al.*, 2006). Preliminary evidence indicates that *Goblet* and the *Blind* gene family might act in the same pathway and further experiments will be performed to test this hypothesis.

Beside the *Blind* and the *Goblet*/*CUC* gene families, there are several additional mutants known that exhibit reduced AM formation and reduced leaf complexity. These tomato mutants are *trifoliate* (Gregor Schmitz, personal communication), *lateral suppressor* (Naomi Ori, personal communication) and the recently discovered mutants *side shoots repressed*, *spoony* and *filomena1* (unpublished).

Moreover, a group of genes known to be involved in establishment of leaf polarity is needed for development of leaf complexity and for AM formation. These are the *Arabidopsis* HD-

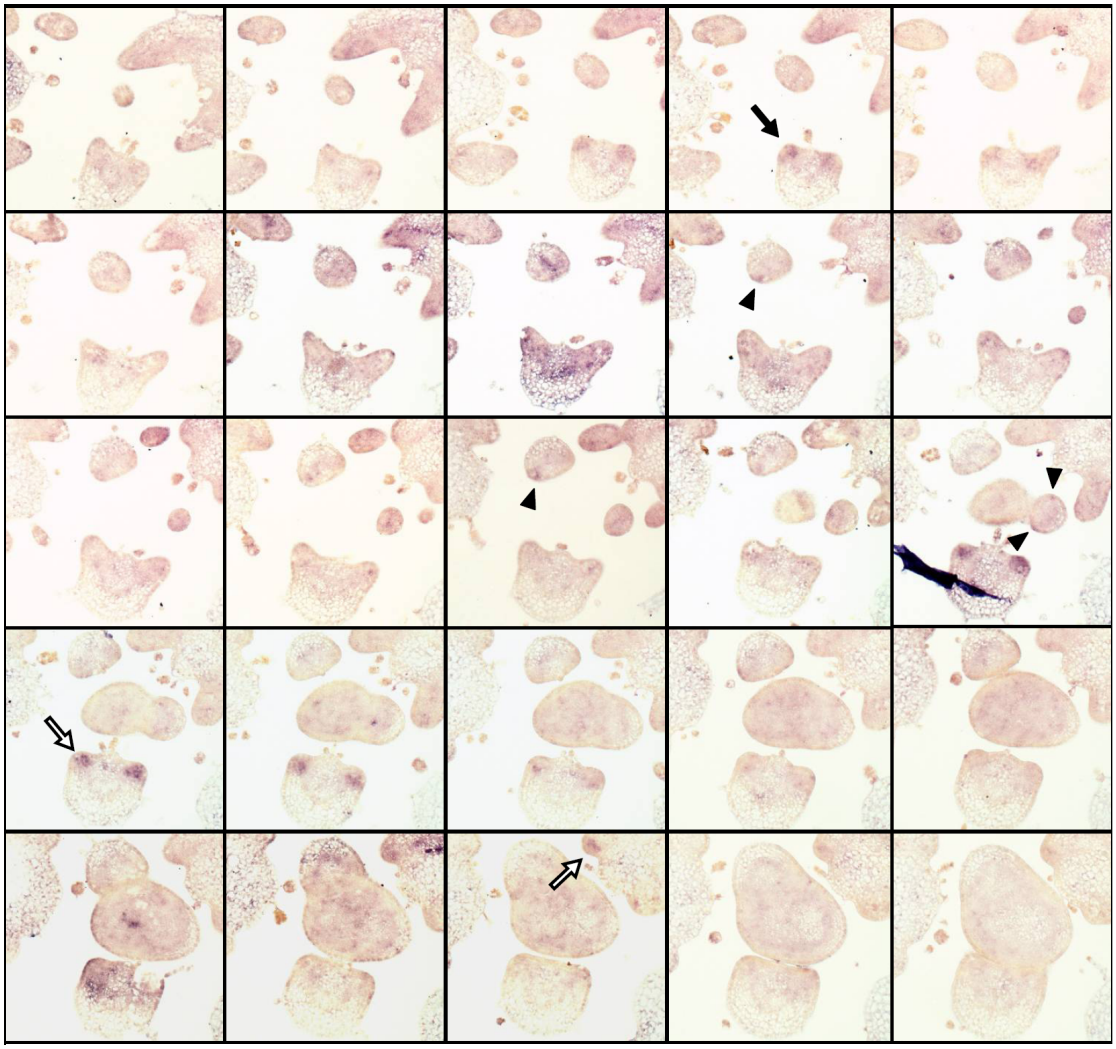
ZIP-Class III transcription factors, *Revoluta, Phabulosa* and *Phavoluta* (Otsuga *et al.*, 2001 and Greb *et al.*, 2003), genes from the *YABBY* family in *Arabidopsis* and Petunia (*YAB1*, Sawa *et al.*, 1999; Yang, 2008; Goltz *et al.,* 2004), the Antirrhinum genes *Phantastica* and *Handlebars* (Waites and Hudson, 2001) and the yet undescribed mutant *e3221*, from the tomato mutant collection "Genes that make tomatoes" (Menda *et al.*, 2004), analysed in this project (data not shown).

As indicated, these mutants probably fall into at least two classes. Those that act as boundary genes, like GOB and C and another class that influences dorso-ventral polarity of leaves, like HD-ZIPIII, *YABBY* and *Phantastica*. Whether these two classes are functionally interconnected or whether they regulate two independent pathways, both needed for AM initiation and leaf complexity development, remains unknown.

In summary, the listed mutants give strong evidence that at least one homologous mechanism exists, that regulates both processes, development of leaf complexity and axillary meristem initiation

5 Appendix

5.A RNA *in-situ* hybridisation of *C*



Appendix Fig. 5.A-1 *Potato Leaf* RNA *in-situ* hybridisation in serial transverse sections
An entire vegetative shoot tip is shown in sections of 8 µm, starting from the tip of the young P3 (picture at the upper left corner) and ending at the axil of P4 (lower right corner). *Potato Leaf* transcript accumulation was detectable at the adaxial flanks of young leaf primordia prior to leaflet formation (arrowheads), in the proximal axils of leaflet primordia (unfilled arrows) and at the distal axil of leaflet primordia (filled arrow).

5.B Sequence flatfiles

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LOCUS       Blind-like3             2218 bp    DNA                     07-MAY-2009

kartiert auf Chr. 4 nahe T0769 (keine Rekombinante)
close above entire
mutants found on TGRC and CROPgenetics maps of chr. 4
see TGRC
http://tgrc.ucdavis.edu/Data/Acc/dataframe.aspx?start=GenSearch.aspx&navstart=nav.html,
all mutants accept di and vg can be exluded due to phentypic descriptions

4ESTs: Library Description Library size (# ESTs) ESTs in this unigene
TUS S. lycopersicum (formerly L. esculentum) Rearrayed collection of L. esculentum
cDNA clones 37722 3
cLEI S. lycopersicum (formerly L. esculentum) whole seedlings 3927 1

FEATURES             Location/Qualifiers
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LOCUS       Blind-like4               2624 bp    DNA                 07-MAY-2009

FEATURES             Location/Qualifiers
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LOCUS   Blind-like5 in C08HBa0239G21-BAC sequence      2300 bp   DNA  7-MAY-2009

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       2281 TTAAGAATTC AAAGCCCCAA
//


LOCUS  Uniflora / SlLax   _genomic   3160 bp    DNA                  07-MAY-2009

map postion: chromosome 9 top; 4 recombinations in 73 chromosomes analysed pointing
north of marker TG18 (~5,5 cM)
BAC end hit in the cds: SL_MboI0013J04_T7_212176

FEATURES             Location/Qualifiers
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                     SKMDTVTMLEEAIHYVKFLKTQIWLHQTMVNLVDINHEMVGYYPLVDDDQNIHKNNIS
                     SMDYQQMQQVQSYDNDAFQQVEFPFEETNISGDVFMYYN"
     variation       1486..1510
                     /note="uf^1"
                     /note="uniflora 25 bp duplication = insertion leading to
                     tandem repeat"
     variation       1720..1722
                     /note="uf^Y"
                     /note="C385T   Caa to Taa leads to Q129stop"
                     /note="same mutation in both lines: e1316 und e1383"
     variation       1336..1338
                     /note="T2C in lax-1 TILLING allele, Moneymaker and Ailsa
                     Craig wild-type, when compared to the accessions M82,
                     Heinz-1706 and Platense wild type"
                     /note="aCg instead of aTg, open reading frame is reduced
                     by 7 amino acids"
     variation       1462..1464
                     /note="lax-2 TILLING allele"
                     /note="C128T - Thr43Ile  - T43I"
                     /note="rather conserved in LAX in 6 species (7 proteins):
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                     /note="no conservation of lax proteins compared to other
                     bHLH family members in 7 species (34 proteins)"
     variation       1702..1704
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                     /note="Thr 123 Lys, T123K, poorly conserved in our bHLH
                     family, several K exist"
                     /note=" In 8 Lax from seven species:  5x T, 2x A, 1x N"
                     /note="C to A Mutation"
     misc_feature    1552..1719
                     /note="bHLH domain, Heim et al"
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                     /note="double SUMO interacting motif:  VxLV and LVxI
                     L,V and I are equivalent"
     misc_feature    2602..3160
                     /note=" Bac end sequence"
                     /note="SL_EcoRI_029_H14 sequence runs SP6 and T7"
                     /note="the two runs are independent but nearly identical"
     repeat_region   2764..3132
                     /note="repeat region"
                     /note="90 percent homology to two unidentified repeats in
                     SGN UniRepeats repeat collection"
     variation       1602..1607
                     /note="CAPS marker possible - restriction site
                     polymorphism in S.penellii"
                     /note="BspCI/MvrI/Ple19I/PvuI do not cut any more in
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ORIGIN
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       61 CCTCTCCAAC TTAGACTCTT AAACTGTGAG CTTCTAAGTG ACGAATTAAT TAGAAATTCT
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     3061 CGAATTGTAT AAAACGAGAA AGAGAGAAAT TATATACAAT TTGAACTTAT ACaAAACGAG
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6 References

Aida, M., Ishida, T., Fukaki, H., Fujisawa, H., and Tasaka, M. (1997). Genes Involved in Organ Separation in Arabidopsis: An Analysis of the cup-shaped cotyledon Mutant. Plant Cell **9,** 841-857.

Bailey, P.C., Martin, C., Toledo-Ortiz, G., Quail, P.H., Huq, E., Heim, M.A., Jakoby, M., Werber, M., and Weisshaar, B. (2003). Update on the Basic Helix-Loop-Helix Transcription Factor Gene Family in Arabidopsis thaliana. Plant Cell **15,** 2497-2502.

Barkoulas, M., Galinha, C., Grigg, S.P., and Tsiantis, M. (2007). From genes to shape: regulatory interactions in leaf development. Current Opinion in Plant Biology **10,** 660-666.

Barkoulas, M., Hay, A., Kougioumoutzi, E., and Tsiantis, M. (2008). A developmental framework for dissected leaf formation in the Arabidopsis relative Cardamine hirsuta. Nat Genet **40,** 1136-1141.

Barthelemy, D., and Caraglio, Y. (2007). Plant Architecture: A Dynamic, Multilevel and Comprehensive Approach to Plant Form, Structure and Ontogeny. Ann Bot **99,** 375-407.

Bennett, T., and Leyser, O. (2006). Something on the side: axillary meristems and plant development. Plant Mol Biol **60,** 843-854.

Berger, Y., Harpaz-Saad, S., Brand, A., Melnik, H., Sirding, N., Alvarez, J.P., Zinder, M., Samach, A., Eshed, Y., and Ori, N. (2009). The NAC-domain transcription factor GOBLET specifies leaflet boundaries in compound tomato leaves. Development **136,** 823-832.

Blein, T., Pulido, A., Vialette-Guiraud, A., Nikovics, K., Morin, H., Hay, A., Johansen, I.E., Tsiantis, M., and Laufs, P. (2008). A Conserved Molecular Framework for Compound Leaf Development. Science **322,** 1835-1839.

Danert, S. (1958). Die Verzweigung der Solanaceen im reproduktiven Bereich. (Abh. Deutsch. Akad. Wiss. Berlin, Math.-Naturwiss. Kl. 1957, 6:,).

Dielen, V., Marc, D., and Kinet, J.M. (1998). Flowering in the uniflora mutant of tomato (Lycopersicon esculentum Mill.): description of the reproductive structure and manipulation of flowering time. Plant Growth Regulation **25,** 149-157.

Dielen, V., Lecouvet, V., Dupont, S., and Kinet, J.-M. (2001). In vitro control of floral transition in tomato (Lycopersicon esculentum Mill.), the model for autonomously flowering plants, using the late flowering uniflora mutant. J. Exp. Bot. **52,** 715-723.

Dielen, V., Quinet, M., Chao, J., Batoko, H., Havelange, A.e., and Kinet, J.-M. (2004). UNIFLORA, a Pivotal Gene That Regulates Floral Transition and Meristem Identity in Tomato (Lycopersicon esculentum). New Phytologist **161,** 393-400.

Doganlar, S., Frary, A., Daunay, M.-C., Lester, R.N., and Tanksley, S.D. (2002). A Comparative Genetic Linkage Map of Eggplant (Solanum melongena) and Its Implications for Genome Evolution in the Solanaceae. Genetics **161,** 1697-1711.

Fehleisen, S. (1967). Uniflora and conjunctiflora: Two new mutants in tomato. TGC Report **17,** 26-28

Gallavotti, A., Zhao, Q., Kyozuka, J., Meeley, R.B., Ritter, M.K., Doebley, J.F., Enrico Pe,

M., and Schmidt, R.J. (2004). The role of barren stalk1 in the architecture of maize. Nature **432,** 630-635.

Golz, J.F., Roccaro, M., Kuzoff, R., and Hudson, A. (2004). GRAMINIFOLIA promotes growth and polarity of Antirrhinum leaves. Development **131,** 3661-3670.

Grbic, B., and Bleecker, A.B. (1996). An altered body plan is conferred on Arabidopsis plants carrying dominant alleles of two genes. Development **122,** 2395-2403.

Greb, T., Clarenz, O., SchÃ¤fer, E., MÃ¼ller, D.r., Herrero, R.n., Schmitz, G., and Theres, K. (2003). Molecular analysis of the LATERAL SUPPRESSOR gene in Arabidopsis reveals a conserved control mechanism for axillary meristem formation. Genes & Development **17,** 1175-1187.

Hareven, D., Gutfinger, T., Pnueli, L., Bauch, L., Cohen, O., and Lifschitz, E. (1994). The floral system of tomato. Euphytica **79,** 235-243.

Hareven, D., Gutfinger, T., Parnis, A., Eshed, Y., and Lifschitz, E. (1996). The making of a compound leaf: Genetic manipulation of leaf architecture in tomato. Cell **84,** 735-744.

Hartmann, U., Sagasser, M., Mehrtens, F., Stracke, R., and Weisshaar, B. (2005). Differential combinatorial interactions of cis-acting elements recognized by R2R3-MYB, BZIP, and BHLH factors control light-responsive and tissue-specific activation of phenylpropanoid biosynthesis genes. Plant Molecular Biology **57,** 155-171.

Heim, M.A., Jakoby, M., Werber, M., Martin, C., Weisshaar, B., and Bailey, P.C. (2003). The Basic Helix-Loop-Helix Transcription Factor Family in Plants: A Genome-Wide Study of Protein Structure and Functional Diversity. Mol Biol Evol **20,** 735-747.

Helm, J. (1951). Vergleichende Betrachtungen über die Entwicklung der Infloreszenz beiLycopersicum esculentumMill. und bei einer Röntgenmutante. TAG Theoretical and Applied Genetics **21,** 89-95.

Hempel, F.D., and Feldman, L.J. (1994). Bidirectional Inflorescence Development in Arabidopsis-Thaliana - Acropetal Initiation of Flowers and Basipetal Initiation of Paraclades. Planta **192,** 276-286.

Hibara, K.-I., Karim, M.R., Takada, S., Taoka, K-I., Furutani, M., Aida, M., and Tasaka, M. (2006). *Arabidopsis CUP-SHAPED COTYLEDON3* regulates postembryonic shoot meristem and organ boundary formation. Plant Cell (in press).

Jasinski, S., Tattersall, A., Piazza, P., Hay, A., Martinez-Garcia, J.F., Schmitz, G., Theres, K., McCormick, S., and Tsiantis, M. (2008). PROCERA encodes a DELLA protein that mediates control of dissected leaf form in tomato. Plant Journal **56,** 603-612.

Jeffrey D. Stevens, E.H.R.M.K.S. (2008). Phylogenetic and expression analysis of the basic helix-loop-helix transcription factor gene family: genomic approach to cellular differentiation. Differentiation **76,** 1006-1022.

Keller, T., Abbott, J., Moritz, T., and Doerner, P. (2006). Arabidopsis REGULATOR OF AXILLARY MERISTEMS1 controls a leaf axil stem cell niche and modulates vegetative development. Plant Cell **18,** 598-611.

Kessler, S., Kim, M., Pham, T., Weber, N., and Sinha, N. (2001). Mutations altering leaf morphology in tomato. International Journal of Plant Sciences **162,** 475-492.

Kidner, C.A., and Martienssen, R.A. . (2004). Spatially restricted microRNA directs leaf polarity through *ARGONAUTE1*. Nature**,** 81–84.

Kimura, S., Koenig, D., Kang, J., Yoong, F.Y., and Sinha, N. (2008). Natural Variation in Leaf Morphology Results from Mutation of a Novel KNOX Gene. Current Biology **18,** 672-677.

Knapp, S., Larondelle, Y., Roßberg, M., Furtek, D., and Theres, K. (1994). Transgenic tomato lines containing Ds elements at defined genomic positions as tools for targeted transposon tagging. Molecular and General Genetics MGG **243,** 666-673.

Komatsu, M., Maekawa, M., Shimamoto, K., and Kyozuka, J. (2001). The LAX1 and FRIZZY PANICLE 2 Genes Determine the Inflorescence Architecture of Rice by Controlling Rachis-Branch and Spikelet Development. Developmental Biology **231,** 364-373.

Komatsu, K., Maekawa, M., Ujiie, S., Satake, Y., Furutani, I., Okamoto, H., Shimamoto, K., and Kyozuka, J. (2003). *LAX* and *SPA*: major regulators of shoot branching in rice. . Proc. Natl. Acad. Sci. USA**,** 11765–11770.

Kuusk, S., Sohlberg, J.J., Magnus Eklund, D., and Sundberg, E. (2006). Functionally redundant SHI family genes regulate Arabidopsis gynoecium development in a dose-dependent manner. Plant J **47,** 99-111.

Li, X.Y., Qian, Q., Fu, Z.M., Wang, Y.H., Xiong, G.S., Zeng, D.L.,, and Wang, X.Q., Liu, X.F., Teng, S., and Hiroshi, F. (2003). Control of tillering in rice. Nature**,** 618–621.

Lifschitz, E., and Eshed, Y. (2006). Universal florigenic signals triggered by FT homologues regulate growth and flowering cycles in perennial day-neutral tomato. Journal of Experimental Botany **57,** 3405-3414.

Lifschitz, E., Eviatar, T., Rozman, A., Shalit, A., Goldshmidt, A., Amsellem, Z., Alvarez, J.P., and Eshed, Y. (2006). The tomato FT ortholog triggers systemic signals that regulate growth and flowering and substitute for diverse environmental stimuli. Proceedings of the National Academy of Sciences of the United States of America **103,** 6398-6403.

Lippman, Z.B., Cohen, O., Alvarez, J.P., Abu-Abied, M., Pekker, I., Paran, I., Eshed, Y., and Zamir, D. (2008). The Making of a Compound Inflorescence in Tomato and Related Nightshades. Plos Biology **6,** 2424-2435.

Long, J., and Barton, M.K. (2000). Initiation of Axillary and Floral Meristems in Arabidopsis. Developmental Biology **218,** 341-353.

Lyndon, R.F. (1990). Plant Development. Topics in plant physiology.

Lynn, K., Fernandez, A., Aida, M., Sedbrook, J., Tasaka, M., Masson, P., and Barton, M.K. . (1999). The *PINHEAD / ZWILLE* gene acts pleiotropically in *Arabidopsis* development and has overlapping functions with the *ARGONAUTE1* gene. Development**,** 469–481.

Mach, J. (2009). Rice Axillary Meristem Formation Requires Directional Movement of LAX PANICLE1 Protein. Plant Cell**,** tpc.109.210410.

Mao, L., Begum, D., Chuang, H.-w., Budiman, M.A., Szymkowiak, E.J., Irish, E.E., and Wing, R.A. (2000). JOINTLESS is a MADS-box gene controlling tomato flower abscission zone development. Nature **406,** 910-913.

Mapelli, S., and Kinet, J.M. (1992). Plant growth regulator and graft control of axillary bud formation and development in the TO-2 mutant tomato. Plant Growth Regulation **11,** 385-390.

McConnell, J.R., and Barton, M.K. . (1998). Leaf polarity and meristem formation in *Arabidopsis*. . Development**,** 2935–2942.

McSteen, P. (2009). Hormonal regulation of branching in grasses. Plant Physiol **149,** 46-55.

Molinero-Rosales, N., Jamilena, M., Zurita, S., Gomez, P., Capel, J., and Lozano, R. (1999). FALSIFLORA, the tomato orthologue of FLORICAULA and LEAFY, controls flowering time and floral meristem identity. Plant Journal **20,** 685-693.

Mueller, L.A., Solow, T.H., Taylor, N., Skwarecki, B., Buels, R., Binns, J., Lin, C., Wright, M.H., Ahrens, R., Wang, Y., Herbst, E.V., Keyder, E.R., Menda, N., Zamir, D., and Tanksley, S.D. (2005). The SOL Genomics Network. A Comparative Resource for Solanaceae Biology and Beyond. Plant Physiol. **138,** 1310-1317.

Müller, D., Schmitz, G., and Theres, K. (2006). Blind Homologous R2R3 Myb Genes Control the Pattern of Lateral Meristem Initiation in Arabidopsis. Plant Cell **18,** 586-597.

Naama Menda, Y.S.D.P.Y.E.D.Z. (2004). In silico screening of a saturated mutation library of tomato. The Plant Journal **38,** 861-872.

Nikovics, K., Blein, T., Peaucelle, A., Ishida, T., Morin, H., Aida, M., and Laufs, P. (2006). The Balance between the MIR164A and CUC2 Genes Controls Leaf Margin Serration in Arabidopsis. Plant Cell **18,** 2929-2945.

Oikawa, T., and Kyozuka, J. (2009). Two-Step Regulation of LAX PANICLE1 Protein Accumulation in Axillary Meristem Formation in Rice. Plant Cell**,** tpc.108.065425.

Ori, N., Cohen, A.R., Etzioni, A., Brand, A., Yanai, O., Shleizer, S., Menda, N., Amsellem, Z., Efroni, I., Pekker, I., Alvarez, J.P., Blum, E., Zamir, D., and Eshed, Y. (2007). Regulation of LANCEOLATE by miR319 is required for compound-leaf development in tomato. Nature Genetics **39,** 787-791.

Otsuga, D., DeGuzman, B., Prigge, M.J., Drews, G.N., and Clark, S.E. (2001). REVOLUTA regulates meristem initiation at lateral positions. Plant Journal **25,** 223-236.

Parnis, A., Cohen, O., Gutfinger, T., Hareven, D., Zamir, D., and Lifschitz, E. (1997). The Dominant Developmental Mutants of Tomato, Mouse-ear and Curl, Are Associated with Distinct Modes of Abnormal Transcriptional Regulation of a Knotted Gene. Plant Cell **9,** 2143-2158.

Perry, J.J.P., Tainer, J.A., and Boddy, M.N. (2008). A SIM-ultaneous role for SUMO and ubiquitin. Trends in Biochemical Sciences **33,** 201-208.

Pnueli, L., Carmel-Goren, L., Hareven, D., Gutfinger, T., Alvarez, J., Ganal, M., Zamir, D., and Lifschitz, E. (1998). The SELF-PRUNING gene of tomato regulates vegetative to reproductive switching of sympodial meristems and is the ortholog of CEN and TFL1. Development **125,** 1979-1989.

Price, H.L., and Drinkard, A.W. (1908). Inheritance in tomato hybrids. Molecular and General Genetics MGG **1,** 402-403.

Prusinkiewicz, P., Erasmus, Y., Lane, B., Harder, L.D., and Coen, E. (2007). Evolution and development of inflorescence architectures. Science **316,** 1452-1456.

Quinet, M., Dielen, V., Batoko, H., Boutry, M., Havelange, A., and Kinet, J.M. (2006). Genetic interactions in the control of flowering time and reproductive structure development in tomato (Solanum lycopersicum). New Phytologist **170,** 701-710.

Quinet, M., Dubois, C., Goffin, M.-C., Chao, J., Dielen, V., Batoko, H., Boutry, M., and Kinet, J.-M. (2006). Characterization of tomato (Solanum lycopersicum L.) mutants affected in their flowering time and in the morphogenesis of their reproductive structure. J.

Exp. Bot. **57,** 1381-1390.

Raman, S., Greb, T., Peaucelle, A., Blein, T., Laufs, P., and Theres, K. (2008). Interplay of miR164, CUP-SHAPED COTYLEDON genes and LATERAL SUPPRESSOR controls axillary meristem formation in Arabidopsis thaliana. Plant J **55,** 65-76.

Ramsay, N.A., and Glover, B.J. (2005). MYB-bHLH-WD40 protein complex and the evolution of cellular diversity. Trends in Plant Science **10,** 63-70.

Reinhardt, D., Frenz, M., Mandel, T., and Kuhlemeier, C. (2005). Microsurgical and laser ablation analysis of leaf positioning and dorsoventral patterning in tomato. Development **132,** 15-26.

Rick, C.M., and Butler, L. (1956). Cytogenetics of the Tomato. Advances in Genetics Incorporating Molecular Genetic Medicine **8,** 267-382.

Ritter, M.K., Padilla, C.M., and Schmidt, R.J. (2002). The maize mutant barren stalk1 is defective in axillary meristem development. Am. J. Bot. **89,** 203-210.

Robinson, R.W., and Rick, C.M. (1954). New Tomato Seedling Characters and Their Linkage Relationships. Journal of Heredity **45,** 241-247.

Romero, I., Fuertes, A., Benito, M.J., Malpica, J.M., Leyva, A., and Paz-Ares, J. (1998). More than 80R2R3-MYB regulatory genes in the genome of Arabidopsis thaliana. Plant Journal **14,** 273-284.

Rozen, S., and Skaletsky, H. (1999). Primer3 on the WWW for General Users and for Biologist Programmers. In Bioinformatics Methods and Protocols, pp. 365-386.

Samach, A., and Lotan, H. (2007). The transition to flowering in tomato. Plant Biotechnol **24,** 71-82.

Sawhney, V.K., and Greyson, R.I. (1972). Initiation of Inflorescence and Floral Organs in Tomato (Lycopersicon-Esculentum). Canadian Journal of Botany **50,** 1493-&.

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

Schmitz, G., Tillmann, E., Carriero, F., Fiore, C., Cellini, F., and, and Theres, K. (2002). The tomato *Blind* gene encodes a *MYB* transcription factor that controls the formation of lateral meristems Proc. Natl. Acad. Sci. USA**,** 1064–1069.

Schmitz, G., and Theres, K. (2005). Shoot and inflorescence branching. Curr Opin Plant Biol **8,** 506-511.

Schumacher, K., Schmitt, T., Rossberg, M., Schmitz, G., and Theres, K. (1999). The Lateral suppressor (Ls) gene of tomato encodes a new member of the VHIID protein family. Proceedings of the National Academy of Sciences of the United States of America **96,** 290-295.

Seeler, J.-S., and Dejean, A. (2003). Nuclear and unclear functions of SUMO. Nat Rev Mol Cell Biol **4**, 690-699.

Sinha, N. (1999). LEAF DEVELOPMENT IN ANGIOSPERMS. Annual Review of Plant Physiology and Plant Molecular Biology **50,** 419-446.

Smith, D.L., and Fedoroff, N.V. (1995). LRP1, a Gene Expressed in Lateral and Adventitious Root Primordia of Arabidopsis. Plant Cell **7,** 735-745.

Soressi, G.P., Gentinetta, E., Odoardi, M., and Salamini, F. (1974). Leaf peroxidase activities in tomato mutants affecting plant morphology. Biochemical Genetics **12,** 181-198.

Stracke, R., Werber, M., and Weisshaar, B. (2001). The R2R3-MYB gene family in Arabidopsis thaliana. Current Opinion in Plant Biology **4,** 447-456.

Stubbe, H. (1960). Mutanten der Wildtomate Lycopersicon pimpinellifolium (Jusl.) Mill. I. Genetic Resources and Crop Evolution **8,** 110-137.

Stubbe, H. (1961). Mutanten der Wildtomate Lycopersicon pimpinellifolium (Jusl.) Mill. II. Genetic Resources and Crop Evolution **9,** 58-87.

Stubbe, H. (1963). Mutanten der Kulturtomate Lycopersicon esculentum Miller IV. Genetic Resources and Crop Evolution **11,** 603-644.

Stubbe, H. (1964). Mutanten der Kulturtomate Lycopersicon esculentum Miller V. Genetic Resources and Crop Evolution **12,** 121-152.

Stubbe, H. (1972). Mutanten der Kulturtomate Lycopersicon esculentum Miller VI. Genetic Resources and Crop Evolution **19,** 185-230.

Sussex, I.M. (1989). Developmental programming of the shoot meristem. Cell **56,** 225-229.

Sussex, I.M., and Kerk, N.M. (2001). The evolution of plant architecture. Curr Opin Plant Biol **4,** 33-37.

Szymkowiak, E.J., and Irish, E.E. (1999). Interactions between jointless and Wild-Type Tomato Tissues during Development of the Pedicel Abscission Zone and the Inflorescence Meristem. Plant Cell **11,** 159-176.

Szymkowiak, E., and Irish, E. (2006). JOINTLESS suppresses sympodial identity in inflorescence meristems of tomato. Planta **223,** 646-658.

Takada, S., Hibara, K., Ishida, T., and Tasaka, M. (2001). The CUP-SHAPED COTYLEDON1 gene of Arabidopsis regulates shoot apical meristem formation. Development **128,** 1127-1135.

Thijs, G., Marchal, K., Lescot, M., Rombauts, S., De Moor, B., RouzÃ©, P., and Moreau, Y. (2002). A Gibbs Sampling Method to Detect Overrepresented Motifs in the Upstream Regions of Coexpressed Genes. Journal of Computational Biology **9,** 447-464.

Tomescu, A.M. (2006). Comment on "something on the side: axillary meristems and plant development." by Tom Bennett and Ottoline Leyser, Plant Molecular Biology 60 (2006) 843-854. Plant Mol Biol **62,** 481-482; author reply 483.

Tucker, D.J. (1977). The effects of far-red light on lateral bud outgrowth in decapitated tomato plants and the associated changes in the levels of auxin and abscisic acid. Plant Science Letters **8,** 339-344.

Tucker, D.J. (1977). Hormonal regulation of lateral bud outgrowth in the tomato. Plant Science Letters **8,** 105-111.

Waites, R., and Hudson, A. (2001). The Handlebars gene is required with Phantastica for dorsoventral asymmetry of organs and for stem cell activity in Antirrhinum. Development **128,** 1923-1931.

Wang, Y.H., and Li, J.Y. (2008). Molecular basis of plant architecture. Annual Review of Plant Biology **59,** 253-279.

White, C.A. (1901). VARIETAL MUTATION IN THE TOMATO. Science **14,** 841-844.

Yang, F. (2008). Identification and characterization of interactors of RAX1 controlling shoot branching in Arabidopsis thaliana. In Mathematisch-Naturwissenschaftliche Fakultät (Universität zu Köln).

Zhang, J.H., Chen, R.G., Xiao, J.H., Qian, C.J., Wang, T.T., Li, H.X., Bo, O.Y., and Ye, Z.B. (2007). A single-base deletion mutation in SlIAA9 gene causes tomato (Solanum lycopersicum) entire mutant. Journal of Plant Research **120,** 671-678.

Abstract

Aerial architecture of seed plants is determined by the development of repeating modules named phytomers. An important factor therein is the formation and fate of axillary meristems (AMs), influencing agronomically important traits, like inflorescence architecture and shoot branching. The present study in tomato represents a reverse genetics approach unveiling the function of genes homologous to the MYB and bHLH proteins, Blind and LAX, two key regulators of axillary meristem initiation.

Public databases and PCR technology enabled the identification of three closely related paralogs of *Blind*, *Blind-like1* (*Bli1*), *Bli2* and *Bli3*. TILLING of *Bli2* led to the identification of the classical tomato mutant *potato leaf* (*c*), a mutant described already more than a hundred years ago. *potato leaf* mutants display leaves with reduced leaflet formation and lack leaf lobing and serration. Nine *potato leaf* alleles were identified, all carrying a mutation in the *Bli2* gene. RNA *in-situ* hybridisation revealed a distinct expression pattern in leaf primordia prior to leaflet formation, at positions axillary of outgrowing leaflets and marking the sinuses of leaf lobes. Ectopic expression of *Blind* (*Bl*) partially complemented the defects of *c*, indicating a conserved function of the two proteins. Silencing of *Bli3* revealed that this gene probably acts in the same process as *C*, because RNAi plants displayed reduced leaf complexity. The protein similarity of Bl and C, the partial complementation of *c* by *Bl* and the phenotype of *Bli3* RNAi plants represent further elements in the rising evidence that leaf dissection and AM initiation employ homologous mechanisms.

Furthermore, silencing of *Bli1* and *Bli3* unveiled that these genes redundantly regulate AM initiation in vegetative and reproductive development. The defect in AM initiation along the shoot axis was complementary to the defect of *blind* mutants. RNA *in-situ* hybridisation showed major expression domains of *Blind* in the shoot apical meristem, at positions of presumptive leaf primordium formation and adaxially of leaf primordia. Additionally, *Blind* mRNA accumulated adaxially of new reproductive and vegetative meristems. Besides AM initiation, *Bl, Bli1 and Bli3* act in the prevention of concaulescent fusions of vegetative and reproductive side-shoots. Moreover, a series of observations disclosed that *Bl*, *Bli1 and Bli3* control the maintenance and reproductive identity of apical and axillary meristems.

In a second project, silencing of the bHLH transcription factor SlLax in tomato, led to the identification of the classical mutant, *uniflora*. *SlLax*/*Uniflora* encodes an ortholog of the AM initiation regulators, *LAX* (rice), *ba1* (maize) and *ROB* (*Arabidopsis*). *Uniflora* (*Uf*) was known to control inflorescence architecture and flowering time in a condition dependent manner. Detailed analysis of organogenesis in different *uniflora* mutant lines, unveiled that *Uf*

controls the initiation of reproductive axillary meristems and regulates reproductive meristem identity. RNA *in-situ* hybridisation indicated that *Uf* mRNA accumulates adaxially of newly initiated reproductive AMs, similar to the expression pattern of *Blind* in inflorescences. Heterodimers of bHLH and MYB proteins regulate several developmental processes. Previous studies showed that the Uf and Blind orthologous proteins ROB and RAX of *Arabidopsis* interact physically. The phenotypic similarities of *Uf* and *Bl*, *Bli1* and *Bli3* loss of function plants, and the overlapping expression patterns of *Uf* and *Bl* strongly suggest that also in tomato these MYB and bHLH proteins may act as heterodimers controlling AM initiation and meristem identity in reproductive development. Together, the observations in tomato, rice, maize and *Arabidopsis* propose that heterodimers of Blind and LAX orthologous MYB and bHLH proteins constitute a fundamental mechanism, controlling axillary meristem formation in flowering plants.

In summary, the present study unveiled the identity of two classical mutants and demonstrated that genes of the *Blind* family and *Uniflora* regulate four important features of aerial plant architecture in tomato: leaf compoundness, organ separation, axillary meristem initiation and the development of apical meristems.

Zusammenfassung

Die oberirdische Architektur von Samenpflanzen wird durch die Entwicklung von sich wiederholenden Modulen, sogenannten Phytomeren, bestimmt. Eine wichtige Rolle spielen hierbei die Bildung und das Schicksal von Achselmeristemen (AM), wodurch bedeutende agronomische Merkmale, wie Infloreszenzarchitektur und Sproßverzweigung beeinflusst werden. Mittels reverser Genetik wurde die Funktion von Genen in Tomaten untersucht, welche homolog zu dem MYB Gen, *Blind*, und dem bHLH Gen, *LAX*, sind, zwei Regulatoren der Initiation von Achselmeristemen.

Öffentliche Datenbanken und PCR Technologie ermöglichten die Identifizierung der drei *Blind* verwandten Gene, *Blind-like1* (*Bli1*), *Bli2* and *Bli3*. TILLING von *Bli2* führte zur Identifikation der klassischen Tomatenmutante *potato leaf* (*c*), die schon vor über einhundert Jahren beschrieben wurde. *potato leaf* entwickelt Blätter mit reduzierter Fiederanzahl, sowie reduzierter Kerbung und Zahnung der Blattränder. Insgesamt konnten neun *potato leaf* Allele identifiziert werden, die alle Mutationen in dem Gen *Bli2* aufzeigen. RNA *in-situ* Hybridisierungen ließen ein spezifisches Expressionsmuster für *C* in Blattprimordien erkennen: vor der Bildung von Blattfiederprimordien, an der Basis auswachsender Blattfiederprimordien sowie an Einbuchtungen der sich entwickelnden Blattränder. Ektopische Expression von *Blind* (*Bl*) komplementierte partiell die Defekte von *c*, was eine konservierte Funktion der beiden Gene vermuten lässt. RNAi Pflanzen, in denen die *Bli3*-Aktivität reduziert wurde, zeigten Blätter mit reduzierter Komplexität, was nahelegt, dass *Bli3* im selben Prozess wirkt wie *C*. Die Ähnlichkeit der Proteine *Bl* und *C*, die partielle Komplementation von *c* durch *Bl* und der Phänotyp von *Bli3*-RNAi-Pflanzen stellen weitere Argumente dar, dass die Entwicklung der Blattkomplexität und die Initiation von Achselmeristemen homologe Mechanismen benutzen.

Weiterhin zeigte die Stilllegung von *Bli1* und *Bli3*, dass diese Gene die Initiation von Achselmeristemen in der vegetativen und reproduktiven Entwicklung in redundanter Weise regulieren. Die Positionen der fehlenden Achselmeristeme entlang der Sprossachse in *Bli1/Bli3*-RNAi-Pflanzen und in *blind* Mutanten waren komplementär. *In-situ* Hybridisierungsexperimente zeigten Expression von *Blind* im Sprossapikalmeristem bei P0 und adaxial von Blattprimordien. Zusätzlich fand sich *Blind* mRNA adaxial von neugebildeten vegetativen und reproduktiven Achselmeristemen. Neben der Funktion in der Anlage von Achselmeristemen wirkten *Bl*, *Bli1* und *Bli3* auch in der Prävention konkauleszenter Fusionen von vegetativen und reproduktiven Seitentrieben. Darüber hinaus

kontrollierten *Bl*, *Bli1* and *Bli3* die Aufrechterhaltung und die reproduktive Identität von apikalen und axillären Meristemen.

In einem zweiten Projekt, führte ein Stilllegen des bHLH Transkriptionsfaktors SlLax in Tomaten zur Identifizierung der klassischen Mutante, *uniflora*. *SlLax*/*Uniflora* kodiert für ein Ortholog der AM-Initiationsregulatoren, *LAX* (Reis), *ba1* (Mais) und *ROB* (*Arabidopsis*). *Uniflora* (*Uf*) wurde als ein Regulator der Infloreszenzarchitektur und des Blühzeitpunkts in Tomate beschrieben. Detaillierte Analysen der Organogenese in verschiedenen *uniflora* Linien, enthüllten, dass *Uf* die Anlage der reproduktiven axillären Meristeme und die reproduktive Identität von Meristemen kontrolliert. RNA *in-situ* Hybridisierungen zeigten, dass *Uf* mRNA ähnlich wie *Blind* adaxial von neu initiierten reproduktiven Achselmeristemen akkumuliert. Heterodimere von bHLH und MYB Proteinen regulieren viele pflanzliche Entwicklungsprozesse. Die phänotypischen Ähnlichkeiten von Pflanzen ohne *Uf* oder *Bl, Bli1* und *Bli3* Genfunktion und die sich überschneidenden Expressionsmuster von *Uf* und *Bl* deuten darauf hin, dass auch in Tomaten diese MYB und bHLH Proteine die Anlage von Achselmeristemen und die reproduktive Identität von Meristemen als Heterodimere kontrollieren. Die Ähnlichkeiten der Gene und der mutanten Phänotypen in Tomate, Reis, Mais und *Arabidopsis* legen die Annahme nahe, dass dies einen grundlegenden Mechanismus in Samenpflanzen darstellt.

Die in dieser Studie charakterisierten Gene der *Blind*-Familie und *Uniflora* regulieren vier wichtige Aspekte der oberirdischen Pflanzenarchitektur von Tomaten: Blattkomplexität, Organtrennung, Achselmeristemanlage und die Entwicklung von apikalen Meristemen.

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Erklärung

Die vorliegende Arbeit wurde am Max-Planck-Institut für Züchtungsforschung in Köln-Vogelsang durchgeführt.

Ich versichere, dass ich die von mir vorgelegte Dissertation selbständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit - einschließlich Tabellen, Karten und Abbildungen -, die anderen Werken im Wortlaut oder dem Sinn nach entnommen sind, in jedem Einzelfall als Entlehnung kenntlich gemachthabe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; dass sie – abgesehen von unten angegebenen Teilpublikationen - noch nicht veröffentlicht worden ist sowie, dass ich eine solche Veröffentlichung vor Abschluss des Promotionsverfahrens nicht vornehmen werde. Die Bestimmungen dieser Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation ist von Professor Dr. Klaus Theres betreut worden.

Köln, 13.05.2009

Bernhard Busch