RESEARCH ARTICLE

Auxin transport inhibitor induced low complexity petiolated leaves and sessile leaf-like stipules and architectures of heritable leaf and stipule mutants in *Pisum sativum* suggest that its simple lobed stipules and compound leaf represent ancestral forms in angiosperms

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Abstract

In angiosperms, leaf and stipule architectures are inherited species-specific traits. Variation in leaf and stipule sizes, and forms result from the interaction between abiotic and biotic stimuli, and gene regulatory network(s) that underlie the leaf and stipule developmental programme(s). Here, correspondence between variation in leaf and stipule architectures described for extant angiosperms and that induced mutationally and by imposition of stress in model angiosperm species, especially in *Pisum sativum*, was detected. Following inferences were drawn from the observations. (i) Several leaf forms in *P. sativum* have origin in fusion of stipule and leaf primordia. Perfoliate (and amplexicaul and connate) simple sessile leaves and sessile adnate leaves are the result of such primordial fusions. Reversal of changes in the gene regulatory network responsible for fusion products are thought to restore original stipule and leaf conditions. (ii) Compound leaf formation in several different model plants, is a result of promotion of pathways for such condition by gene regulatory networks directed by *KNOX1* and *LEAFY* transcription factors or intercalation of the gene networks directed by them. (iii) Gene regulatory network for compound leaves in *P. sativum* when mutated generates highly complex compound leaves on one hand and simple leaves on other hand. These altered conditions are mutationally reversible. (vi) Simple leaves in model plants such as *Arabidopsis thaliana* despite overexpression of *KNOX1* orthologues do not become compound. (v) All forms of leaves, including simple leaf, probably have origins in a gene regulatory network of the kind present in *P. sativum*.

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Introduction

Leaves and stipules are determinate lateral organs of flowering plants (angiosperms), born orderly on their stem nodes. Only about one-third of the angiosperm species produce stipules or are stipulated (Lubbock 1891; Tyler 1897; Bell and Bryan 2008). Stipules are formed in pairs, one on either side of the leaf. Angiosperms demonstrate considerable diversity in size and architecture of leaves and stipules (Cronquist 1988; Nicotra *et al.* 2011). They may occur as simple or compound structures (Charlton 1998; Sambamurty 2005; Kumar *et al.* 2013). Simple leaves or subunits (pinnae) in compound leaves and stipules are generally bifacial. Species with unifacial type leaves and or stipules are rare (Sharma and Kumar 2012). Simple leaves are entire or variously lobed and compound leaves are palmately or pinnately dissected to different degrees (Geeta *et al.* 2012). Stipules are generally simple, of lobed architecture (Lubbock 1891; Bierhorst 1971). In species bearing compound stipules, the stipule architecture is similar to that of the associated leaf (Sharma *et al.* 2012a; Kumar *et al.* 2013). In most species, leaves are the

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principal photosynthetic organs. They intercept light, drive the plant hydraulics, exchange gases with atmosphere and dissipate heat (Nicotra *et al.* 2011). In a few species, stipules are the main organs to perform the above functions of leave (Sharma and Kumar 2012). Generally, stipules complement the leaf functions (Sharma *et al.* 2012b). Their common role is in the protection of apex and or differentiating coleaf, axillary bud and secondary inflorescences at the node of their occurrence (Ryder 1954; Eames 1961; Foster and Gifford 1974; Stein 1982; Jackson 1996). It is believed that the wide variation in leaf and stipule morphologies manifested in the extant angiosperm species is an outcome of the interactions between environment and species in the course of their evolution (Givnish 1987; Niklas 1994; Marks and Lechowicz 2006; Boyce *et al.* 2009; Efroni *et al.* 2010; Peppe *et al.* 2011; Geeta *et al.* 2012). Important questions in plant morphology include what is the nature of relationship between leaf and stipules (Rutishauser and Isler 2001), what was the ancestral leaf form (Geeta *et al.* 2012) and how did the extant variation in leaf and stipule morphologies originate from the ancestral condition (Floyd and Bowman 2006; Barkoulas *et al.* 2007; Blein *et al.* 2008; Nicotra *et al.* 2011).

Two views are prevalent about stipules *vis-a-vis* leaves. One is that stipules are a part of leaf (Colomb 1887; Sinnott and Bailey 1914; Parkin 1948) and the other is that stipules are independent organs (Rutishauser and Sattler 1986; Rutishauser and Dickison 1989; Rutishauser 1999; Rutishauser and Isler 2001; Kumar *et al.* 2009). Also there are two views about the ancestory of leaf forms. First, simple leaf was ancestral (Cronquist 1988; Doyle 2007; Geeta *et al.* 2012). Secondly, the ancestral form was compound leaf (Sinha 1997; Bharathan *et al.* 2002; Busch and Gleissberg 2003). The resolution of alternate possibilities is expected from the molecular genetic analysis of leaf and stipule variants in a variety of model plant systems (Townsley and Sinha 2012). Such analyses has been in progress in the simple leaved species *A. thaliana* (Byrne *et al.* 2000; Eshed *et al.* 2004; Hu *et al.* 2011), *Antirrhinum majus* (Waites and Hudson 1995), *Juncus prismatocarpus* (Yamaguchi and Tsukaya 2010), *Nicotiana tabacum* (Ahearn *et al.* 2001), *Oryza sativa* (Yan *et al.* 2008) and *Zea mays* (Candela *et al.* 2008; Xing *et al.* 2011), exstipulate compound leaved species *Cardamine hirsuta* (Hay and Tsiantis 2006), *Eschscholzia californica* (Busch and Gleissberg 2003; Bartholmes *et al.* 2012) and *Solanum lycopersicon* (Koenig *et al.* 2009), stipulate non-IRL (inverted repeat loss) leguminous species *Glycine max* (Champagne *et al.* 2007) and *Lotus japonicus* (Luo *et al.* 2005), and stipulate IRL leguminous species *Medicago truncatula* (Peng *et al.* 2011) and *Pisum sativum* (Hofer *et al.* 1997; Kumar *et al.* 2010). Here, answers to the two questions posed above have been searched in the variant leaf and stipule forms induced by ATI (auxin transport inhibitors) and present in mutants of known genotypes in the model plant *P. sativum*.

P. sativum serving as a useful model system for molecular genetical analysis of gene regulatory networks determines all lateral organs (Hofer *et al.* 1997; Wang et al. 2008; Kumar *et al.* 2011). It is the only system where both stipules and leaf are being investigated (Gourlay *et al.* 2000; Yaxley *et al.* 2001; Kumar *et al.* 2009; Sharma *et al.* 2012b). In *P. sativum* there is heteroblasty; the leaves and stipules of highest complexity are formed on the first flowering node and a few nodes immediately below and above it (Yaxley *et al.* 2001; Mishra *et al.* 2009; Kumar *et al.* 2009). The fully formed wild-type leaf comprises of a petiole which extends into rachis which has 15 pinnae on it: three pairs of leaflets on petiole proximal side, four pairs of tendrils on side distal to petiole and a terminal/apical tendril. Natural and/or induced alleles are known in six genes that determine leaf morphology. Both loss of function (*uni*) and hypomorphic (*uni-tac*; lesser in expression) alleles are known in *UNIFOLIATA* (*UNI*) gene and only loss-of-function alleles in *AFILA* (*AF*), *CRISPA* (*CRI*), *INSECATUS* (*INS*), *MULTIFOLIATE PINNA* (*MFP*), *STAMINA-PISTILLOIDA* (*STP*) and *TENDRIL-LESS* (*TL*) genes (de Vilmorin and Bateson 1911; White 1917; Eriksson 1929; Lamprecht 1933, 1959; Kujala 1953; Goldenberg 1965; Sharma 1972; Hofer *et al.* 2001; Taylor *et al.* 2001; Smirnova 2002; Kumar *et al.* 2004; Tattersall *et al.* 2005; Hofer *et al.* 2009). *UNI*, *CRI*, *STP* and *TL* are known to be transcription factors or coregulators of transcription factors; *UNI*, *CRI* and *STP* are respectively orthologues of *LEAFY*/*FLORICAULA*, *ASYMMETRIC LEAVES 1*/*PHANTASTICA* and *UNUSUAL FLORAL ORGANS*/*FIMBRIATA* (Hofer *et al.* 1997; Taylor *et al.* 2001; Tattersall *et al.* 2005; Hofer *et al.* 2009). Whereas *uni* (or *uni-tac*) and *stp* mutations reduce the leafblade complexity, *af*, *ins*, *mfp* and *tl* mutations increase leaf ramification (Taylor *et al.* 2001; DeMason 2005a; Mishra *et al.* 2009; Kumar *et al.* 2010). Table 1 lists phenotypes of 38 available *P. sativum* leaf mutant genotypes. Together they encompass large variation in leaf architecture varying from single leaflet and single tendril respectively in *uni* and *uni af* to more than 600 leaflets arranged in bi-pinnate, tri-pinnate and higher order-pinnate arrangements in *af mfp tl* triple mutant. Leaves produced on shoots grown *in vitro* are generally less complex than the leaves formed in *in vivo* shoots (Bai and DeMason 2006; Kumar *et al.* 2013). The *in vitro* shoots grown in the presence of auxins and gibbrellins have marginally more complex leaves than those present on untreated shoots (DeMason 2005b; DeMason and Chawla 2006; DeMason and Chetty 2011). The leaves formed in *in vitro* shoots treated with ATI (Scanlon 2003) produce leaves with lower complexity (Gould *et al.* 1991; DeMason and Chawla 2004a, b; DeMason 2005b). In such shoots, nodes barren of leaf or bearing single leafleted leaves have been observed. Wild-type stipules in *P. sativum* are of large size (foliaceous). They are mediolaterally asymmetrical. The petiole-side or inner side of stipule is smaller than the other side which bears a toothed lobe (Sharma *et al.* 2012b). The lobes of the stipule overlap to produce a peltate structure around the stem. Both sides of stipule have more than one primary vein, all of which have reticulate venation, thus

Table 1. Compound leaf phenotypes of wild-type, single *uni* (and *uni-tac*), *af*, *tl*, *mfp* and *ins* leaf mutants and various combinations of leaf mutants in *Pisum sativum*.

Serial no.	Genotype						Properties of adult plant leaves (in mutants, only the features different from wild-type are described)	Reference
	UNI	STP	AF	TL	MFP	INS		
1	+	+	+	+	+	+	Petiolated, petiole extended into rachis that has three domains, the domain proximal to petiole has three pairs of simple leaflets, that distal to petiole has three or four pairs of simple tendrils and the apical/terminal domain has a simple tendril. In total there are 15 pinnae on the leafblade	Mishra *et al.* (2009)[a]
2	− (Loss of function)	+	+	+	+	+	Decurrent simple, simple lobed or compound with two or three leaflets	As above
3	− (Loss of function)	+	−	+	+	+	Simple tendril	Unpublished observation
4	− (Loss of function)	+	+	−	+	+	Decurrent simple	As above
5	− (Hypomorphic)	+	+	−	+	+	Curtailed distal domain and leafleted terminal/apical domain	Mishra *et al.* (2009)[a]
6	+	−	+	+	+	+	Curtailed distal domain	As above
7	+	+	−	+	+	+	Compound tendril replaces each of proximal leaflets (entirely tendriller)	As above
8	+	+	+	−	+	+	Simple leaflet replaces each of distal tendril and terminal/apical tendril	As above
9	+	+	+	+	−	+	Each distal tendril is replaced by multifoliate leafblade like structures comprising of tendrilled leaflets	As above
10	+	+	+	+	+	−	Proximal most leaflets are apically incised and truncated midrib ectopically bears distal part of wild-type leafblade	Kumar *et al.* (2010)
11	− (Loss of function)	−	+	+	+	+	Decurrent simple	Taylor *et al.* (2001)
12	− (Hypomorphic)	−	+	+	+	+	Trifoliate, terminal leaflet lobed	As above
13	+	−	−	+	+	+	Simple tendrils in all domains	As above
14	+	−	+	−	+	+	Entirely leafleted with curtailed distal domain	As above
15	+	−	−	−	+	+	Proximal and distal leaflets replaced by compound rachis, the rachis branches bear tiny elliptic leaflets at their terminal positions; less complex than *af tl*	As above
16	− (Hypomorphic)	+	−	+	+	+	Proximal pinnae are compound tendrils and terminal tendril is replaced by leaflet; leaflets can occur at the terminal positions proximal compound tendrils	Mishra *et al.* (2009)[a]
17	− (Hypomorphic)	+	+	−	+	+	Entirely leafleted; distal domain curtailed	As above
18	− (Hypomorphic)	+	+	+	−	+	One or two pairs of pinnablades of tendrilled leaflets or asymmetrical tendrilled leaflets replace distal domain tendrils	As above
19	− (Hypomorphic)	+	+	+	+	−	*uni-tac* phenotype; *ins* adventitious blade formation on proximal most pinnae rare; occasional splitting of main rachis into two branches from distal domain onwards	Unpublished observations
20	+	+	−	−	+	+	Compound rachis replaces each pinna, the rachis branches bear tiny elliptic leaflets at their apical positions; bipinnate to tripinnate configuration is mimicked	Mishra *et al.* (2009)[a]
21	+	+	−	+	−	+	Compound rachis replaces each pinna, the rachis branches bear tiny tendrilled leaflets at their apical positions; bipinnate to tripinnate configuration is mimicked	As above
22	+	+	−	+	+	+	Only the *af* phenotype is seen on proximal pinnae; occasionally rachis is seen split into two distal domain onwards producing branched leafblade	Kumar *et al.* (2010)[a]

Table 1 (*contd.*)

Serial no.	Genotype						Properties of adult plant leaves (in mutants, only the features different from wild-type are described)	Reference
	UNI	STP	AF	TL	MFP	INS		
23	+	+	+	−	−	+	Each tendril of distal domain is replaced by multifoliate leafblades; each of such leafblade comprises of small leaflets in region proximal to leaf rachis and tendrilled leaflets in region distal to rachis	Mishra *et al.* (2009)[a]
24	+	+	+	−	+	−	Proximal most leaflets are incised; the intercalary truncated midrib bears a tiny leafblade comprising of leaflets	Kumar *et al.* (2010)[a]
25	+	+	+	+	−	−	Adventitious/ectopic blade borne by proximal leaflets has the morphology of distal-cum-terminal domain of the main leafblades	As above
26	+	+	−	−	−	+	All pinnae are replaced by compound rachis, more ramified than in *af tl*, the rachis branches bear tiny lanceolate leaflets at their ends; tripinnate to higher order configuration is mimicked	Mishra *et al.* (2009)[a]
27	+	+	+	−	−	−	Proximal domain is enlarged, distal-cum-terminal domain is split and proximal most leaflets produce adventitious blade like the distal-cum-terminal domain of *af tl mfp*	Kumar *et al.* (2010)[a]
28	+	+	−	+	−	−	Only the *af mfp* phenotype is seen; occasional splitting of main leafblade axis is seen beyond proximal domain	As above; unpublished observations
29	+	+	−	−	−	−	Only the *at tl mfp* phenotype is seen; splitting of main leaf rachis beyond proximal domain is seen in some leaves	As above
30	− (Hypomorphic)	+	−	−	+	+	Small leaflet bearing compound blades replace proximal leaflets and distal and terminal leaflets are small and simple; leafblade mimicks bipinnate-cum-unipinnate morphology	Mishra *et al.* (2009)[a]
31	− (Hypomorphic)	+	−	+	−	+	Proximal and distal pinnae are replaced by compound blades bearing symmetrical tendrilled leaflets; simple leaflets occupy terminal positions in the main and secondary rachis. Leafblade has bipinnate-cum-unipinnate configuration	As above
32	− (Hypomorphic)	+	−	+	+	−	*uni-tac af* phenotype except that the leaflets formed on proximal most compound blades were inflected/bilobed	Kumar *et al.* (2010)[a]
33	− (Hypomorphic)	+	+	−	−	+	Distal domain consists of asymmetrical lanceolate leaflets whereas terminal position has leaflet	Mishra *et al.* (2009)[a]
34	− (Hypomorphic)	+	+	−	+	−	*ins* phenotype less frequent than in *tl ins*	Kumar *et al.* (2010)[a]
35	− (Hypomorphic)	+	+	+	−	−	*ins* phenotype less frequent than in *mfp ins*	As above
36	− (Hypomorphic)	+	−	−	−	+	Proximal and distal pinnae are compound and bear small leaflets, asymmetrical leaflets accompany small leaflets at distal positions of main rachis and secondary rachis of proximal pinnae; leafblaed has bipinnate morphology	Mishra *et al.* (2009)[a]
37	− (Hypomorphic)	+	+	−	−	−	*ins* phenotype is not seen; splitting of main leaf rachis beyond proximal domain is seen in some leaves	Kumar *et al.* (2010)[a]
38	− (Hypomorphic)	+	−	+	−	−	Adventitious blade seen as tendrilled leaflet at low frequency	As above
39	− (Hypomorphic)	+	−	−	−	−	*uni-tac af tl mfp* phenotype is seen, *ins* phenotype is not seen	As above

[a]All the relevant previous references are cited in these papers.

stipules have improved hydraulics (Sharma *et al.* 2012b). Loss-of-function mutation in three different genes is known to affect stipule morphology; in the mutants of two of these genes plants are fertile, plants of third mutant are infertile. Mutations in *STIPULE-REDUCED* (*ST*) gene make the stipules narrower (Pellew and Sverdrup 1923). *st* stipules have knife-blade-like morphology and do not overlap with each other or are free (Kumar *et al.* 2009). *COCHLEATA* (*COCH*) mutants (*coch*) bear stipules that have wild-type leaf-like morphology (Blixt 1967, 1972). *coch* stipule morphology depends on the allelic structure of leaf morphology genes for example in *af coch*, *tl coch*, *mfp coch*, *af tl coch* and *af tl mfp coch* mutants the stipules respectively have *af*-, *tl*-, *mfp*-, *af tl*- and *af tl mfp*- leaf-like morphologies (Yaxley *et al.* 2001; Kumar *et al.* 2009). The *uni-tac coch* mutants have simple leaflet-like stipules (Kumar *et al.* 2009). Stipules are generally absent in nodes of *coch st* double mutants (Blixt 1967; Marx 1987; Gourlay *et al.* 2000; Yaxley *et al.* 2001; Kumar *et al.* 2009). In *coch* plants one or both stipules may be absent from occasional nodes and on the same node one stipule may be simple and the other leaf-like compound stipule (Kumar *et al.* 2009). In *cist* (*circular stipule*) mutant, perfoliate structure was present at each node which were barren of leaves. The mature *cist* node produce secondary inflorescences that bore sterile flowers (Kumar and Sharma 1975). *In vitro* grown wild-type shoots produce normal stipules (DeMason and Chawla 2004a). 1-N-naphthylpthalmic acid (NPA), an ATI, treated wild-type shoots formed all the different kinds of known laminated stipules–adnate, intrapetiolar, interpetiolar, opposite and ochreate stipules (Kumar *et al.* 2013).

In view of the observations that in *P. sativum* auxin ATI treated shoots produced phenocopies of mutant leaves of lower complexity and stipules of all the known types in angiosperms, it was desired to experimentally examine if more varied leaf and stipule types could be recovered in auxin and ATI treated shoots. In the experiments reported here novel leaf and stipule types were indeed recovered. These observations together with existing information about gene regulatory network for leaf and stipule morphogenesis and range of leaf and stipule morphologies in heritable mutants in *P. sativum* and lessons from other model systems have been discussed here to seek answers about ancestral type of leaf and relationship between stipules and leaf.

Materials and methods

Plant material

The *P. sativum* lines of the genotypes *COCH ST UNI AF TL*, and *COCH ST uni-tac AF TL* (*uni-tac* mutant), *COCH ST UNI af TL* (or *af* mutant) *COCH ST UNI AF tl* (or *tl* mutant), and *coch st UNI AF TL* (or *coch st* double mutant) used here were in the background of Skp1 (Prajapati and Kumar 2002). The construction of genotypes in a constant genetic background has been described earlier (Mishra *et al.* 2009; Kumar *et al.* 2009). The *coch*, *st*, *af* and *tl* mutant alleles were from Blixt collection (Blixt 1972). The line *Cicer arietinum* cv. Radhey was obtained from the National Bureau of Plant Genetic Resources, New Delhi. *Lathyrus aphaca* and *L. sativus* accessions were of wild origin collected from the NIPGR campus. All genotypes used here are being maintained by growing them in the experimental field at NIPGR every winter season (November–March/April). Culture of each genotype was started with a single seed. Subculturing was done with single-node explants. The explants formed were multiplied by subculturing.

Tissue culture technique

MS medium containing Gamborg vitamins, 11 μM 6-benzylaminopurine (both from Sigma-Aldrich, Berlin, Germany), 3% sucrose and 0.8% agar (both from Hi-Media Laboratories, Mumbai, India) was used throughout. Any supplements were added to autoclaved medium cooled to 75°C before dispensing it to the sterilized culture jars. Cultures were raised in 375 mL glass bottles (Allied Scientific Sales, New Delhi, India) which accommodated up to 15 explants on 70 mL medium. The explants were grown at 25°C and exposed to white light at the rate of 3000 Lux for 16 h and to darkness for 8 h at 25°C. Under these conditions, explants on basal medium produced more than 10 nodes in five weeks times. The supplements used were NPA and gibberellic acid (GA_3). Their stock solutions were prepared at 100 mM in dimethyl sulphoxide (DMSO) (Qualigens, Mumbai). The concentration of NPA and GA were 40 μM. Each treatment was repeated six times.

Recording of observations

The control, NPA and GA treated cultures were phenotyped respectively after six weeks and four weeks of incubation (began to dry later). Each shoot was individually observed and photographed, using Nikon COOLPIX L24 digital camera (14 megapixels) and/or by using Nikon Digital Sight DS-Ri1 camera (Tokyo, Japan) at 0.5× magnification in AZ-100 Nikon multi objectives stereozoom microscope. Each shoot was measured for its length and number of nodes borne on it. The leaves born on second node onwards were characterized for the number of pinnae, structure of each of pinna and sizes of petiole and rachis. The sizes of leafblade and its components were measured either manually or by using Nikon Digital Sight DS-Ri1 camera at 0.5× magnification in AZ-100 Nikon multi objectives stereozoom microscope. The leafblades and leaflets were weighed on TE64 (Sartorius, New Delhi, India) to determined their fresh weights. For each treatment per genotype atleast 60 leaves formed on 20 shoots were examined.

Gene expression studies

Transcript levels of *UNI* and *TL* genes whose sequences are known were studied. The primer sequences are given

Table 2. Primer sequences for the semi-quantitive PCR (RT-PCR) assays.

Gene	Forward primer	Reverse primer
UNIFOLIATA (*UNI*)	CTACGCGGTTACCCCTACAA	ATTTCTCACCGCGCTCTTTA
ACTIN-9	ATGGTTGGAATGGGACAAAA	GCAGTTTCCAACTCCTGCTC

UNI, GenBank accession no. AF0101902; *ACTIN-9*, GenBank accession no. U81047.

Table 3. Primer sequences for the quantitative real time PCR (qRT-PCR) assays.

Genes	Forward primer	Reverse primer
UNIFOLIATA (*UNI*)	CAACCGCCCCGATG	CCTCCAAGCCTCCTAGTTCTCTT
TENDRIL-LESS (*TL*)	GTTCCTCCTCCAGGTTCTTCTTA	GCTTCACTTCGCTTCAATTCC
ACTIN-9	TTGTAGCACCACCAGAGAGGAA	TTGCAATCCACATCTGTTGGA

TENDRIL-LESS, GenBank accession no. EU938524.

in the table 1. The apices for measuring transcript levels were resourced from 14 days old *in vitro* grown shoots. Several apices per genotype per treatment were pooled and frozen in liquid nitrogen and stored at −70°C as a sample. From each sample total RNA was extracted by using the RNeasy Plant Mini kit (Qiagen, Hilden, Germany). The RNA was quantified by Nanodrop-1000 (Wilmington, USA) spectrophotometer and its integrity was checked by resolving on 1.5% agarose gel made in 10× MOPS buffer with formaldehyde. First-strand cDNAs were generated by using the oligo (dT8) primer of the Revert Aid H Minus first strand cDNA synthesis kit (Fermentas, Massachusetts, USA) according to manufacturer's instructions. Semi-quantitative PCR (RT-PCR) was performed by using 400 ng first strand cDNA and gene primers, enzyme and dNTP mix at 0.4 μM, 0.05 units/μL and 0.2 mM concentration, respectively in 25 μL final volume.

The temperature profile for RT-PCR was: one 3 min cycle of initial denaturation at 95°C, 35 cycles of 30 s at 94°C, annealing at 59°C for 30 s, extension at 72°C for 1 min, and final extension at 72°C for 10 min and final hold at 4°C for infinity. PCR product was separated on 0.8% agarose gel in 1× TBE buffer containing 0.5 μL/mL ethidium bromide and quantified in 1-D-analysis tool by using Vision Works Image Acquisition and Analysis software in GelDoc-it imaging system (UVP, Los Angeles, USA).

Real time PCR (qRT-PCR) assays were performed on 20 ng cDNA on Step one Real Time PCR Detection System (Life Technologies, formerly Applied Biosystem, Carlsbad, USA) according to manufacturer's instructions. The relative expression levels of *UNI* and *TL* were compared by calculating the relative quantity values (RQ) by using comparative C_t method also referred to as the $2_T^{-\Delta\Delta C}$ method. The pea *ACTIN9* gene was used as the internal control. Two biological and three technical replicates were used for qRT-PCR. The primers used for the studied genes for RT-PCR and qRT-PCR are given in tables 2 and 3.

Results

Morphological studies

Exogenous auxin treatments: To study the effects of auxin supplementation on shoot morphology, the explants of wild type and *uni-tac*, *af* and *tl* mutants were grown on the medium containing 0.5, 1.5 or 4.5 μM concentrations of each of three auxins. The auxins individually added to the medium were indole-3-acetic acid (IAA), ∝-napthaleneacetic aid (NAA) and methyl indole-3-acetic-acid (m-IAA). The auxin-wise results are presented in tables 4–6. In all genotypes, the exogenous treatments with IAA and NAA affected only marginally the shoot size and the number of stipule pairs and leaves born (nodes) on shoot only marginally. Treatment with m-IAA on wild-type, *af* and *tl* explants led to increase in the numbers of pinnae and rachis, and petiole length in leaves together with reduction in shoot length and number of leaves. Increase in pinnae number was not observed in m-IAA treated *uni-tac* shoots. The genotypewise structures of the leaves, in the wild type and mutants, remained morphologically unaffected by the exogenous application of auxin. Also, the stipule structures did not demonstrate any significant changes on auxin supplementation. These results indicated that the shoots of all four genotypes were not deficient in inborn auxin.

Auxin transport inhibitor treatments: The explants of wild type and *uni-tac*, *af* and *tl* mutants were grown in presence of different concentrations of four ATIs of different molecular mechanisms. The ATIs used were NPA, P-chlorophenoxyisobutyric acid (PCIB) and l-napthoxyacetic acid (NOA) at 20, 40 and 80 μM concentration and 2,3,5-triiodobenzoic acid (TIBA) at 10, 20 and 40 μM concentration. The effects of NPA, TIBA, PCIB and NOA on nine quantitative parameters of shoot morphology are shown respectively in tables 7, 8, 9 and 10 and figure 1. In comparison with other ATIs, TIBA showed strongest effects

Table 4. Effect of the auxin indole-3-acetic acid (IAA) on the growth of shoots grown *in vitro* from explants of wild-type, *tl*, *uni-tac* and *af* genotypes and on morphology of leaves formed on the second and subsequent nodes of the shoots.

			Leaf						
Genotype	Treatment*	Shoot length	Number	Petiole length (cm)	Rachis length (cm)	Blade length (cm)	Leaflet number	Tendril number	Pinna (leaflet + tendril) number
A	B	C	D	E	F	G = E + F	H	I	J = H + I
UNI-TAC AF TL	Control	6.521 ± 0.432[a]	11.236 ± 0.723[a]	0.363 ± 0.024[a]	0.214 ± 0.024[a]	0.568 ± 0.042[a]	2.00 ± 0.00[a]	2.620 ± 0.100[a]	4.620 ± 0.070[a]
	0.5 μM IAA	7.312 ± 0.92[a]	11.832 ± 0.614[b]	0.394 ± 0.034[b]	0.224 ± 0.053[b]	0.612 ± 0.041[b]	2.018 ± 0.024[a]	2.616 ± 0.232[a]	4.636 ± 0.122[a]
	1.5 μM IAA	7.601 ± 0.563[b]	11.124 ± 0.764[b]	0.393 ± 0.024[a]	0.249 ± 0.018[a]	0.642 ± 0.024[b]	2.00 ± 0.00[a]	2.78 ± 0.047[b]	4.673 ± 0.076[b]
	4.5 μM IAA	6.021 ± 0.421[b]	11.046 ± 1.124[a]	0.40 ± 0.062[a]	0.252 ± 0.022[b]	0.623 ± 0.064[b]	2.00 ± 0.00[a]	2.644 ± 0.124[a]	4.62 ± 0.01[a]
UNI-TAC AF tl	Control	7.462 ± 0.245[a]	7.00 ± 0.087[a]	0.637 ± 0.046[a]	0.172 ± 0.018[b]	0.768 ± 0.177[a]	3.929 ± 0.13[a]	0[a]	3.929 ± 0.13[a]
	0.5 μM IAA	6.682 ± 0.324[b]	6.802 ± 0.048[b]	0.612 ± 0.057[b]	0.202 ± 0.038[a]	0.813 ± 0.124[a]	3.957 ± 0.113[a]	0[a]	3.957 ± 0.113[a]
	1.5 μM IAA	7.134 ± 0.276[b]	7.102 ± 0.059[b]	0.653 ± 0.042[b]	0.221 ± 0.021[a]	0.868 ± 0.137[b]	3.887 ± 0.156[b]	0[a]	3.887 ± 0.156[b]
	4.5 μM IAA	6.465 ± 0.237[b]	5.903 ± 0.046[b]	0.563 ± 0.084[a]	0.148 ± 0.009[b]	0.714 ± 0.158[b]	3.947 ± 0.139[b]	0[a]	3.947 ± 0.139[b]
uni-tac AF TL	Control	5.775 ± 0.086[a]	8.79 ± 0.243[a]	0.312 ± 0.017[a]	0[a]	0.312 ± 0.017[a]	3.015 ± 0.032[a]	0[a]	3.015 ± 0.032[a]
	0.5 μM IAA	5.902 ± 0.135[b]	9.247 ± 0.656[a]	0.286 ± 0.019[b]	0[a]	0.286 ± 0.019[b]	3.032 ± 0.019[a]	0[b]	3.032 ± 0.019[b]
	1.5 μM IAA	5.578 ± 0.376[a]	9.012 ± 0.417[a]	0.283 ± 0.018[b]	0[a]	0.283 ± 0.018[b]	3.000 ± 0.000[a]	0[b]	3.000 ± 0.000[b]
	4.5 μM IAA	5.467 ± 0.214[b]	8.47 ± 0.057[b]	0.314 ± 0.016[b]	0[a]	0.314 ± 0.014[b]	3.000 ± 0.000[a]	0[b]	3.000 ± 0.000[b]
UNI-TAC af TL	Control	8.798 ± 0.413[a]	9.754 ± 0.359[a]	0.787 ± 0.012[a]	0.379 ± 0.018[a]	1.166 ± 0.063[a]	0[a]	9.528 ± 0.231[a]	9.528 ± 0.231[a]
	0.5 μM IAA	8.767 ± 0.415[b]	9.121 ± 0.228[b]	0.771 ± 0.038[a]	0.368 ± 0.047[a]	1.139 ± 0.059[b]	0[a]	9.454 ± 0.269[a]	9.454 ± 0.269[a]
	1.5 μM IAA	8.634 ± 0.323[b]	9.758 ± 0.357[b]	0.778 ± 0.039[a]	0.387 ± 0.019[b]	1.165 ± 0.095[a]	0[a]	9.582 ± 0.264[a]	9.582 ± 0.264[a]
	4.5 μM IAA	8.147 ± 0.268[b]	9.124 ± 0.268[a]	0.762 ± 0.042[a]	0.404 ± 0.024[b]	1.156 ± 0.072[a]	0[a]	9.768 ± 0.087[b]	9.768 ± 0.187[b]

[a,b]For a genotype and parameter, the values of treatment effects that carry the same letter as superscript are not different from the respective control.
The parameter values in columns C to J are based on measurements made on 50–75 shoots.

Table 5. Effect of the auxin α-napthaleneacetic acid (NAA) on the growth of shoots grown *in vitro* from explants of wild-type, *tl*, *uni-tac* and *af* genotypes and on morphology of leaves formed on the second and subsequent nodes of the shoots.

			Leaf						
Genotype	Treatment*	Shoot length	Number	Petiole length (cm)	Rachis length (cm)	Blade length (cm)	Leaflet number	Tendril number	Pinna (leaflet + tendril) number
A	B	C	D	E	F	G = E + F	H	I	J = H + I
UNI-TAC AF TL	Control	6.521 ± 0.432^{a}	11.236 ± 0.723^{a}	0.363 ± 0.024^{a}	0.214 ± 0.024^{a}	0.568 ± 0.042^{a}	2.00 ± 0.00^{a}	2.620 ± 0.106^{a}	4.620 ± 0.071^{a}
	0.5 μM NAA	6.414 ± 0.437^{b}	10.367 ± 0.895^{b}	0.378 ± 0.025^{b}	0.206 ± 0.012^{b}	0.584 ± 0.046^{b}	2.000 ± 0.00^{a}	2.380 ± 0.108^{b}	4.380 ± 0.124^{b}
	1.5 μM NAA	6.174 ± 0.157^{a}	11.342 ± 0.123^{a}	0.399 ± 0.028^{b}	0.244 ± 0.009^{a}	0.643 ± 0.038^{b}	2.00 ± 0.00^{a}	2.560 ± 0.101^{a}	4.500 ± 0.118^{b}
	4.5 μM NAA	5.847 ± 0.143^{b}	9.684 ± 0.473^{b}	0.416 ± 0.016^{a}	0.212 ± 0.014^{b}	0.628 ± 0.022^{b}	2.00 ± 0.00^{a}	2.644 ± 0.103^{b}	4.56 ± 0.084^{a}
UNI-TAC AF tl	Control	7.462 ± 0.245^{a}	7.000 ± 0.087^{a}	0.637 ± 0.046^{a}	0.172 ± 0.018^{a}	0.768 ± 0.177^{a}	3.929 ± 0.130^{b}	0^{a}	3.929 ± 0.130^{a}
	0.5 μM NAA	6.385 ± 0.183^{b}	6.173 ± 0.063^{b}	0.617 ± 0.022^{a}	0.196 ± 0.047^{a}	0.813 ± 0.097^{a}	3.124 ± 0.167^{b}	0^{a}	3.124 ± 0.167^{b}
	1.5 μM NAA	5.973 ± 0.137^{b}	6.385 ± 0.047^{b}	0.583 ± 0.033^{a}	0.214 ± 0.034^{b}	0.797 ± 0.125^{a}	3.135 ± 0.215^{b}	0^{a}	3.135 ± 0.215^{b}
	4.5 μM NAA	7.126 ± 0.286^{b}	6.483 ± 0.067^{b}	0.601 ± 0.055^{b}	0.137 ± 0.058^{a}	$0.738 \pm 0.0.115^{a}$	3.428 ± 0.246^{b}	0^{a}	3.428 ± 0.246^{b}
uni-tac AF TL	Control	5.775 ± 0.086^{a}	8.79 ± 0.243^{a}	0.312 ± 0.017^{a}	0.016 ± 0.016^{a}	0.328 ± 0.017^{a}	3.015 ± 0.032^{a}	0.049 ± 0.012^{a}	3.065 ± 0.017^{a}
	0.5 μM NAA	5.638 ± 0.048^{b}	9.264 ± 0.268^{b}	0.306 ± 0.012^{a}	0^{a}	0.306 ± 0.012^{b}	3.000 ± 0.000^{a}	0^{b}	3.000 ± 0.000^{b}
	1.5 μM NAA	5.284 ± 0.069^{b}	8.326 ± 0.169^{b}	0.289 ± 0.032^{a}	0^{a}	0.289 ± 0.032^{b}	3.000 ± 0.000^{a}	0^{b}	3.000 ± 0.000^{b}
	4.5 μM NAA	5.125 ± 0.085^{b}	7.125 ± 0.133^{b}	0.242 ± 0.045^{b}	0^{a}	0.242 ± 0.045^{b}	3.000 ± 0.000^{a}	0^{b}	3.000 ± 0.000^{b}
UNI-TAC af TL	Control	8.798 ± 0.413^{a}	9.754 ± 0.359^{a}	0.787 ± 0.012^{a}	0.379 ± 0.018^{a}	1.166 ± 0.063^{a}	0^{a}	9.528 ± 0.231^{a}	9.528 ± 0.231^{a}
	0.5 μM NAA	8.274 ± 0.248^{b}	10.254 ± 0.275^{b}	0.749 ± 0.018^{b}	0.367 ± 0.022^{b}	1.116 ± 0.054^{a}	0^{a}	9.947 ± 0.146^{b}	9.545 ± 0.146^{a}
	1.5 μM NAA	8.034 ± 0.241^{b}	9.953 ± 0.242^{a}	0.716 ± 0.042^{b}	0.475 ± 0.016^{b}	1.191 ± 0.039^{a}	0^{a}	10.133 ± 0.129^{b}	9.764 ± 0.129^{a}
	4.5 μM NAA	7.657 ± 0.375^{b}	8.573 ± 0.187^{b}	0.703 ± 0.015^{b}	0.289 ± 0.026^{b}	0.992 ± 0.046^{b}	0^{a}	9.847 ± 0.115^{b}	9.535 ± 0.115^{a}

[a,b]For a genotype and parameter, the values of treatment effects that carry the same letter as superscript are not different from the respective control.
The parameter values in columns C to J are based on measurements made on 50–75 shoots.

Table 6. Effect of the auxin methyl indole-3-acetic acid (m-IAA) on the growth of shoots grown *in vitro* from explants of wild-type, *tl*, *uni-tac* and *af* genotypes and on morphology of leaves formed on the second and subsequent nodes of the shoots.

			Leaf						
Genotype	Treatment*	Shoot length	Number	Petiole length (cm)	Rachis length (cm)	Blade length (cm)	Leaflet number	Tendril number	Pinna (leaflet + tendril) number
A	B	C	D	E	F	G = E + F	H	I	J = H + I
UNI-TAC AF TL	Control	6.521 ± 0.432^{a}	11.236 ± 0.723^{a}	0.363 ± 0.024^{a}	0.214 ± 0.024^{a}	0.568 ± 0.042^{a}	2.00 ± 0.00^{a}	2.620 ± 0.106^{a}	4.620 ± 0.071^{a}
	0.5 μM m-IAA	8.223 ± 0.071^{b}	9.832 ± 0.067^{b}	0.387 ± 0.056^{b}	0.238 ± 0.028^{a}	0.637 ± 0.087^{a}	2.00 ± 0.000^{a}	2.656 ± 0.084^{b}	4.656 ± 0.057^{b}
	1.5 μM m-IAA	7.374 ± 0.057^{b}	9.657 ± 0.048^{b}	0.487 ± 0.068^{a}	0.343 ± 0.048^{a}	0.957 ± 0.057^{b}	2.00 ± 0.00^{a}	3.357 ± 0.047^{a}	5.286 ± 0.190^{b}
	4.5 μM m-IAA	6.021 ± 0.421^{b}	11.046 ± 1.124^{a}	0.40 ± 0.062^{b}	0.252 ± 0.022^{a}	0.726 ± 0.064^{b}	2.00 ± 0.00^{b}	2.644 ± 0.124^{b}	4.62 ± 0.01^{b}
UNI-TAC AF tl	Control	7.462 ± 0.245^{a}	7.00 ± 0.087^{a}	0.637 ± 0.046^{a}	0.172 ± 0.018^{a}	0.768 ± 0.177^{a}	3.929 ± 0.130^{a}	0^{a}	3.929 ± 0.130^{a}
	0.5 μM m-IAA	6.942 ± 0.227^{b}	6.802 ± 0.048^{b}	0.753 ± 0.016^{b}	0.243 ± 0.068^{b}	$0.996 \pm 0.0.113^{b}$	3.867 ± 0.104^{b}	0^{a}	3.867 ± 0.104^{b}
	1.5 μM m-IAA	6.768 ± 0.266^{b}	7.102 ± 0.059^{b}	0.883 ± 0.026^{a}	0.352 ± 0.041^{b}	1.235 ± 0.237^{b}	3.964 ± 0.126^{b}	0^{a}	3.964 ± 0.126^{b}
	4.5 μM m-IAA	5.357 ± 0.325^{b}	4.538 ± 0.352^{b}	0.658 ± 0.079^{b}	0.238 ± 0.094^{a}	0.896 ± 0.076^{b}	3.958 ± 0.134^{b}	0^{a}	3.958 ± 0.134^{b}
uni-tac AF TL	Control	5.775 ± 0.086^{a}	8.79 ± 0.243^{a}	0.312 ± 0.017^{a}	0.016 ± 0.016^{a}	0.328 ± 0.017^{a}	3.015 ± 0.032^{a}	0.049 ± 0.012^{a}	3.065 ± 0.017^{a}
	0.5 μM m-IAA	5.850 ± 0.177^{a}	7.750 ± 0.310^{b}	0.330 ± 0.023^{b}	0^{a}	0.330 ± 0.023^{a}	3.000 ± 0.000^{a}	0^{a}	3.000 ± 0.000^{b}
	1.5 μM m-IAA	6.500 ± 0.366^{b}	7.625 ± 0.323^{b}	0.338 ± 0.018^{b}	0^{a}	0.340 ± 0.023^{b}	3.000 ± 0.000^{a}	0^{a}	3.000 ± 0.000^{b}
	4.5 μM m-IAA	5.075 ± 0.169^{b}	6.875 ± 0.037^{b}	0.340 ± 0.022^{a}	0^{a}	0.340 ± 0.022^{b}	3.000 ± 0.000^{a}	0^{a}	3.000 ± 0.000^{a}
UNI-TA af TL	Control	8.798 ± 0.413^{a}	9.754 ± 0.359^{a}	0.787 ± 0.012^{a}	0.379 ± 0.018^{a}	1.166 ± 0.063^{a}	0^{a}	9.528 ± 0.231^{a}	9.528 ± 0.231^{a}
	0.5 μM m-IAA	6.753 ± 0.335^{b}	7.435 ± 0.328^{b}	0.790 ± 0.043^{a}	0.400 ± 0.028^{b}	1.140 ± 0.058^{b}	0^{a}	9.760 ± 0.272^{b}	9.760 ± 0.272^{b}
	1.5 μM m-IAA	5.879 ± 0.157^{b}	6.858 ± 0.101^{b}	0.928 ± 0.053^{b}	0.493 ± 0.032^{b}	1.604 ± 0.074^{b}	0^{a}	9.852 ± 0.244^{b}	9.952 ± 0.214^{b}
	4.5 μM m-IAA	4.564 ± 0.278^{b}	5.848 ± 0.347^{b}	0.858 ± 0.051^{a}	0.476 ± 0.031^{b}	1.314 ± 0.081^{b}	0^{a}	9.674 ± 0.252^{b}	9.674 ± 0.252^{b}

[a,b]For a genotype and parameter, the values of treatment effects that carry the same letter as superscript are not different from the respective control.
The parameter values in columns C to J are based on measurements made on 50–75 shoots.

Table 7. Effect of the auxin transport inhibitor 1-N-naphthylpthalamic acid (NPA) on the growth of shoots grown *in vitro* from explants of wild-type, *tl*, *uni-tac* and *af* genotypes and on morphology of leaves formed on the second and subsequent nodes of the shoots.

			Leaf							
Genotype	Treatment*	Shoot length (cm)	Number	Petiole length (cm)	Rachis length (cm)	Blade length (cm)	Leaflet number	Tendril number	Pinna (leaflet + tendril) number	Frequency of shoot apex conversion into leaf
			D	E	F	G = E + F	H	I	J = H + I	K
A	B	C	Figure 1c, g and p	Figure 1c, g and o	Figure 1, c, h and o	Figure 1d, i and p	Figure 1c, g and o	Figure 1c, o and q	Figure 1c, g, k and p	Figure 1e, i and s
UNI-TAC AF TL	Control	6.052 ± 0.431^{a}	$13.00 \pm f0.721^{a}$	0.356 ± 0.024^{a}	0.211 ± 0.024^{a}	0.565 ± 0.042^{a}	2.00 ± 0.000^{a}	2.613 ± 0.106^{a}	4.615 ± 0.031^{a}	0^{a}
	20 μM NPA	5.833 ± 0.045^{a}	9.66 ± 0.146^{b}	0.255 ± 0.023^{b}	0.112 ± 0.010^{b}	0.346 ± 0.031^{b}	1.965 ± 0.135^{a}	1.613 ± 0.221^{b}	3.578 ± 0.022^{b}	0.072 ± 0.035^{b}
	40 μM NPA	5.011 ± 0.031^{b}	8.101 ± 0.102^{b}	0.185 ± 0.018^{b}	0.033 ± 0.011^{b}	0.255 ± 0.032^{b}	1.702 ± 0.232^{a}	0.903 ± 0.122^{b}	2.605 ± 0.231^{b}	0^{a}
	80 μM NPA	3.952 ± 0.075^{b}	6.000 ± 0.059^{b}	0.146 ± 0.013^{a}	0.112 ± 0.005^{b}	0.151 ± 0.010^{b}	1.944 ± 0.0140^{a}	0.315 ± 0.052^{b}	2.259 ± 0.176^{b}	0.081 ± 0.032^{b}
UNI-TAC AF tl	Control	7.502 ± 0.252^{a}	7.025 ± 0.095^{a}	0.642 ± 0.052^{a}	0.174 ± 0.024^{a}	0.824 ± 0.182^{a}	3.934 ± 0.134^{a}	0^{a}	3.934 ± 0.135^{a}	0^{a}
	20 μM NPA	6.524 ± 0.294^{b}	5.723 ± 0.152^{b}	0.346 ± 0.026^{b}	0.035 ± 0.014^{b}	0.373 ± 0.024^{b}	2.709 ± 0.139^{b}	0^{a}	2.709 ± 0.139^{b}	0^{a}
	40 μM NPA	5.232 ± 0.232^{b}	4.531 ± 0.261^{b}	0.284 ± 0.0224^{b}	0.033 ± 0.012^{b}	0.306 ± 0.036^{b}	2.386 ± 0.121^{b}	0^{a}	2.386 ± 0.126^{b}	0^{a}
	80 μM NPA	4.042 ± 0.184^{b}	4.145 ± 0.215^{b}	0.243 ± 0.013^{b}	0.033 ± 0.012^{b}	0.254 ± 0.026^{b}	2.432 ± 0.114^{b}	0^{a}	2.432 ± 0.114^{b}	0.0533 ± 0.026^{b}
uni-tac AF TL	Control	5.781 ± 0.092^{a}	8.822 ± 0.253^{a}	0.312 ± 0.022^{a}	0.012 ± 0.012^{a}	0.324 ± 0.024^{a}	3.016 ± 0.016^{a}	0.050 ± 0.025^{a}	3.116 ± 0.016^{a}	0^{a}
	20 μM NPA	5.622 ± 0.183^{a}	6.631 ± 0.181^{b}	0.245 ± 0.24^{a}	0.012 ± 0.012^{a}	0.302 ± 0.024^{a}	2.554 ± 0.123^{b}	0.030 ± 0.030^{b}	2.584 ± 0.141^{b}	0^{a}
	40 μM NPA	5.302 ± 0.092^{a}	6.322 ± 0.252^{b}	0.192 ± 0.032^{b}	0^{a}	0.203 ± 0.022^{a}	2.164 ± 0.182^{b}	0^{b}	2.164 ± 0.182^{b}	0^{a}
	80 μM NPA	3.273 ± 0.221^{b}	4.24 ± 0.244^{b}	0.134 ± 0.014^{b}	0^{a}	0.132 ± 0.014^{b}	2.153 ± 0.122^{b}	0^{b}	2.153 ± 0.123^{b}	0^{a}
UNI-TAC af TL	Control	8.902 ± 0.412^{a}	8.44 ± 0.364^{a}	0.792 ± 0.012^{a}	0.382 ± 0.023^{a}	1.172 ± 0.062^{a}	0^{a}	9.54 ± 0.264^{a}	9.54 ± 0.264^{a}	0^{a}
	20 μM NPA	5.972 ± 0.332^{b}	5.62 ± 0.224^{b}	0.624 ± 0.034^{b}	0.092 ± 0.012^{b}	0.504 ± 0.044^{b}	1.204 ± 0.164^{b}	4.762 ± 0.346^{b}	5.966 ± 0.362^{b}	0^{a}
	40 μM NPA	5.904 ± 0.334^{b}	5.52 ± 0.224^{b}	0.583 ± 0.103^{b}	0.094 ± 0.014^{b}	0.502 ± 0.042^{b}	1.292 ± 0.152^{b}	4.112 ± 0.352^{b}	5.404 ± 0.374^{b}	0^{a}
	80 μM NPA	2.892 ± 0.222^{b}	3.14 ± 0.414^{b}	0.402 ± 0.052^{b}	0.163 ± 0.043^{b}	0.533 ± 0.083^{b}	0.686 ± 0.260^{b}	4.686 ± 0.357^{b}	5.372 ± 0.421^{b}	0.022 ± 0.022^{a}

[a,b]For a genotype and parameter, the values of treatment effects that carry the same letter as superscript are not different from the respective control.
The parameter values in columns C to K are based on measurements made on 50–75 shoots.

Table 8. Effect of the auxin transport inhibitor 2, 3, 5-triidobenzoic acid (TIBA) on the growth of shoots grown *in vitro* from explants of wild-type, *tl*, *uni-tac* and *af* genotypes and on morphology of leaves formed on the second and subsequent nodes of the shoots.

			Leaf							
Genotype	Treatment*	Shoot length (cm)	Number	Petiole length (cm)	Rachis length (cm)	Blade length (cm)	Leaflet number	Tendril number	Pinna (leaflet + tendril) number	Frequency of shoot apex conversion into leaf
A	B	C	D	E	F	G = E + F	H	I	J = H + I	K[c]
UNI-TAC AF TL	Control	6.102 ± 0.132^{a}	11.112 ± 0.126^{a}	0.451 ± 0.032^{a}	0.239 ± 0.047^{a}	0.690 ± 0.062^{a}	2.00 ± 0.000^{a}	2.743 ± 0.054^{a}	4.743 ± 0.046^{a}	0^{a}
	10 μM TIBA	4.212 ± 0.075^{a}	6.246 ± 0.103^{b}	0.209 ± 0.041^{b}	$0.1O8 \pm 0.032^{b}$	0.317 ± 0.058^{b}	1.835 ± 0.087^{b}	1.428 ± 0.117^{b}	3.263 ± 0.056^{b}	0.0545 ± 0.031^{a}
	20 μM TIBA	3.162 ± 0.083^{b}	5.203 ± 0.072^{b}	0.152 ± 0.011^{b}	0.089 ± 0.041^{b}	0.241 ± 0.037^{b}	1.428 ± 0.158^{a}	0.842 ± 0.085^{b}	2.270 ± 0.076^{b}	0^{a}
	40 μM TIBA	2.153 ± 0.025^{b}	4.763 ± 0.046^{b}	0.115 ± 0.042^{a}	0.038 ± 0.017^{b}	0.153 ± 0.042^{b}	1.323 ± 0.0128^{a}	0.689 ± 0.075^{b}	2.012 ± 0.116^{b}	0^{a}
UNI-TAC AF tl	Control	7.875 ± 0.129^{a}	8.126 ± 0.0042^{a}	0.672 ± 0.032^{a}	0.193 ± 0.024^{a}	0.865 ± 0.084^{a}	3.763 ± 0.126^{a}	0^{a}	3.763 ± 0.126^{a}	0^{a}
	10 μM TIBA	5.375 ± 0.148^{b}	5.128 ± 0.132^{b}	0.326 ± 0.022^{b}	0.042 ± 0.011^{b}	0.368 ± 0.028^{b}	2.634 ± 0.166^{b}	0^{a}	2.634 ± 0.166^{b}	0^{a}
	20 μM TIBA	4.167 ± 0.157^{b}	3.246 ± 0.125^{b}	0.257 ± 0.054^{b}	0.026 ± 0.018^{b}	0.283 ± 0.058^{b}	2.124 ± 0.085^{b}	0^{a}	2.124 ± 0.085^{b}	0^{a}
	40 μM TIBA	3.058 ± 0.162^{b}	3.137 ± 0.154^{b}	0.216 ± 0.018^{b}	0.020 ± 0.046^{b}	0.236 ± 0.066^{b}	2.145 ± 0.124^{b}	0^{a}	2.145 ± 0.124^{b}	0^{a}
uni-tac AF TL	Control	5.298 ± 0.082^{a}	7.921 ± 0.143^{a}	0.367 ± 0.046^{a}	0^{a}	0.367 ± 0.046^{a}	3.000 ± 0.000^{a}	0^{b}	3.000 ± 0.000^{a}	0^{a}
	10 μM TIBA	5.428 ± 0.138^{a}	5.432 ± 0.161^{b}	0.276 ± 0.189^{a}	0^{a}	0.276 ± 0.189^{a}	2.048 ± 0.132^{b}	0^{b}	2.048 ± 0.132^{b}	0^{a}
	20 μM TIBA	4.312 ± 0.052^{a}	4.243 ± 0.169^{b}	0.168 ± 0.022^{b}	0^{a}	0.168 ± 0.022^{b}	1.894 ± 0.143^{b}	0^{b}	1.894 ± 0.143^{b}	0^{a}
	40 μM TIBA	2.203 ± 0.081^{b}	3.187 ± 0.175^{b}	0.111 ± 0.018^{b}	0^{a}	0.111 ± 0.018^{b}	1.324 ± 0.117^{b}	0^{b}	1.324 ± 0.117^{b}	0^{a}
UNI-TAC af TL	Control	10.231 ± 0.157^{a}	9.897 ± 0.174^{a}	0.804 ± 0.012^{a}	0.312 ± 0.023^{a}	1.116 ± 0.024^{a}	0^{a}	9.840 ± 0.148^{a}	9.840 ± 0.148^{a}	0^{a}
	10 μM TIBA	4.676 ± 0.237^{b}	7.865 ± 0.158^{b}	0.436 ± 0.056^{b}	0.126 ± 0.012^{b}	0.562 ± 0.043^{b}	1.101 ± 0.127^{b}	4.126 ± 0.127^{b}	5.227 ± 0.135^{b}	0.079 ± 0.031
	20 μM TIBA	4.686 ± 0.279^{b}	6.164 ± 0.118^{b}	0.486 ± 0.112^{b}	0.094 ± 0.014^{b}	0.580 ± 0.065^{b}	0.787 ± 0.113^{b}	2.227 ± 0.112^{b}	3.014 ± 0.127^{b}	0
	40 μM TIBA	2.127 ± 0.086^{b}	2.187 ± 0.064^{b}	0.387 ± 0.072^{b}	0.091 ± 0.063^{b}	0.478 ± 0.058^{b}	0^{a}	1.894 ± 0.168^{b}	1.894 ± 0.168^{b}	0.052 ± 0.026

The parameter values in columns C to K are based on measurements made on 50–75 shoots.

[a,b]For a genotype and parameter, the values of treatment effects that carry the same letter as superscript are not different from the respective control.

[c]The concerned shoot morphologies, also seen in NPA treated shoots, are referred in table 7.

Table 9. Effect of the auxin influx carrier inhibitor parachlorophenoxy isobutyric acid (PCIB) on the growth of shoots grown *in vitro* from explants of wild-type, *tl*, *uni-tac* and *af* genotypes and on morphology of leaves formed on the second and subsequent nodes of the shoots.

			Leaf							
Genotype	Treatment*	Shoot length (cm)	Number	Petiole length (cm)	Rachis length (cm)	Blade length (cm)	Leaflet number	Tendril number	Pinna (leaflet + tendril) number	Frequency of shoot apex conversion into leaf
A	B	C	D	E	F	G = E + F	H	I	J = H + I	K
UNI-TAC AF TL	Control	6.052 ± 0.431^{a}	13.00 ± 0.721^{a}	0.356 ± 0.024^{a}	0.211 ± 0.024^{a}	0.565 ± 0.042^{a}	2.00 ± 0.000^{a}	2.613 ± 0.106^{a}	4.615 ± 0.031^{a}	0^{a}
	20 μM PCIB	6.113 ± 0.125^{a}	9.962 ± 0.148^{b}	0.409 ± 0.007^{b}	0.190 ± 0.022^{b}	0.599 ± 0.039^{b}	2.036 ± 0.067^{b}	2.054 ± 0.138^{b}	4.090 ± 0.022^{b}	0^{a}
	40 μM PCIB	5.485 ± 0.112^{a}	8.126 ± 0.121^{b}	0.257 ± 0.021^{b}	0.112 ± 0.021^{b}	0.039 ± 0.038^{b}	2.145 ± 0.068^{b}	1.455 ± 0.132^{b}	3.600 ± 0.141^{b}	0^{a}
	80 μM PCIB	4.363 ± 0.046^{b}	6.273 ± 0.048^{b}	0.227 ± 0.017^{b}	0.105 ± 0.026^{b}	0.333 ± 0.033^{b}	2.236 ± 0.008^{b}	1.400 ± 0.490^{b}	3.636 ± 0.113^{b}	0^{a}
UNI-TAC AF tl	Control	7.502 ± 0.252^{a}	7.025 ± 0.095^{a}	0.642 ± 0.052^{a}	0.174 ± 0.024^{a}	0.824 ± 0.182^{a}	3.934 ± 0.134^{a}	0^{a}	3.934 ± 0.135^{a}	0^{a}
	20 μM PCIB	6.200 ± 0.138^{b}	$6.78 \pm 0.0.148^{b}$	0.613 ± 0.048^{b}	0.164 ± 0.047^{b}	0.777 ± 0.114^{a}	3.166 ± 0.079^{b}	0^{a}	3.166 ± 0.079^{b}	0^{a}
	40 μM PCIB	5.597 ± 0.153^{b}	4.531 ± 0.261^{b}	0.519 ± 0.038^{b}	0.149 ± 0.056^{b}	0.668 ± 0.095^{b}	3.116 ± 0.032^{b}	0^{a}	3.116 ± 0.032^{b}	0^{a}
	80 μM PCIB	4.875 ± 0.086^{b}	4.145 ± 0.215^{b}	0.423 ± 0.067^{b}	0.138 ± 0.012^{b}	0.561 ± 0.108^{b}	3.000 ± 0.014^{b}	0^{a}	2.432 ± 0.114^{b}	0^{a}
uni-tac AF TL	Control	5.781 ± 0.092^{a}	8.822 ± 0.253^{a}	0.312 ± 0.022^{a}	0.012 ± 0.012^{a}	0.324 ± 0.024^{a}	3.016 ± 0.016^{a}	0.050 ± 0.025^{a}	3.116 ± 0.016^{a}	0^{a}
	20 μM PCIB	5.218 ± 0.248^{b}	6.900 ± 0.107^{b}	0.298 ± 0.025^{b}	0^{a}	0.298 ± 0.025^{b}	3.000 ± 0.000^{b}	0^{a}	3.000 ± 0.000^{b}	0^{a}
	40 μM PCIB	5.000 ± 0.024^{b}	6.541 ± 0.012^{b}	0.280 ± 0.019^{b}	0^{a}	0.280 ± 0.019^{b}	2.833 ± 0.041^{b}	0^{a}	2.833 ± 0.041^{b}	0^{a}
	80 μM PCIB	3.754 ± 0.201^{b}	5.727 ± 0.056^{b}	0.251 ± 0.016^{b}	0^{a}	0.251 ± 0.016^{b}	2.900 ± 0.057^{b}	0^{a}	2.900 ± 0.057^{b}	0^{a}
UNI-TAC af TL	Control	8.902 ± 0.412^{a}	8.44 ± 0.364^{a}	0.792 ± 0.012^{a}	0.382 ± 0.023^{a}	1.172 ± 0.062^{a}	0^{a}	9.572 ± 0.264^{a}	9.572 ± 0.262^{a}	0^{a}
	20 μM PCIB	9.214 ± 0.136^{a}	8.253 ± 0.264^{b}	0.725 ± 0.016^{b}	0.392 ± 0.018^{b}	1.117 ± 0.059^{a}	0^{a}	10.143 ± 0.168^{b}	10.143 ± 0.168^{b}	0^{a}
	40 μM PCIB	7.895 ± 0.242^{b}	9.124 ± 0.164^{b}	0.647 ± 0.053^{b}	0.327 ± 0.014^{b}	0.974 ± 0.039^{b}	0^{a}	8.023 ± 0.096^{b}	8.023 ± 0.096^{b}	0^{a}
	80 μM PCIB	7.231 ± 0.147^{b}	6.376 ± 0.179^{b}	0.538 ± 0.089^{b}	0.308 ± 0.054^{b}	0.841 ± 0.056^{b}	0^{a}	7.897 ± 0.114^{b}	7.897 ± 0.114^{b}	0^{a}

The parameter values in columns C to K are based on measurements made on 50–75 shoots.

[a,b]For a genotype and parameter, the values of treatment effects that carry the same letter as superscript are not different from the respective control.

Table 10. Effect of the auxin efflux carrier inhibitor (1-napthoxy) acetic acid (NOA) on the growth of shoots grown *in vitro* from explants of wild-type, *tl*, *uni-tac* and *af* genotypes and on morphology of leaves formed on the second and subsequent nodes of the shoots.

			Leaf							
Genotype	Treatment*	Shoot length (cm)	Number	Petiole length (cm)	Rachis length (cm)	Blade length (cm)	Leaflet number	Tendril number	Pinna (leaflet + tendril) number	Frequency of shoot apex conversion into leaf
A	B	C	D	E	F	G = E + F	H	I	J = H + I	K
UNI-TAC AF TL	Control	6.092 ± 0.471[a]	11.00 ± 0.821[a]	0.396 ± 0.034[a]	0.261 ± 0.024[a]	0.657 ± 0.053[a]	2.000 ± 0.000[a]	2.735 ± 0.095[a]	4.735 ± 0.046[a]	0[a]
	20 μM NOA	6.089 ± 0.395[a]	10.00 ± 0.212[b]	0.327 ± 0.012[b]	0.213 ± 0.027[a]	0.567 ± 0.048[a]	2.036 ± 0.067[a]	1.986 ± 0.097[b]	4.594 ± 0.034[b]	0[a]
	40 μM NOA	5.938 ± 0.325[a]	10.265 ± 0.08[a]	0.279 ± 0.063[b]	0.198 ± 0.031[a]	0.477 ± 0.041[b]	2.145 ± 0.068[b]	2.486 ± 0.142[b]	4.637 ± 0.089[a]	0[a]
	80 μM NOA	4.765 ± 0.185[b]	9.375 ± 0.065[b]	0.248 ± 0.019[b]	0.147 ± 0.034[b]	0.395 ± 0.065[b]	2.236 ± 0.008[b]	1.879 ± 0.083[b]	3.874 ± 0.075[b]	0[a]
UNI-TAC AF tl	Control	7.842 ± 0.274[a]	7.628 ± 0.083[a]	0.681 ± 0.052[a]	0.198 ± 0.012[a]	0.879 ± 0.143[a]	4.126 ± 0.152[a]	0[a]	4.126 ± 0.152[a]	0[a]
	20 μM NOA	6.285 ± 0.179[b]	6.937 ± 0.114[b]	0.635 ± 0.058[a]	0.168 ± 0.016[a]	0.803 ± 0.156[b]	3.236 ± 0.043[b]	0[a]	3.236 ± 0.043[b]	0[a]
	40 μM NOA	6.372 ± 0.141[b]	6.486 ± 0.076[b]	0.612 ± 0.069[a]	0.144 ± 0.042[a]	0.756 ± 0.053[b]	3.275 ± 0.026[b]	0[a]	3.275 ± 0.026[b]	0[a]
	80 μM NOA	5.754 ± 0.157[b]	5.487 ± 0.087[b]	0.523 ± 0.084[b]	0.148 ± 0.039[b]	0.671 ± 0.078[b]	3.000 ± 0.000[b]	0[a]	3.000 ± 0.000[b]	0[a]
uni-tac AF TL	Control	5.848 ± 0.074[a]	8.885 ± 0.247[a]	0.382 ± 0.042[a]	0[a]	0.382 ± 0.042[a]	3.000 ± 0.000[a]	0[a]	3.000 ± 0.000[b]	0[a]
	20 μM NOA	5.453 ± 0.042[b]	7.463 ± 0.142[b]	0.298 ± 0.046[a]	0[a]	0.298 ± 0.046[a]	3.000 ± 0.000[a]	0[a]	3.000 ± 0.000[b]	0[a]
	40 μM NOA	5.428 ± 0.019[b]	7.386 ± 0.153[b]	0.273 ± 0.054[b]	0[a]	0.273 ± 0.054[a]	3.000 ± 0.000[a]	0[a]	3.000 ± 0.000[b]	0[a]
	80 μM NOA	4.647 ± 0.126[b]	6.784 ± 0.138[b]	0.248 ± 0.033[b]	0[a]	0.248 ± 0.033[b]	3.000 ± 0.000[a]	0[a]	3.000 ± 0.000[a]	0[a]
UNI-TAC af TL	Control	7.902 ± 0.242[a]	9.440 ± 0.384[a]	0.799 ± 0.016[a]	0.402 ± 0.043[a]	1201 ± 0.084[a]	0[a]	10.452 ± 0.283[a]	10.452 ± 0.283[a]	0[a]
	20 μM NOA	8.985 ± 0.214[a]	8.433 ± 0.322[a]	0.785 ± 0.024[a]	0.332 ± 0.058[b]	1.117 ± 0.067[b]	0[a]	9.683 ± 0.184[a]	9.683 ± 0.184[a]	0[a]
	40 μM NOA	8.375 ± 0.327[b]	8.174 ± 0.185[a]	0.687 ± 0.067[b]	0.319 ± 0.065[b]	1.006 ± 0.053[b]	0[a]	9.148 ± 0.169[b]	9.148 ± 0.169[b]	0[a]
	80 μM NOA	7.768 ± 0.353[b]	7.248 ± 0.047[b]	0.626 ± 0.059[b]	0.269 ± 0.093[b]	0.8995 ± 0.068[b]	0[a]	8.143 ± 0.227[b]	8.143 ± 0.227[b]	0[a]

The parameter values in columns C to K are based on measurements made on 50–75 shoots.

[a,b]For a genotype and parameter, the values of treatment effects that carry the same letter as superscript are not different from the respective control.

on the length of shoots and number of nodes produced on them. However, NPA proved to be more potent than TIBA at the concentrations used, in terms of negative effects on the six parameters of leaf morphology and on the continuation of growth at the shoot apex. All the four ATIs at their highest concentration used in these experiments demonstrated inhibitory effects on shoot growth measured in terms of shoot height, number of nodes formed and size of leaves in terms of petiole and rachis sizes and complexity of leafblade. The average size of leaf and its complexity in four genotypes studied was reduced up to 50% or less by ATIs at their highest concentration. In *af* mutant, treatments with NPA and TIBA led to production of shoots in which leaflets occurred in place of tendrils at 19% and 18% frequency, respectively. This phenomenon was not observed in *af* shoots formed in the presence of PCIB and NOA. The pathways of reduction in the complexity of leaves by ATI treatments were investigated further by morphological analysis of affected leaves. The observations on various kinds of structures found on shoots of wild-type and *uni-tac*, *af* and *tl* mutants treated with NPA, TIBA, PCIB, and NOA are respectively summarized in tables 11, 12, 13, 14 and figures 2 and 3. In all the treated shoots, there was preponderance of unipinnate leaves. In wild-type, *uni-tac*, *af* and *tl* treated shoots large majority of leaves had at least two normal domains, proximal and distal, like in control counterpart shoots. Several NPA treated shoots of wild-type produced leaves in which proximal domain pinnae were respectively leaflets and tendril pairs, but the apical pinna had aborted into a stub-like structure. Such a leaf structure was not seen in NPA treated shoots of the *uni-tac*, and *tl* mutants. In several NPA treated *af* shoots (9%), a trident-shaped stub-like structure was noticed in place of leaf. Several leaves of wild-type (9%), *uni-tac* and *tl* (9%), shoots which had been treated with NPA produced only a pair of leaflets. The apical domain was entirely missing from such leaves. Similarly two pinnae leaves were also seen in shoots

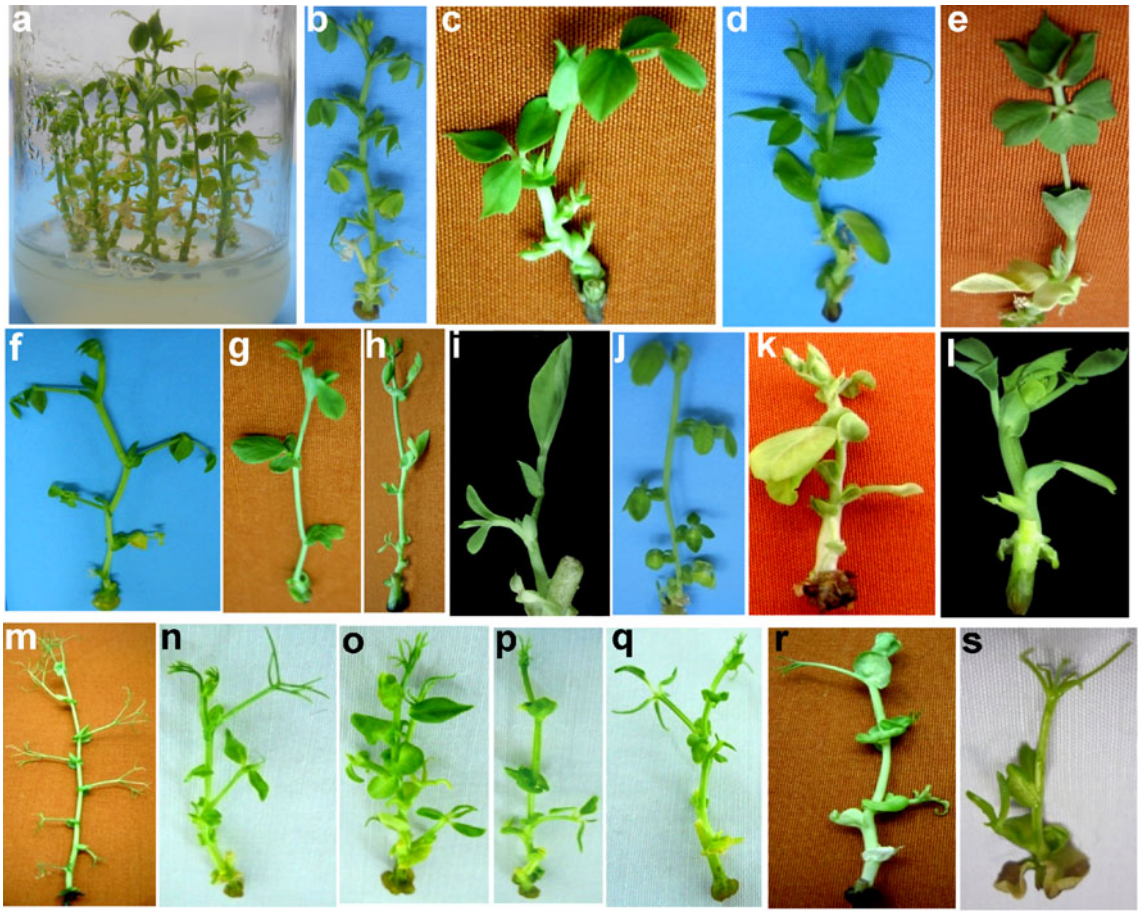

Figure 1. Thirty-five to 40 day-old shoots of *Pisum sativum* wild-type (WT) and *tl*, *uni-tac* and *af* mutant genotypes grown *in vitro* on the beds of basal medium (BS) in the absence and presence of the auxin transport inhibitor 1, N-naphthylpthalmic acid (NPA) (tables 4–7). a, Wild-type shoots on BS; b, f, j and m wild-type, *tl*, *uni-tac* and *af* shoots, respectively on BS; c, d and e, wild-type shoots respectively on BS + 40 μM NPA and BS + 80 μM NPA; g, h and i, *tl* shoots respectively on BS + 40 μM NPA and BS + 80 μM NPA; k and l, *uni-tac* shoots on BS + 40 μM NPA; and n to r and s *af* shoots on BS + 40 μM NPA and BS + 80 μM NPA. A perfoliately fused stipule is seen on fifth node from below in o. In e, I and s, the shoot apical meristem is seen consumed by the apicl leaf. Some variant forms of shoots seen on NPA medium were also seen on TIBA, PCIB and NOA containing media.

Table 11. Effect of the auxin transport inhibitor 1-N-napthylpthalamic acid (NPA) on the architecture of leafblades formed on the second and subsequent nodes of shoots arising from explants grown *in vitro*.

Genotype	Treatment*	Bladeless leaf** Figure 3c–f	Simple leaf Figure 2i and p	Leaflet pair/tendril pair Figures 2h and o and 3b and h	Leaflet pair/ tendril pair + stub Figure 2e	Leaflet/tendril pair + leaflet/tendril Figure 2d, g and n	Leaflet/tendril pair + leaflet/tendril pair + tendril/leaflet Figure 2a, c, j and i
UNI-TAC AF TL	Control	0^{a}	0^{a}	0^{a}	0^{a}	0.162 ± 0.022^{a}	0.841 ± 0.053^{a}
	20 μM NPA	0.122 ± 0.047^{b}	0.042 ± 0.023^{a}	0.037 ± 0.032^{a}	0.037 ± 0.032^{a}	0.221 ± 0.064^{b}	0.544 ± 0.072^{b}
	40 μM NPA	0.202 ± 0.052^{b}	0.225 ± 0.074^{b}	0.118 ± 0.036^{b}	0.118 ± 0.036^{b}	0.123 ± 0.043^{b}	0.223 ± 0.073^{b}
	80 μM NPA	0.243 ± 0.054^{b}	0.162 ± 0.032^{b}	0.082 ± 0.042^{b}	0.082 ± 0.042^{b}	0.322 ± 0.072^{b}	0.062 ± 0.032^{b}
UNI-TAC AF tl	Control	0^{a}	0^{a}	0^{a}	0^{a}	0.623 ± 0.092^{a}	0.421 ± 0.012^{a}
	20 μM NPA	0.033 ± 0.025^{a}	0.116 ± 0.041^{b}	0^{a}	0^{a}	0.517 ± 0.066^{b}	0.150 ± 0.046^{b}
	40 μM NPA	0.052 ± 0.023^{b}	0.022 ± 0.052^{a}	0^{a}	0^{a}	0.483 ± 0.058^{b}	0.150 ± 0.046^{b}
	80 μM NPA	0.016 ± 0.016^{a}	0.116 ± 0.041^{b}	0^{a}	0^{a}	0.433 ± 0.064^{b}	0.116 ± 0.041^{b}
uni-tac AF TL	Control	0^{a}	0^{a}	0^{a}	0^{a}	0.956 ± 0.022^{a}	0.054 ± 0.034^{a}
	20 μM NPA	0.051 ± 0.022^{b}	0.112 ± 0.047^{b}	0.051 ± 0.022^{b}	0^{a}	0.751 ± 0.053^{b}	0.031 ± 0.031^{b}
	40 μM NPA	0.112 ± 0.047^{b}	0.174 ± 0.042^{b}	0.103 ± 0.031^{b}	0^{a}	0.623 ± 0.065^{b}	0^{a}
	80 μM NPA	0.03 ± 0.03^{a}	0.28 ± 0.05^{b}	0.23 ± 0.05^{b}	0^{a}	0.454 ± 0.063^{b}	0^{a}
UNI-TAC af TL	Control	0^{a}	0^{a}	0^{a}	0^{a}	0.026 ± 0.018^{a}	0.974 ± 0.018^{a}
	20 μM NPA	0.221 ± 0.051^{b}	$0.031 \pm 0.011^{b,c}$	0.043 ± 0.057^{a}	0.043 ± 0.057^{a}	0.212 ± 0.052^{b}	0.493 ± 0.052^{b}
	40 μM NPA	0.298 ± 0.052^{b}	$0.044 \pm 0.022^{b,c}$	0.063 ± 0.056^{a}	0.063 ± 0.056^{a}	0.182 ± 0.042^{b}	0.407 ± 0.057^{b}
	80 μM NPA	0.152 ± 0.022^{b}	0^{a}	0.157 ± 0.042^{b}	0.157 ± 0.042^{b}	0.214 ± 0.044^{b}	0.474 ± 0.058^{b}

*Each value in a row is a fraction of the total.

**The term bladeless was given by DeMason and Chawla (2004a, b) for the absence of any structure representing leafblade beyond the distal apex of fused stipules.

[a,b]For a genotype and parameter, the values of treatment effects that carry the same letter as superscript are not different from the respective control.

[c]These values indicates both simple leaves and tendrils.

Table 12. Effect of the auxin transport inhibitor 2, 3,-5-triidobenzoic acid (TIBA) on the architecture of leafblades formed on the second and subsequent nodes of shoots arising from explants grown *in vitro*.

Genotype	Treatment*	Bladeless leaf** Figure 3e	Simple leaf Figure 2i	Leaflet pair/tendril pair Figure 2h	Leaflet pair/ tendril pair + stub Figure 2e	Leaflet/tendril pair + leaflet/tendril Figure 2d, g and n	Leaflet/tendril pair + leaflet/tendril pair + tendril/leaflet Figure 2a–c, j and i
UNI-TAC AF TL	Control	0^a	0^a	0^a	0^a	0.200 ± 0.056^a	0.300 ± 0.00^a
	10 μM TIBA	0^a	0.094 ± 0.017^b	0.218 ± 0.060^a	0.090 ± 0.039^b	0.254 ± 0.060^b	0.436 ± 0.067^b
	20 μM TIBA	0^a	0.116 ± 0.049^b	0.272 ± 0.061^b	0.163 ± 0.049^b	0.272 ± 0.006^b	0.272 ± 0.061^b
	40 μM TIBA	0^a	0.043 ± 0.013^b	0.054 ± 0.031^b	0.200 ± 0.053^b	0.318 ± 0.066^b	0.363 ± 0.065^b
UNI-TAC AF tl	Control	0^a	0^a	0^a	0^a	0.706 ± 0.052^a	0^a
	10 μM TIBA	0^a	0^a	0.266 ± 0.051^b	0^a	0.733 ± 0.051^b	0^a
	20 μM TIBA	0^a	0^a	0.240 ± 0.049^b	0^a	0.760 ± 0.049^b	0^a
	40 μM TIBA	0^a	0^a	0.093 ± 0.032^a	0^a	0.900 ± 0.033^b	0^a
uni-tac AF TL	Control	0^a	0^a	0^a	0^a	1.000 ± 000^a	0^a
	10 μM TIBA	0^a	0.290 ± 0.006^b	0.277 ± 0.006^b	0^a	0.436 ± 0.067^b	0^a
	20 μM TIBA	0^a	0.345 ± 0.063^b	0.200 ± 0.053^b	0^a	0.454 ± 0.061^b	0^a
	40 μM TIBA	0^a	0.218 ± 0.055^b	0.145 ± 0.067^a	0^a	0.636 ± 0.065^b	0^a
UNI-TAC af TL	Control	0^a	0^a	0^a	0^a	0^a	1.000 ± 0.000^a
	10 μM TIBA	0.145 ± 0.047^b	0^a	0^a	0.218 ± 0.055^b	0.254 ± 0.067^b	0.381 ± 0.066^b
	20 μM TIBA	0^a	0^a	0^a	0.009 ± 0.039^a	0.290 ± 0.061^b	0.681 ± 0.066^b
	40 μM TIBA	0.054 ± 0.030^a	0^a	0^a	0.145 ± 0.047^b	0.388 ± 0.066^b	0.413 ± 0.067^b

*Each value in a row is a fraction.

**The term bladeless was given by DeMason and Chawla (2004a, b) for the absence of any structure representing leafblade beyond the distal apex of fused stipules.

[a,b]For a genotype and parameter, the values of treatment effects that carry the same letter as superscript are not different from the respective control.

Table 13. Effect of the auxin influx carrier inhibitor parachlorophenoxy isobutyric acid (PCIB) on the architecture of leafblades formed on the second and subsequent nodes of shoots arising from explants grown *in vitro*.

Genotype	Treatment*,[c]	Bladeless leaf**	Simple leaf	Leaflet pair/tendril pair	Leaflet pair/ tendril pair + stub	Leaflet/tendril pair + leaflet/tendril	Leaflet/tendril pair + leaflet/ tendril pair + tendril/leaflet
UNI-TAC AF TL	Control	0^a	0^a	0^a	0^a	0.162 ± 0.022^a	0.841 ± 0.053^a
	20 μM PCIB	0^a	0^a	0^a	0^a	0.301 ± 0.059^b	0.673 ± 0.061^b
	40 μM PCIB	0^a	0^a	0^a	0^a	0.509 ± 0.067^b	0.472 ± 0.057^b
	80 μM PCIB	0^a	0^a	0.181 ± 0.011^b	0^a	0.551 ± 0.066^b	0.0481 ± 0.064^b
UNI-TAC AF tl	Control	0^a	0^a	0^a	0^a	0.623 ± 0.092^a	0.421 ± 0.012^a
	20 μM PCIB	0^a	0^a	0^a	0^a	0.783 ± 0.053^b	0.216 ± 0.050^b
	40 μM PCIB	0^a	0^a	0.050 ± 0.028^b	0^a	0.800 ± 0.052^b	0.150 ± 0.046^b
	80 μM PCIB	0^a	0^a	0.083 ± 0.036^b	0^a	0.850 ± 0.109^b	0.005 ± 0.028^b
uni-tac AF TL	Control	0^a	0^a	0^a	0^a	0^a	0^a
	20 μM PCIB	0^a	0^a	0^a	0^a	0^a	0^a
	40 μM PCIB	0^a	0^a	0.100 ± 0.038^b	0^a	0.899 ± 0.039^b	0^a
	80 μM PCIB	0^a	0^a	0.116 ± 0.041^b	0^a	0.886 ± 0.054^b	0^a
UNI-TAC af TL	Control	0^a	0^a	0^a	0^a	0.171 ± 0.043^a	0.828 ± 0.004^a
	20 μM PCIB	0^a	0^a	0^a	0^a	0.210 ± 0.047^b	0.789 ± 0.056^a
	40 μM PCIB	0^a	0^a	0^a	0^a	0.263 ± 0.051^b	0.736 ± 0.051^b
	80 μM PCIB	0^a	0^a	0^a	0^a	0.206 ± 0.052^b	0.763 ± 0.049^a

*Each value in a row is a fraction.

**The term bladeless was given by DeMason and Chawla (2004a, b) for the absence of any structure representing leafblade beyond the distal apex of fused stipules.

[a,b]For a genotype and parameter, the values of treatment effects that carry the same letter as superscript are not different from the respective control.

[c]The relevant figures of leafblades are referred to in the tables 11 and 12.

Table 14. Effect of the auxin efflux carrier inhibitor (1-napthoxy) acetic acid (NOA) on the architecture of leafblades formed on the second and subsequent nodes of shoots arising from explants grown *in vitro*.

Genotype	Treatment*,[c]	Bladeless leaf**	Simple leaf	Leaflet pair/ tendril pair	Leaflet pair/tendril pair + stub	Leaflet/tendril pair + leaflet/tendril	Leaflet/tendril pair + leaflet/tendril pair + tendril/leaflet
UNI-TAC AF TL	Control	0[a]	0[a]	0[a]	0[a]	0.162 ± 0.022[a]	0.841 ± 0.053[a]
	20 μM NOA	0[a]	0[a]	0[a]	0[a]	0.303 ± 0.061[a]	0.717 ± 0.062[b]
	40 μM NOA	0[a]	0[a]	0[a]	0[a]	0.464 ± 0.067[b]	0.537 ± 0.068[b]
	80 μM NOA	0[a]	0[a]	0[a]	0[a]	0.518 ± 0.068[b]	0.481 ± 0.068[b]
UNI-TAC AF tl	Control	0[a]	0[a]	0[a]	0[a]	0.623 ± 0.092[a]	0.421 ± 0.012[a]
	20 μM NOA	0[a]	0[a]	0[a]	0[a]	0.724 ± 0.068[b]	0.276 ± 0.038[b]
	40 μM NOA	0[a]	0[a]	0[a]	0[a]	0.786 ± 0.036[b]	0.214 ± 0.045[b]
	80 μM NOA	0[a]	0[a]	0[a]	0[a]	0.984 ± 0.015[b]	0.016 ± 0.08[b]
uni-tac AF TL	Control	0[a]	0[a]	0[a]	0[a]	0.949 ± 0.123[a]	0.050 ± 0.028[a]
	20 μM NOA	0[a]	0[a]	0[a]	0[a]	0.921 ± 0.036[a]	0.083 ± 0.035[b]
	40 μM NOA	0[a]	0[a]	0[a]	0[a]	0.932 ± 0.034[a]	0.066 ± 032[a]
	80 μM NOA	0[a]	0[a]	0[a]	0[a]	1.000 ± 0.000[a]	0[a]
UNI-TAC af TL	Control	0[a]	0[a]	0[a]	0[a]	0.171 ± 0.043[a]	0.828 ± 0.040[a]
	20 μM NOA	0[a]	0[a]	0[a]	0[a]	0.184 ± 0.045[b]	0.816 ± 0.045[b]
	40 μM NOA	0[a]	0[a]	0[a]	0[a]	0.223 ± 0.048[b]	0.776 ± 0.049[b]
	80 μM NOA	0[a]	0[a]	0[a]	0[a]	0.197 ± 0.046[b]	0.803 ± 0.047[b]

*Each value in a row is a fraction.

**The term bladeless was given by DeMason and Chawla (2004a, b) for the absence of any structure representing leafblade beyond the distal apex of fused stipules.

[a,b]For a genotype and parameter, the values of treatment effects that carry the same letter as superscript are not different from the respective control.

[c]The relevant figures of leafblades are referred to in the tables 11 and 12.

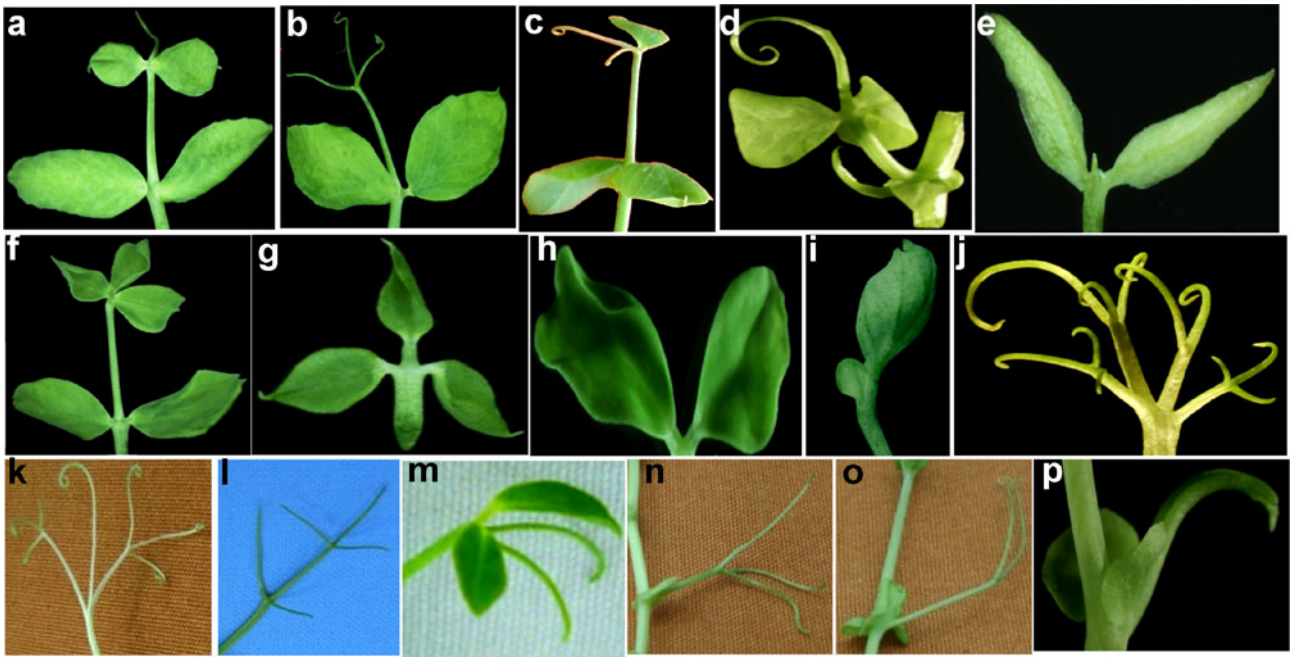

Figure 2. Morphologies of leaves borne on the shoots of *Pisum sativum* WT, *tl*, *uni-tac* and *af* genotypes grown *in vitro* in the presence of NPA (table 8). a and b, f, g and j, leaves of normal morphology, respectively, in WT, *tl*, *uni-tac* and *af* genotypes. a–e leaves of varying complexity observed in WT shoots; f–I, leaves of varying architecture (including adnate leaf presumably formed by fusion of stipule and leaf primordia, at i) seen in WT, *tl* and *uni-tac* shoots; and j–p leaves of varying architecture seen on *af* shoots (in m, a leaf is seen in which tendrils are replaced by leaflets and in p, o and n respectively leaves are seen consisting of only 1, 2 and 3 tendrils. Some of the leaf forms seen with NPA treatments, were also seen with TIBA, PCIB and/or NOA treatments (tables 9–11).

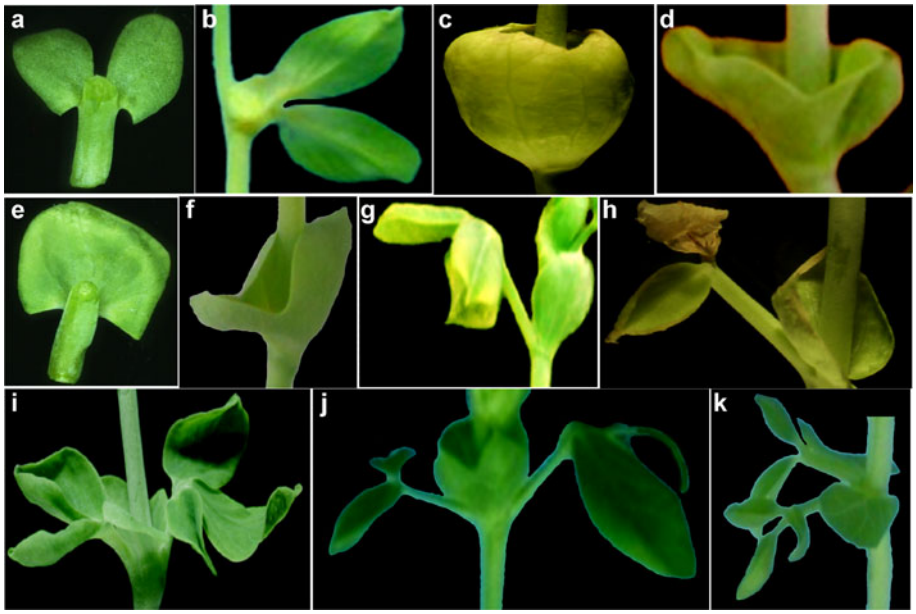

Figure 3. Variation in stipule morphology and arrangement of stipule in respect to the associated leaf seen in shoots of *Pisum sativum*, WT, *tl*, *uni-tac* and *af* genotypes grown in the presence of NPA, PCIB, TIBA and/or NOA (tables 12 and 13). a, Stipules of normal WT morphology; b, adnate binnate leaf presumably resulting from fusion of stipule inner margins with leaf petiole; c, ochreate fused stipules, associated leaf absent; d, fused stipules forming connate leafy structure; associated leaf absent; e, fused stipules forming a amplexicaul leafy structure, associated leaf absent; f, fused stipules forming a sheathing leaf structure, associated leaf absent; g, stipule in which outer margins are fused and is arranged opposite to leaf; h, stipule in which inner margins are fused and leaf is placed intrapetiolarly; I, two adnate compound leaves arranged opposite to each other with fusion of outer margins of stipule parts of adnate leaves (called interpetiolarly fused on side in table 12); j, opposite leaves arranged on two sides of ochreately fused stipules (called interpetiolarly fused on both sides in table 12); k, one of the two stipules is adnate trifoliated compound similar in apical/distal morphology to the associated leaf.

Table 15. Effect of the auxin transport inhibitor 1-N-naphthylpthalamic acid (NPA) on the morphology of stipules formed on the second and subsequent nodes produced on shoots arising from in *vitro* grown explants of wild-type, *tl*, *uni-tac* and *af* genotypes.

		Stipules										
				Without leaf			With leaf					
Genotype	Treatment	Free	Adnately fused	Ochreately fused	Fused at both inner and outer margins	Fused at inner margins	Oppositely fused	Intra-petiolarly fused	Inter-petiolarly fused on one side	Inter-petiolarly fused on both sides	Petiolated	Compound leaf like
		Figure 3a, k and l	Figures 2i and 3b, and i	Figure 3c	Figure 3c, d, f and j	Figure 3e and h	Figure 3g and j	Figure 3h	Figure 3i	Figure 3j	Figure 3k	Figure 3l
UNI-TAC AF TL	Control	1^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	20 μM NPA	0.721 ± 0.066^{b}	0.159 ± 0.026^{b}	0.039 ± 0.018^{b}	0^{a}	0.077 ± 0.038^{b}	0^{a}	0^{a}	0.039 ± 0.017^{b}	0^{a}	0^{a}	0^{a}
	40 μM NPA	0.403 ± 0.068^{b}	0.138 ± 0.063^{b}	0.121 ± 0.037^{b}	0^{a}	0.079 ± 0.036^{b}	0.2 ± 0.05^{b}	0.02 ± 0.02^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	80 μM NPA	0.422 ± 0.072^{b}	0.112 ± 0.037^{b}	0.058 ± 0.028^{b}	0^{a}	0.182 ± 0.029^{b}	0.2 ± 0.02^{b}	0^{a}	0^{a}	0^{a}	0.02 ± 0.02^{a}	0.02 ± 0.02^{a}
UNI-TAC AF tl	Control	1^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	20 μM NPA	0.452 ± 0.061^{b}	0.200 ± 0.046^{b}	0^{a}	0^{a}	0.033 ± 0.018^{a}	0.118 ± 0.002^{b}	0^{a}	0.07 ± 0.03^{b}	0.133 ± 0.048^{b}	0^{a}	0^{a}
	40 μM NPA	0.514 ± 0.058^{b}	0.228 ± 0.059^{b}	0^{a}	0^{a}	0.033 ± 0.018^{a}	0^{a}	0^{a}	0.10 ± 0.034^{b}	0.133 ± 0.048^{b}	0^{a}	0^{a}
	80 μM NPA	0.634 ± 0.055^{b}	0.222 ± 0.021b	0^{a}	0^{a}	0.022 ± 0.021^{a}	0^{a}	0^{a}	0.033 ± 0.018^{a}	0.1 ± 0.03^{b}	0^{a}	0^{a}
uni-tac AF TL	Control	1^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	20 μM NPA	0.612 ± 0.066^{b}	0.223 ± 0.046^{b}	0.052 ± 0.025^{b}	0.071 ± 0.026^{b}	0.051 ± 0.026^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	40 μM NPA	0.544 ± 0.073^{b}	0.228 ± 0.053^{b}	0.032 ± 0.027^{a}	0^{a}	0.183 ± 0.047^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	80 μM NPA	0.562 ± 0.067^{b}	0.367 ± 0.057^{b}	0.028 ± 0.032^{a}	0^{a}	0.033 ± 0.023^{b}	0.033 ± 0.023^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
UNI-TAC af TL	Control	1^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	20 μM NPA	0.671 ± 0.057^{b}	0.066 ± 0.028^{b}	0.040 ± 0.016^{b}	0.026 ± 0.018^{a}	0.168 ± 0.057^{b}	0.026 ± 0.011^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	40 μM NPA	0.609 ± 0.056^{b}	0.092 ± 0.046^{b}	0.043 ± 0.026^{a}	0.039 ± 0.023^{a}	0.212 ± 0.048^{b}	0.013 ± 0.012^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	80 μM NPA	0.763 ± 0.048^{b}	0.157 ± 0.049^{b}	0.013 ± 0.012^{a}	0.013 ± 0.012^{a}	0.039 ± 0.022^{a}	0.013 ± 0.012^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}

[a,b]For a genotype and parameter, the values of treatment effects that carry the same letter as superscript are not different from the respective control.

Table 16. Effect of the auxin transport inhibitor 2,3,5- Triidobenzoic acid (TIBA) on the morphology of stipules formed on the second and subsequent nodes produced on shoots arising from *in vitro* grown explants of wild-type, *tl*, *uni-tac* and *af* genotypes*.

		Stipules								
				Without leaf			With leaf			
Genotype	Treatment**	Free	Adnately fused	Ochreately fused	Fused at both inner and outer margins	Fused at inner margins	Oppositely fused	Intrapetiolarly fused	Interpetiolarly fused on one side	Interpetiolarly fused on both sides
UNI-TAC AF TL	Control	1^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	10 μM TIBA	0.963 ± 0.025^{b}	0.036 ± 0.025^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	20 μM TIBA	0.745 ± 0.059^{b}	0.254 ± 0.059^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0	0^{a}
	40 μM TIBA	0.781 ± 0.055^{b}	0.218 ± 0.055^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
UNI-TAC AF tl	Control	1^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	10 μM TIBA	0.745 ± 0.059^{b}	0.254 ± 0.059^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	20 μM TIBA	0.563 ± 0.067^{b}	0.436 ± 0.067^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	40 μM TIBA	0.854 ± 0.047^{b}	0.145 ± 0.019^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
uni-tac AF TL	Control	1^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	10 μM TIBA	0.763 ± 0.057^{b}	0.236 ± 0.057^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	20 μM TIBA	0.636 ± 0.065^{b}	0.363 ± 0.065^{b}	0^{a}	0	0^{b}	0^{a}	0^{a}	0^{a}	0^{a}
	40 μM TIBA	0.745 ± 0.059^{b}	0.245 ± 0.057^{b}	0^{a}	0	0^{b}	0^{a}	0^{a}	0^{a}	0^{a}
UNI-TAC af TL	Control	1^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	10 μM TIBA	0.727 ± 0.006^{b}	0.127 ± 0.004^{b}	0^{a}	0^{a}	0.145 ± 0.047^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	20 μM TIBA	0.836 ± 0.049^{b}	0.163 ± 0.021^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	40 μM TIBA	0.727 ± 0.006^{b}	0.218 ± 0.056^{b}	0^{a}	0^{a}	0.054 ± 0.053^{b}	0^{a}	0^{a}	0^{a}	0^{a}

*The shoots of treatments with PCIB and NOA showed no fused stipules.

**The concerned stipule morphologies, also seen in the NPA treated shoots, are referred to in the table 12.

[a,b]For a genotype and parameter, the values of treatment effects that carry the same letter as superscript are not different from the respective control.

of wild-type (18%), *uni-tac* (21%) and *tl* (27%) genotypes treated with high concentrations of PCIB. This kind of leaf structure was also seen in *af* mutant shoots treated with NPA albeit, less frequently (9%). Also NPA treated shoots of all genotypes also produced simple leaves: 14% in wild-type, 8% in *tl* and 19% in *uni-tac* and 3% in *af*. The frequency of leaves bearing a pair of opposite tendrils was 9% in NPA treated *af* shoots. Adnate stipules-cum-leaves were visualized in shoots of all genotypes treated with all ATIs (tables 15 and 16; figures 2 and 3). These structures comprised of fusion between inner margins of stipules with leaf petiole such that the node produced only one compound structure made of both stipules and leaf. They occurred in a frequency of about 18% in NPA treated shoots and 24% in TIBA treated shoots. The NPA treated shoots of all genotypes also produced nodes on which leaf distinct from stipule(s) could not be visualized. The absence of leaf from nodes has been earlier called bladeless leaf. The structures formed at such nodes were most likely the fusion products of stipule and leaf primordia. These nodes were designated here as stipule-leaf. The frequency of stipule-leaf nodes varied genotypewise, it was 19% in wild-type, 3% in *tl*, 6% in *uni-t*ac and 22% in *af*. The stipule-leaf structures formed had ochreate, perfoliate, sheathing or gamophyllous and amplexicaul shapes. Simple tendril also occurred on NPA treated *af* shoots but with very low frequency (~0.01%).

Like in case of leaves, the stipules produced on ATI treated shoots were also affected in their structure (tables 15 and 16 and figures 2 and 3). Among the ATIs used, the stipule abnormalities were observed in highest frequency on the shoots of all the genotypes treated with NPA. The ATIs could be arranged in the following order in terms of their negative effects on stipules development: NPA > TIBA > PCIB and NOA. While bulk of stipule pairs formed on shoots of all the genotypes treated with NPA were of normal morphology and free as expected for their *COCH ST* genotype, about 43% or more stipule pairs were produced in fused forms. The most common form of stipule fusions observed on NPA treated shoots produced adnate structures which have been described above. Relatively less frequent stipules were fused at inner margins, outer margins or both inner and outer margins. Whereas stipules fused at inner margins were seen in all genotypes; the stipules fused at both inner and outer margins were mostly seen in NPA treated shoots of *uni-tac* and *af* mutant genotypes and have been described above as stipule-leaf. The ochreately fused stipules were seen in treated shoots of all genotypes, except in *tl* mutant genotype. Frequencies of innerpetiolarly and intrapetiolarly fused stipule were very low. Intrapetiolarly fused stipules were seen in wild-type treated shoots. Interpetiolarly stipules fused on one side were seen in wild-type and *tl* mutants. Interpetiolarly stipules fused on both inner and outer margins were seen in wild-type and *tl*. The interpetiolarly fused stipules led to opposite arrangement of associated leaves thus leading to change in phyllotaxy.

Effects of NPA on the expressions of UNI (UNI-TAC) and TL genes: The apices of control, NPA and IAA treated shoots of all the genotypes were studied for the transcriptional expression of *UNI* and *TL* genes by using qRT-PCR and or RT-PCR procedures. The results are shown in figures 4–6. It will be seen from figures 4 and 5 that *UNI* transcript level was lower in *uni-tac* and more than two-fold higher in *tl* and *af* as compared to wild-type. Lower transcription in *uni-tac* is in agreement with the mutational lesion affecting transcription

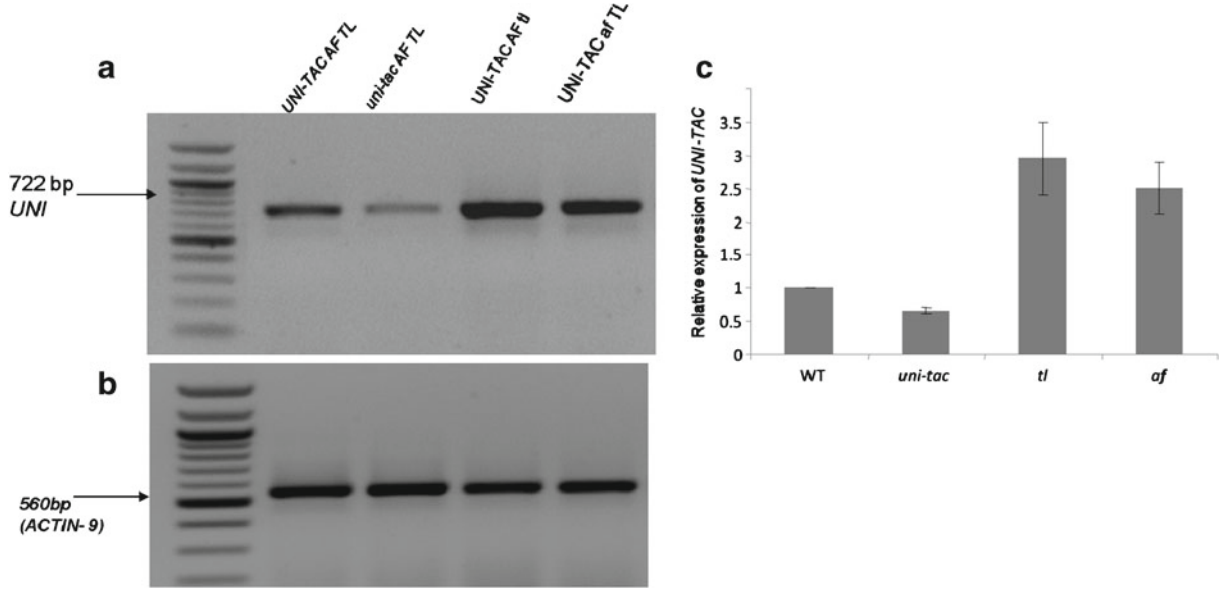

Figure 4. *UNI* transcript levels in the apices of *Pisum sativum* wild-type , *uni-tac*, *tl* and *af* shoots grown *in vitro* in basal medium (a). *ACTIN-9* served as the control (b). The transcript levels were estimated by semi-quantitative RT- PCR procedure. The relative transcript levels are shown in (c).

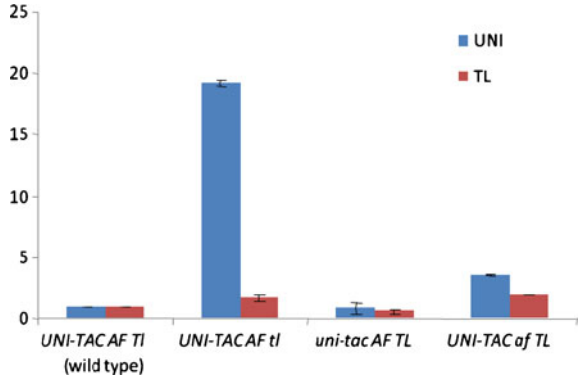

Figure 5. Transcriptional expression of *UNI* (*UNI-TAC*) and *TL* genes in the apices of the *in vitro* grown shoots of wild-type and *uni-tac*, *af* and *tl* mutants in *Pisum sativum*. The transcriptional levels are expressed with reference to those in wild-type, taken as 1.

initiation or stability of UNI m-RNA in the mutant (DeMason and Chetty 2011). Higher transcription levels in *af* and *tl* are in agreement with the observation that both *AF* and *TL* genes downregulate UNI in wild-type (Mishra *et al.* 2009). *TL* transcript level was also lower in *uni-tac* than in wild-type (figure 5). The observations summarized in figure 6 show that NPA significantly lowered the transcriptional expression of both *UNI* and *TL* genes in all genotypes. Further, IAA increased expression of *UNI* in wild-type, *af* and *tl* shoots, but not in *uni-tac* shoots. IAA also increased the expression of *TL* in wild-type and *af* shoots. These results suggested that the site that responded to promotion of *UNI* transcription by IAA was missing in *uni-tac* mutant and that stimulation of *TL* transcription required *UNI* product.

Discussion

In the results reported above, leaves and stipules of variant morphologies born on *P. sativum* shoots grown in presence of ATIs were described. Their significance with reference to leaves and stipules of corresponding structures described in angiosperm species in general, and leaf and stipule phenotypes described for the stable mutants of model plants are discussed below.

Variation in leaf and stipule forms in ATI treated P. sativum shoots

High degree of correspondence was noticed between stipules and leaves of *P. sativum* mutants and of certain angiosperm species and ATI induced abnormal stipules and leaves. Among the four genotypes of *P. sativum* investigated here, the responses of wild-type, *uni-tac* and *tl*, that bear tendril(s)

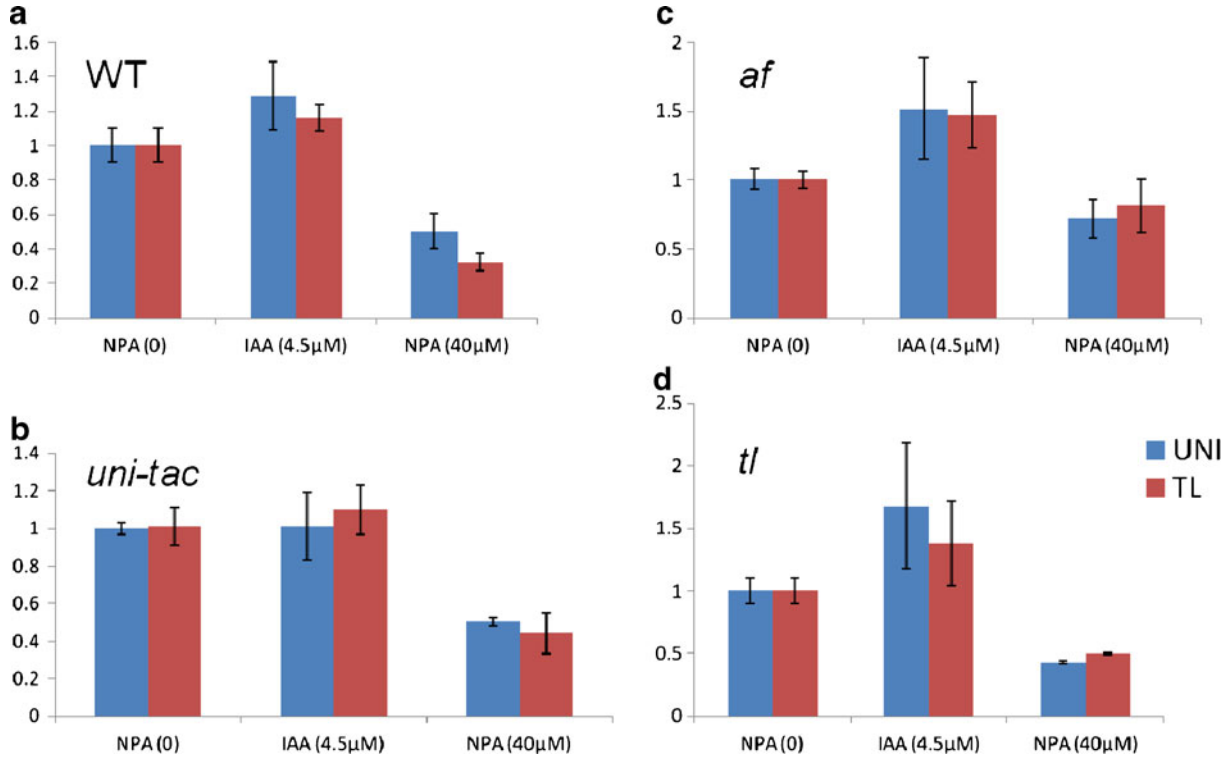

Figure 6. Transcriptional expression of *UNI* (*UNI-TAC*) and *TL* genes in the apices of the shoots of wild-type (a) and *uni-tac* (b), *af* (c) and *tl* (d) grown *in vitro* in the absence of any elicitor (control) and presence of 40 μM NPA or 4.5 μM IAA. The transcriptional levels for each genotype are expressed with reference to the respective control.

Table 17. Definitions and examples of some forms of leaves described among angiosperms.

Leaf type		Description	Plant species in which reported/identified	Family	Reference(s)
Tripinnate leaf[a]		Pinnately compound leaf with pinnae which are bipinnately compound	*Oenanthe phellandrium*	Apiaceae	Lindley (1848)
			Moringa oleifera	Moringaceae	Roloff *et al.* (2009)
			Thalictrum minus	Ranunculaceae	Lindley (1848)
Bipinnate leaf[b]		Leaf is divided twice and each pinna is subdivided into smaller leaflets	*Aralia spinosa*	Araliaceae	www.floridasnature.com
			Mimosa julibrissin	Fabaceae	Lindley (1848)
			Melia azederach	Meliaceae	Ruschenberger (1854)
Unipinnate leaf	Multifoliate leaf[c]	Lamina is divided only once in a pinnate manner; rachis has more than four pinnae (leaflets or combination of leaflets and tendril(s))	*Azadirachta indica*	Meliaceae	Verma (2011)
			Robinia pseudoacacia	Fabaceae	Davy (1902)
			Cleome viscosa	Cleomaceae	Pandey and Mishra (2008)
			Pisum sativum	Fabaceae	Mishra *et al.* (2009)
	Quadrifoliate leaf	Rachis with two pairs of leaflets/tendrils	*Arachis hypogea*	Fabaceae	Maheshwari (1963)
			Cassia absus	Fabaceae	Ruschenberger (1854)
			Paris quadrifolia	Melanthiaceae	www.botanical.com/botanical
	Trifoliate leaf[d]	Leaf rachis with three leaflets/tendrils	*Commiphora wightii*	Burseraceae	George (1832)
			Phaseolus vulgaris	Fabaceae	Maheshwari (1963)
			Acer triflorum	Sapindaceae	www.efloras.org
			Aegle marmelos	Rutaceae	Chauhan (1999)
	Binate leaf	Leaf rachis with two leaflets/tendrils	*Jeffersonia diphylla*	Berberidaceae	www.ontariowildflowers.com
			Zornia diphylla	Fabaceae	www.fao.org
			Cliffortia pulchella	Rosaceae	Rees (1820)
			Cattleya violacea	Orchidaceae	www.orchidsaustralia.com
			Balanites aegyptiaca	Zygophyllaceae	Don (1831)
Simple leaf	Tendril[e]	Leaf is a tendril	*Lathyrus aphaca*	Fabaceae	Goebel and Balfour (1900) Sharma and Kumar (2012)
	Leaflet[f]	Single bifacial structure sessile or petiolated	*Alliaria petiolata*	Brassicaceae	www.efloras.org
			Euonymus fortune	Celastraceae	Petrides (1973)
			Cercis canadensis	Fabaceae	Viertel (1970)
	Amplexicaul leaf	Simple leaf with its base clasping the stem	*Dracaena sanderiana*	Asparagaceae	www.zipcodezoo.com/Plants
			Lamium amplexicaule	Lamiaceae	Thornton and Lee (1818)
			Pandanus spinulosus	Pandanaceae	John (1963)
			Plantago orzuiensis	Plantaginaceae	www.bioone.org
	Perfoliate leaf	Base of simple leaf surrounds the stem; leaf appears to be pierced by the stem	*Bupleurum rotundifolium*	Apiaceae	Anonymous (1854)
			Uvularia perfoliata	Colchicaceae	Wood (1855)
			Baptisia perfoliata	Fabaceae	Gray (1870)
	Connate leaf	Base of the opposite leaves fused together around the stem; the structure appears like a simple leaf	*Mimulus androsaceus*	Caprifoliaceae	Comstock (1836)
			Diplosoma retroversum	Mesembryanthemaceae	Hartmann (2002)
			Lonicera flava	Phrymaceae	Gray (1870)
	Sheathing leaf	Base of simple leaf entirely wrapped around stem	*Pontederia cordata*	Pontederiaceae	Magee and Ahles (2007)
			Zostera marina	Zosteraceae	Magee and Ahles (2007)
			Najas gracillima	Najadaceae	Magee and Ahles (2007)

[a–f]These leaf forms are seen in Mendelian mutants of *Pisum sativum*. a *af tl*; b *af tl uni-tac*; c wild-type; d *stp tl* or *uni-tac stp*; e *uni af*; f *uni*. All other leaf forms are seen on shoots of *P. sativum* grown *in vitro* in the presence of the auxin transport inhibitor 1, N-naphthylpthalmic acid.

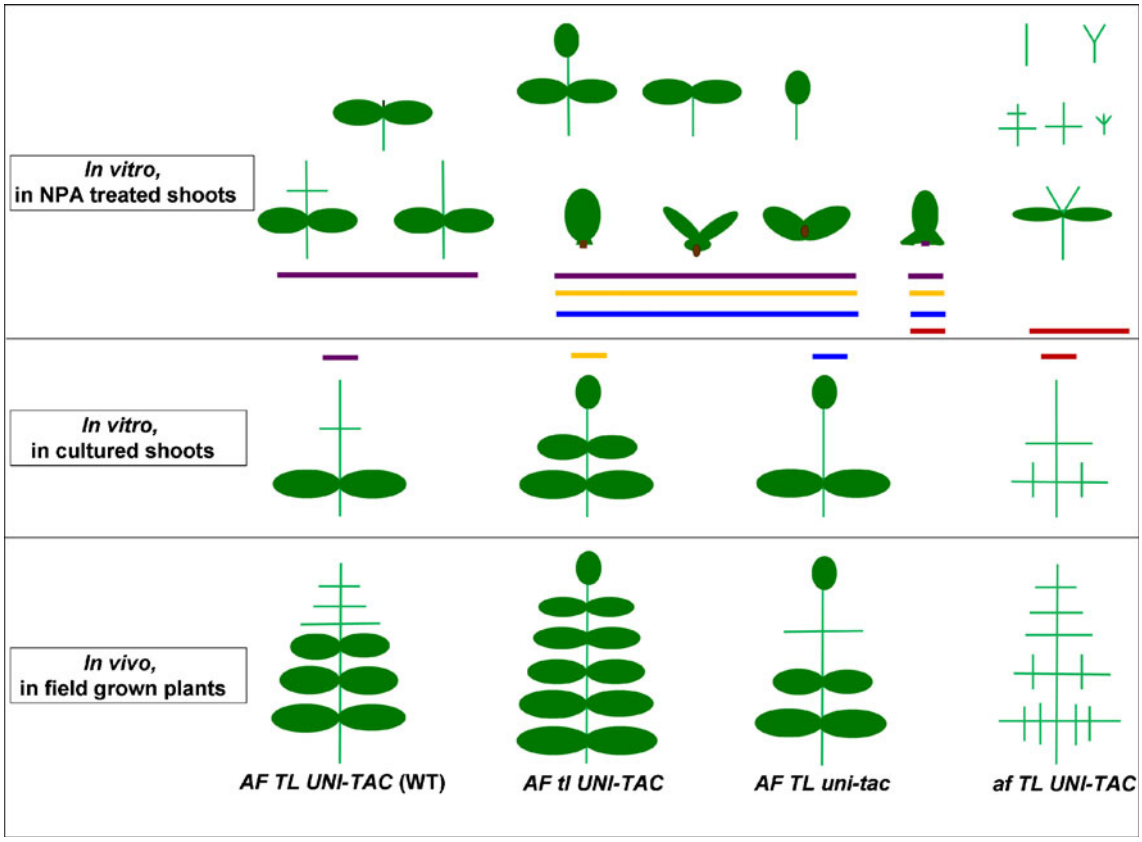

Figure 7. Diagrammatic depiction of the origins of various forms of leaves borne in plants/shoots of wild-type, *tl*, *uni-tac* and *af* genotypes of *Pisum sativum* grown in soil and *in vitro* in the presence and absence of auxin transport inhibitor(s) (NPA, TIBA, NOA, or PCIB). The leaves formed on soil grown plants are larger and more complex than those formed on shoots grown *in vitro* on basal medium. Pinnate leaves bearing both leaflets and tendrils but of less complexity are formed on *in vitro* grown shoots of WT grown in presence of NPA (purple colour). Trifoliate, binate and simple leaves and adnate simple and binnate leaves and connate and perfoliate leaves arising from fusion of stipules with and without involvement of leaf are formed on NPA^+ *in vitro* grown shoots of WT (purple), *tl* (yellow) and *uni-tac* (blue) genotypes. The *af* NPA^+ (red) *in vitro* grown shoots form leaves bearing one to five simple tendrils, leaves in which a few leaflets replace tendrils and perfoliate leaf arising out of fusion of stipules and leaf primordium.

and or leaflets on their leaves, to ATI treatments were similar. Leaves of single, binate and three leaflet (trifoliate) compositions were formed along with those of other configurations, but all of them were of less than normal complexity. In the ATI treated *af* shoots, leaves bearing single, two or three simple tendrils were observed together with tendriller leaves of higher complexity. The compound leaves formed in the presence of ATI maintained their imparipinnate character, except in the binately leafleted or tendrilled leaves. Unipinnate leaves of *ins* or *mfp* type and those possessing higher levels of complexity such as that in *af tl*, *af tl uni-tac* and *af tl mfp* were not observed on any of the auxin or ATI treated shoots. The aberrant leaf differentiation observed in the presence of ATI was associated with downregulation of *UNI* as well as *TL* expression in shoot apices of all the four genotypes investigated. Some leaf architectures corresponding to ATI induced structures are known in model species and angiosperms in general. Leaves are trifoliate in wild-type *M. truncatula*, and *uni* and *sgl1* mutant leaves respectively of *P. sativum* and *M. truncatula* are single leafleted

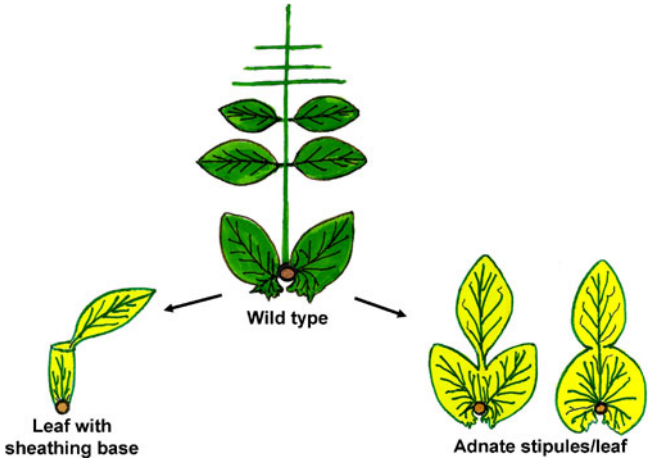

Figure 8. Induced somatic variation in the structure of stipules and leaves in *Pisum sativum*. A study of the reported experiments has shown that the wild-type *P. sativum* has the genetic potential for adnate and sheathing base type of leaves which are formed by fusion of stipules and leaf. Sheathed leaves are common among monocotyledons and adnate leaves among dicotyledons.

Table 18. Definitions and examples of different kinds of laminated stipules described among angiosperms.

Stipule type	Description	Plant species in which reported/identified	Family	Reference
Foliaceous	Stipule is leaf-like of large size. The pair is attached to stem, one on either side of leaf petiole, they overlap to produce a peltate structure at the node	*Pisum sativum*	Fabaceae	Sambamurty (2005); Kumar *et al.* (2013)[b]
		Medicago lupulina	Fabaceae	www.missouriplants.com
		Tetrathylacium johnsenii	Flacourtiaceae/Salicaceae	Croat (1978)
		Passiflora subpeltata	Passifloraceae	Fascicle (1958)
		Chaenomeles speciosa	Rosaceae	www.hsu.edu
		Psychotria capitata	Rubiaceae	Croat (1978)
		Salix lasiandra	Salicaceae	www.floras.org
Free lateral	Stipules are of relatively smaller size, the pair is attached to stem node, one on either side of leaf petiole attachment site, such that they do not overlap with each other	*Sauropus bacciformis*	Euphorbiaceae	www.efloras.org
		st mutants of *Pisum sativum*	Fabaceae	Pellew and Sverdrup (1923); Kumar *et al.* (2013)
		Gossypium hirsutum	Malvaceae	Sambamurty (2005)
		Melianthus major	Melianthaceae	Society for the Diffusion of Useful Knowledge (1842)
		Morus alba	Moraceae	Verma (2011)
		Obetia radula	Urticaceae	Verdcourt (1989)
		Urtica angustifolia	Urticaceae	www.efloras.org
		Viola tricolor	Violaceae	Moll (1934)
Leaf-like	The stipules have compound structure, architecturally similar to that of the accompanying leaf	*Delonix regia*	Caesalpinioideae	Sharma *et al.* (2012b)
		Caesalpinia bonduc	Caesalpinioideae	Sharma *et al.* (2012b)
		coch mutants of *Pisum sativum*	Fabaceae	Blixt (1967)
		Azara microphylla	Flacourtiaceae/Salicaceae	Charlton (1991, 1998)
		Talisia croatii	Sapindaceae	Condit *et al.* (2010)
Adnate[a]	Stipules on each side of leaf get fused to petiole up to a part or whole of it	*Drosera rotundifolia*	Droseraceae	www.omnisterra.com/botany
		Arachis hypogaea	Fabaceae	Grisebach (1864)
		Pelargonium crassipes	Geraniaceae	Harvey and Sonder (1860)
		Potamogeton bicupulatus	Potamogetonaceae	www.michiganflora.net
		Ranunculus subrigidus	Ranunculaceae	www.nature.ca/aaflora
		Rosa centifolia	Rosaceae	Shah (2009)
		Viola decumbens	Violaceae	Harvey and Sonder (1860)
Intrapetiolar[a]	Stipule pair fuses along their inner margins. The fused stipules now become axillary to the leaf petiole	*Cryptosepalum maraviense*	Apocynaceae	www.zimbabweflora.co.zw
		Anthocleista grandiflora	Caesalpinioideae	www.zimbabweflora.co.zw
		Saraca indica	Loganiaceae	Mitra (1950)
		Bersama tysoniana	Melianthaceae	Wyk and Wyk (1997)
		Platanus occidentalis	Platanaceae	Darlington (1853)
		Leucosidea sericea	Rosaceae	Kubitzki (2004)
		Prunus padus	Rosaceae	Barton (1804)
		Gardenia jasminoide	Rubiaceae	Mitra (1950)

Table 18 (*contd.*)

Stipule type	Description	Plant species in which reported/identified	Family	Reference
Opposite[a]	Stipules at a node fuse along their outer margins. The fusion product now lies opposite to the attachment side of the petiole on the stem	*Ricinus communis*	Euphorbiaceae	Moll (1934)
		Astragalus onobrychis	Leguminosae	www.colinherb.com
		Alchemilla vulgaris	Rosaceae	Moll (1934)
Interpetiolar[a]	These are formed by the oppositely placed leaves on a node. One stipule of each of the two leaves on a side fuse. Such fusion also occurs on the other side. Two joint stipules are formed. These are present on either side of the two petioles of the opposite leaves	*Schefflera actinophylla*	Araliaceae	www.plantnet.rbgsyd.nsw.gov.au
		Buddleja salviifolia	Buddlejaceae	Wyk and Wyk (1997)
		Macrocarpaea zophoflora	Gentianaceae	Harvard University (2002)
		Spigelia anthelmia	Loganiaceae	www.plantnet.rbgsyd.nsw.gov.au
		Cassipourea elliptica	Rhizophoraceae	Wyk and Wyk (1997)
		Cephalanthus occidentalis	Rubiaceae	De Jussieu (1849)
		Galium cruciata	Rubiaceae	Strasburger *et al.* (1976)
		Pauridiantha symplocoides	Rubiaceae	www.zimbabweflora.co.zw
Ochreate[a]	The two stipules fuse with each other along both the sides to form a tube which encircles the internode stem to some distance	*Potentilla ochreata*	Fruticosae	Shah and Wilcock (1991)
		Ficus elastica	Moraceae	Moll (1934)
		Platanus occidentalis	Platanaceae	Moll (1934)
		Rheum rhaponticum	Polygonaceae	Moll (1934)
		Galium cruciata	Rubiaceae	Strasburger *et al.* (1976)
		Dentella repens	Rubiaceae	Strasburger *et al.* (1976)
		Calamus daemonorops	Sparidae	Sharma (1993)
Exstipulate	Stipules are absent from the nodes that continue to bear leaf	*coch st* mutants of *Pisum sativum*	Fabaceae	Kumar *et al.* (2009)
		Sophora tomentosa	Fabaceae	www.efloras.org
		Scolopia oreophila	Salicaceae	Schmidt *et al.* (2002)

[a]These kinds of stipules are seen on 1, N-naphthylphthalamic acid treated *in vitro* grown shoots of *Pisum sativum*; [b]This paper gives all the other related references.

(Wang et al. 2008). Single tendrilled leaves are formed on *uni af* double mutant (table 1). Angiosperm species that bear one, two or three leaflets and single tendril on their leaves have been described (table 17).

Unifoliate and binately compound adnate leaves in which inner margins of the stipules had fused with petiole were seen in ATI treated shoots, especially of wild-type, *unitac* and *tl* genotypes (figures 2, 7 and 8). Such structures are not known among mutants of model plants. However, angiosperm species that bear unifoliate and compound adnate leaves are known (table 18). Stipules in which inner, outer or both margins had fused to give rise to intrapetiolar, opposite and ochreate stipule arrangements with respect to leaf were observed in ATI treated shoots (figure 3). Also we observed oppositely placed leaves whose stipules had fused to form interpetiolar arrangements between stipules and leaves. All different kinds of fused stipules retained the stipule characteristic of multiple primary veins (figures 3, 7 and 9). Angiosperm species possessing corresponding structures are known (table 18).

ATI treated shoots produced nodes in which stipules were fused and associated leaf was absent. The fused stipules had assumed the amplexicaul, perfoliate and gamophyllous (or connate) -like sessile leaf configurations. These stipule-leaf structures had also retained the multi-primary-vein character of stipules (figures 3 and 9). A mutant is known in *P. sativum* in which structure corresponding to perfoliate fused stipules described above was present (Kumar and Sharma 1975). Angiosperm species with connate, perfoliate and amplexicaul type of sessile leaves have been described (table 17). These leaves also possess the multi-primary-vein feature. It is highly significant that ATI induced fused stipular structure(s) occur naturally and inheritably in angiosperms in general and in *P. sativum* mutant.

The observations altogether suggest that angiosperms have three kinds of leaves. Decurrent or petiolated simple or variously compounded leaves. Laminae of these leaves or of their subunits have only one primary vein. These are the conventional angiosperm leaves. The second kind of leaves are the adnate stipule-leaf types which get formed by fusion of stipules and leaf. The adnate stipule-leaf kinds of leaves have both stipular and leaf-like venation in the respective structural domains of such leaves. The third kind of leaf is the stipule(s). Stipules are being called leaf(ves) here because ATI treatment turned them in to amplexicauly, perfoliately and connately sessile leaf-like structures. Since all kinds of

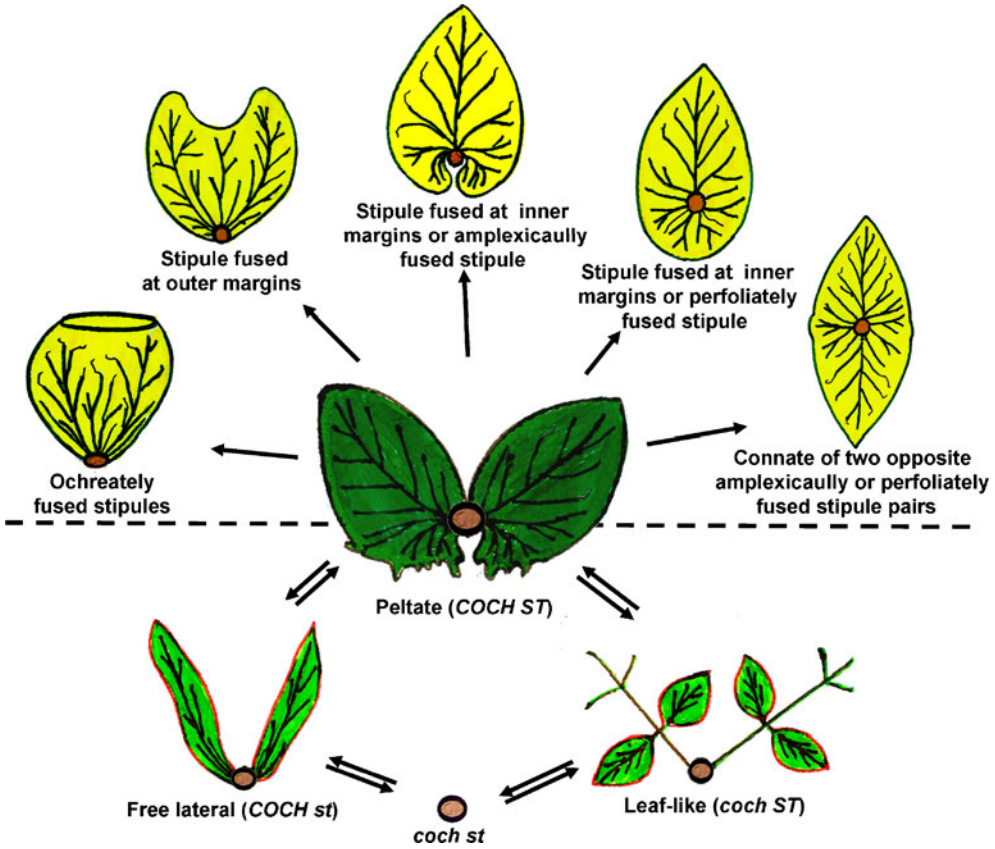

Figure 9. Genetic and somatic induced variation in the structure of stipules in *Pisum sativum*. The observed variation has shown that the wild-type *P. sativum* has gene regulatory network for all the eight morphologies and also for the absence of stipules. Therefore, wild-type stipules have the genetical potential to evolve into ochreate, lobed, amplexicaul, perfoliate and connate sessile leaves (yellow coloured), in addition to the *st* and *coch* stipules (green coloured).

stipules and simple to highly compound leaves are represented among stipule and leaf mutants and somatic variants formed on ATI treated shoots of *P. sativum*, it is possible to conclude that the basic gene regulatory network responsible for a variety of simple and compound forms of leaves is present in wild-type *P. sativum*. Changes in the pathways of expression of the concerned genes and in concentrations of gene products at site of their action in meristem have resulted in leaves and stipules assuming different forms.

Gene regulatory networking in P. sativum leaf and stipule morphology variants

Previous and present genetic experiments in *P. sativum* have permitted assignment of functions to known genes involved in development of compound leaf and foliaceous stipules. How do the deficiency of their activities, caused by heritable mutations or perturbations induced by ATI, affect leaf and stipule development processes is discussed below.

Leaf morphology variants

In *P. sativum*, primordia for two stipules and a leaf are produced laterally at adjacent sites where a node is formed, in the upward growing shoot apical meristem (Kumar *et al.* 2013). Activated by the complex of UNI and STP, meristem of leaf primordium grows acropetally to produce 15 pinnae sub-primordia on a rachis (Taylor *et al*. 2001; V. Sharma and S. Kumar unpublished observations). This *UNI*-led proximo-distal growth is made determinate by the *AF*, *INS*, *TL* and *MFP* major genes (Mishra *et al.* 2009; Kumar *et al.* 2010). Medio-lateral growth of sub-primordia (um) (i) in proximal domain is activated by *UNI*, *AF* and *INS* to form leaflets, (ii) in distal domain it is activated by *UNI*, *TL* and *MFP* to form tendrils, and (iii) at terminal position by *UNI*, *AF*, *TL* and *MFP* to form tendril (Mishra *et al.* 2009; Kumar *et al.* 2010). *UNI* acts as the master regulator of compound leaf gene network because it activates the synthesis of STP, AF, INS, TL and MFP and also acts in complex with them (Kumar *et al.* 2010). In consonance with the current ideas, each leaf and pinna primordium is formed at the site of auxin maxima created in meristem by the auxin transport efflux *PIN-FORMED 1* (*PIN1*) family of proteins (Aida *et al.* 2002; Benkova *et al.* 2003; Reinhardt *et al.* 2003; Barkoulas *et al.* 2008; Veit 2009). Primordium is separated from the surrounding cells/tissues by the activity of *CUP-SHAPED COTYLEDON* (*CUC*) class of transcription factors which make the separating cells relatively quiescent (Aida *et al.* 1999, 2002; Benkova *et al.* 2003; Furutani *et al.* 2004; Blein *et al.* 2008; Veit 2009; Hasson *et al.* 2010). Bifaciality in leaflets, similar to that in simple leaves, is attained by differentiation of some cell layers in developing leaf into adaxial type promoted by class III *HOMEODOMAIN-LEUCINE ZIPPER* (*HD-ZIP* III) transcription factors (McConnel *et al.* 2001; Xu *et al.* 2003; Prigge *et al.* 2005). Concurrently, differentiation of juxtaposed cell layers into abaxial type is promoted by *KANAD1* (*KAN*) family transcription factors and class I *AUXIN RESPONSE FACTORS* (*ARF*) such as *ETT* (*ARF3*) and *ARF4* (Pekker *et al.* 2005; Hunter *et al.* 2006; Garcia *et al.* 2006; Fahlgren *et al.* 2006; Chitwood *et al.* 2007; Kidner 2010). Accumulation of *HD-ZIP III* transcripts in abaxialized cells is curtailed by microRNA 165/166 and of *ARF* in adaxial cells by trans-acting small interfering RNA (ta-siRNA) (Williams *et al.* 2005; Nogueira *et al.* 2007; Zhou *et al.* 2007; Chitwood *et al.* 2009). YABBY group of transcription factors act in adaxialized/abaxialized region and promote lamina development (Eshed *et al.* 2004; Hasson *et al.* 2010).

The reduction in leaf complexity following ATI treatment was in agreement with the known strong inhibitory effect of ATI such as NPA on *PIN1* expression (Geldner *et al.* 2001; Scanlon 2003). ATI treatment generally decreased *PIN1* facilitated auxin in meristematic cells such that the capacity of stem cells to divide and thereby the size of meristem were reduced. In leaves bearing single leaflet or a tendril, the leaf primordium meristem itself got consumed. Leaves of binate leaflets or tendrils apparently developed from division of leaf primordium meristem into two pinna primordia. By analogy, in trifoliate leaves, the meristematic cells of the leaf primordia had been consumed by the subprimordia for three pinnae. The structures of leaves formed under ATI treatments suggest that compound leaf growth occurs in a manner similar to that of the shoot (Vercruyssen *et al.* 2011). Rachis growth occurs like that of stem; it occurs downward below the site of differentiation of primordia for organs at rachis node, like growth of stem internode (Bryan *et al.* 2012). Compound leaves formed under ATI treatments were imparipinnately or paripinnately compound. Treatment with auxins did not change the uni-imparipinnate architecture of leaves, it only marginally increased the pinna number. Leaf architecture is changed by recessive loss-of-function mutations (figures 10 and 11) in *AF*, *INS*, *TL* and *MFP* genes; their combinations make leaf more ramified than in wild-type (Mishra *et al.* 2009; Kumar *et al.* 2010). Against upto 15 pinnae in wild-type leaves, *af tl*, *af mfp* and *af tl mfp* leaves, respectively produce upto 200, 319 and 641 leaflets, arranged in tetra-pinnately or tri-pinnately in proximal domain and tri-pinnately or bi-pinnately in distal domain (Mishra *et al.* 2009). The *stp* recessive mutation and *uni-tac* semidominant mutation reduce the complexity of leaf (Taylor *et al.* 2001; Mishra *et al*. 2009; S. Kumar, unpublished observations). Recessive *uni* mutation(s) make the leaf simple (Hofer *et al.* 1997). Some of leaf structures, less complex than the compound wild-type leaf structure are seen in ATI treatments (DeMason and Chawla 2004a, b; present study). This indicates correspondence between the *UNI* deficient pathways of leaf development arising from mutation(s) on one hand and perturbations caused by ATI on the other hand (figure 10). This means that *UNI* promoted meristematic activity for acropetal leaf growth is negatively controlled, by the ATI effect on *PIN1* activity. Formation of simple leaf in *uni* perhaps means that UNI

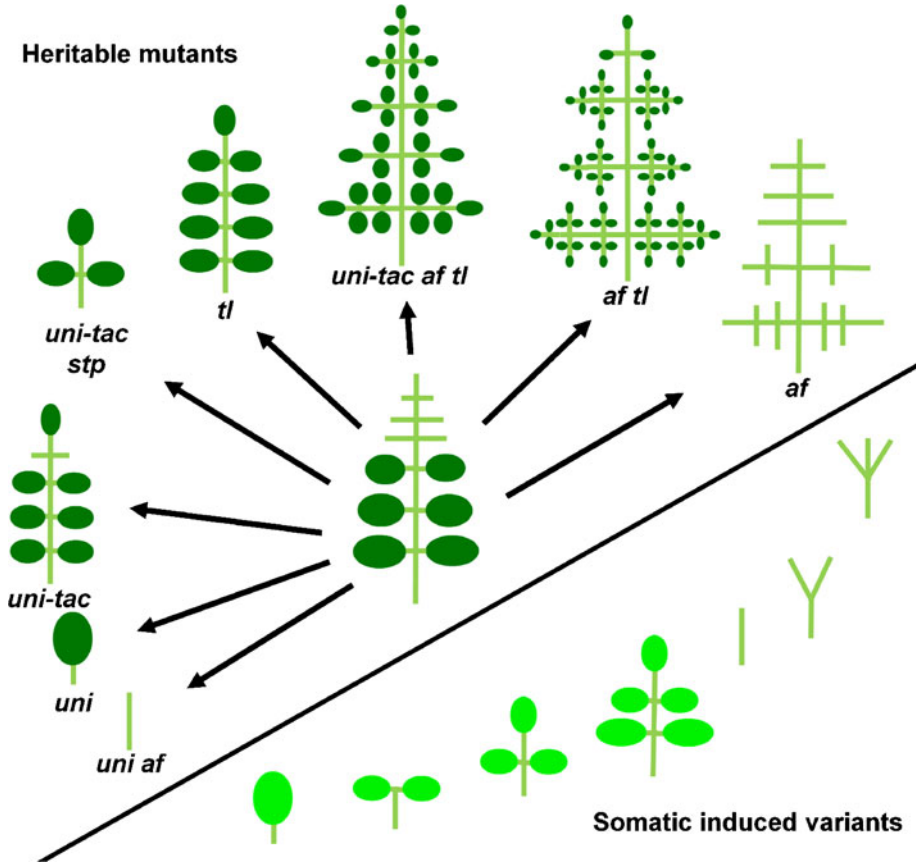

Figure 10. Some of the genetic and induced somatic variation in leaf architecture of *Pisum sativum*. Wild-type has the genetic potential for all the different kinds of leaf forms shown here and more complex forms.

function, downstream of PIN1 and CUC functions, that normally activates the compound leaf rachis growth is unavailable in required concentration. This finds experimental support in the underexpression of UNI in the presence of AT1 in present study. In ATI treatments, on some nodes leaf was absent although stipules were present, albeit in fused form. This perhaps means that stipule primordium formation precedes leaf primordium formation. At such nodes the stock of stem cells afforded by shoot apical meristem for primordia formation was exhausted by stipule primordia and stem cells for leaf primordium formation were not available in sufficient numbers.

Stipule morphology variants

The stipules are largely similar to leaflets in morphology and anatomy, and their development is affected by ATI (Sharma *et al.* 2012a; Kumar *et al.* 2013). Therefore, the genetic mechanisms of stipule and leaf primordia development may be similar. Since stipules are bifacial-like leaflets the two types of structures may also share the process of lamina formation.

Nodes in ATI treatments often produced variously fused stipules (stipules fused along inner margins, outer margins, both margins, and inner margins with outer margins of intervening leaf primordium). This suggested that primordia for stipule pair were formed together and ATI interfered with NAC type (*CUC*) transcription factor functions required for primordia delimitation (Blein *et al.* 2008). Thus there must be a time interval between primordia initiation and their delimitation in the presence of ATI. This allowed fusion of inner sides of stipule primordia with the margins of accompanying leaf primordium which had already separated leaflet primordium (ia) to finally produce an adnate stipule-leaf. An infertile-induced mutant in which stipules were perfoliately fused has been reported (Kumar and Sharma 1975). True breeding mutations in three major genes *CIST*, *COCH* and *ST* are known to change the regulation of stipule development (Kumar and Sharma 1975; Kumar *et al.* 2009). *cist* stipules are perfoliate structures unaccompanied by leaf. The *st* stipules are of much smaller in size, shaped as knife-like blades and arranged on stem free from each other, unlike the foliaceous peltately arranged wild-type stipules of large size. Leafblade like compound stipules are formed in *coch* mutants. *coch* stipules respectively assume the structure of *af*, *tl*, *mfp*, *af tl*, *af mfp*, *af tl mfp* leaves in *coch af*, *coch tl*, *coch mfp*, *coch af tl*, *coch af mfp* and *coch af tl mfp* genotypes. *COCH* performs several functions, including an

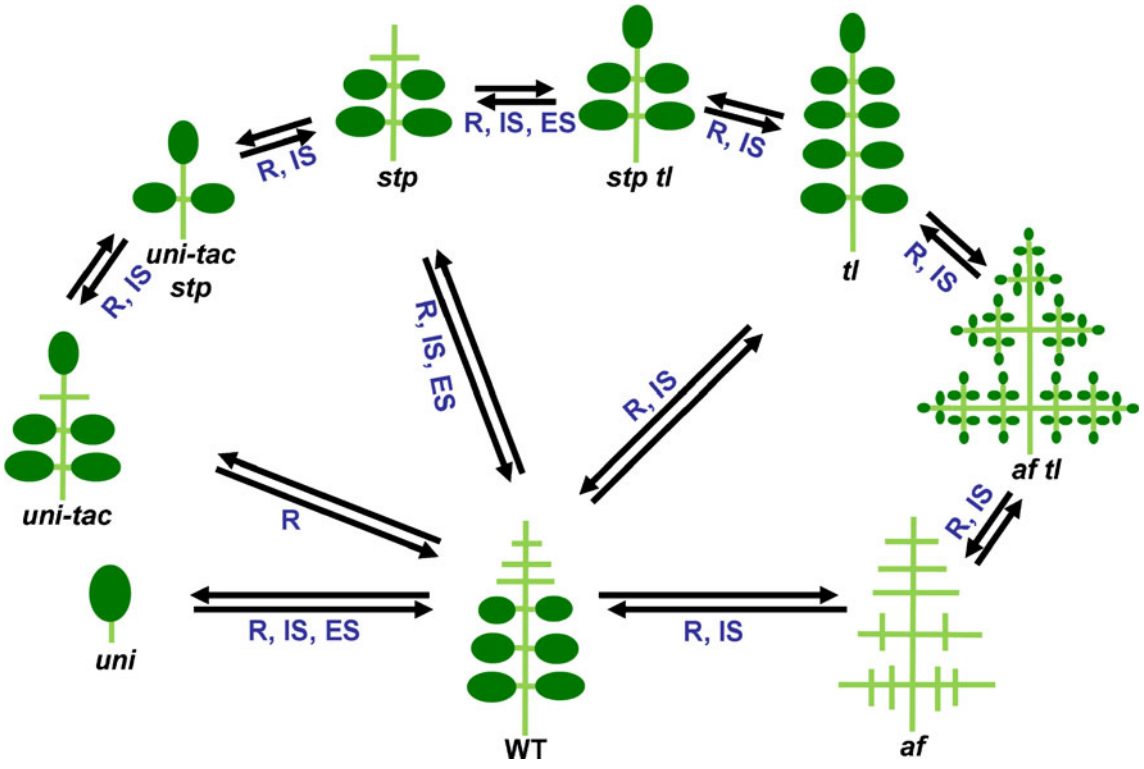

Figure 11. Genetically stable wild-type and mutant leaf architectures in *Pisum sativum*. Single gene or promoter mutations decrease or increase complexity of leaf architecture. Two loss of function forward mutations *af* and *tl* convert unipinnately compound leaf into bi-/tri-pinnately compound leaf. *uni* loss of function forward mutation converts compound leaf into simple leaf. Reversion mutations (R), intragenic suppressor mutations (IR) or extragenic suppressor mutations (ES) in respect of *uni* gene mutation can revert simple leaf to compound leaf condition. Another forward mutation in *UNI* or the gene having homologous function activated by ES mutation can bring back simple leaf phenotype. This process can be repeated; environmental conditions will provide the selective conditions. Knowledge about gene regulatory network for compound leaf morphogenesis in *P. sativum* suggests a gene network of its kind represented the ancestral type for leaf morphology evolution.

essential role in initiation of stipule and together with *ST* promotion of growth and development of stipules, repression of *UNI*-pathway of compound leaf development in the stipule primordia and downregulation of *UNI* in leaf, inflorescence and flower primordia (Kumar *et al.* 2009; Sharma *et al.* 2012c). Compound stipule(s) are observed at very low frequency in ATI treatments (figure 3k), but the mode of their origin is not clear.

Stipule vis-a-vis leaf

P. sativum wild-type and *st* stipule are only qualitatively similar anatomically to *P. sativum* leaflet (Kumar *et al.* 2013; Sharma *et al.* 2012a,b). Venation is denser in stipules than in leaflet. Morphologically wild-type stipule demonstrates palmate features by possessing a lobe, toothed outer margin and several primary veins, proximal to stem. *st* stipules also have several primary veins. Wild-type and *st* stipules are laminated like leaflet but have superior hydraulics. Essentially, each *P. sativum* wild-type or *st* stipule is a sessile simple leaf/leaflet.

P. sativum coch mutant plants often produce nodes on which one stipule is compound and the other petiolated simple and other nodes in which one side of leaf is stipulate and the other side barren of stipule (Kumar *et al.* 2009). *uni coch* plants produce sessile simple leaflet-like stipules (Sharma *et al.* 2012c). In *coch st* double mutant plants stipules are absent from large majority of nodes (Kumar *et al.* 2009). These observations seem to agree with the suggestion that stipules are organs distinct from leaf (Rutishauser and Isler 2001).

Fused stipules of a variety of morphologies are formed on ATI treated *P. sativum* shoots (Kumar *et al.* 2013). Some of the fused stipules mimick the structures of amplexicaul, perfoliate and connate (gamophyllous) leaves which are sessile and bear more than one primary vein or palmate features on their laminae (figure 9). Another type of fusion product are adnate structures in which stipules are fused with the leaf upto a part or entire height of petiole (figure 8). Adnate, amplexicaul, perfoliate and connate leaf bearing angiosperm species are known (tables 17 and 18). Perfoliate stipules have been observed in *cist* mutant of *P. sativum* (Kumar and Sharma 1975).

Following inference is reached from the above observations. (i) In addition to laminate simple and compound organs conventionally called leaves, stipules comprise another form

of leaf because these may have given rise to sessile adnate, amplexicaul, perfoliate and connate leaves which on account of loss of separate leaf entity retain capacity to produce a bud or inflorescence in their axil a property of leaf. (ii) Stipules lack axillary bud because they originated from sessile leaves (perfoliate, amplexicaul, connate) when restitution of leaf occurred by the process of genetic reversion. (iii) Stipules and compound leaf in *P. sativum* are not distinct organs; foliaceous stipules and leaf together comprise a kind of stipulated leafy lateral organ (stipulate leaf) and they share a common gene regulatory network for their development.

Ancestral leaf

Angiosperm species bear leaves of variant forms, unlobed or lobed simple leaves and divided, dissected or compound complex leaves (complex leaves have been called as compound leaves) (Bharathan and Sinha 2001; Bharathan *et al.* 2002; Champagne *et al.* 2007; Geeta *et al.* 2012). Stipule and leaf morphological variation in the extant angiosperm species can be considered as the spectrum of forms selected for their bearers survival and widest distribution in the environment of their habitation. The ancestral leaf form could be simple or compound. Phylogenetic analyses of angiosperms have shown that simple leaved species were progenitors of compound leaved species more often than vice versa (Hickey and Doyle 1977; Cronquist 1988; Doyle 2007; Geeta *et al.* 2012). This has been interpreted to mean that simple leaf form is the ancestral form. The molecular genetic analysis of leaf forms in model species however, suggests that compound leaf was most probably the ancestral form.

It can be argued that the ancestral form will have the gene regulatory network for both simple and compound leaves. In simple leaved ancestral species the compound leaf regulatory network will be turned off by some mutation. Conrariwise the network for simple leaf will be mutationally blocked in favour of compound leaf formation. Simple leaved model systems have been studied in considerable detail. Various genetic manipulations including overexpression of genes assigned important roles in compound leaf formation have produced leaves that are atmost heavily serrated, toothed or lobed. But no variants with compound leaf have been observed (Cho *et al.* 2007; Efroni *et al.* 2010; Uchida *et al.* 2010). These observations imply that simple leaves of the types investigated are not the ancestral leaf forms.

Certain compound leaved species meet the above requirement in principle. In *P. sativum* and *M. truncatula*, a single loss-of-function mutation in *LFY* orthologs *UNI* and *SGL1* results in replacement of compound leaves by simple leaves (Hofer *et al.* 1997; Wang *et al.* 2008). Reversion or intragenic suppressor mutations will bring back the compound leaf morphology in *uni* and *sgl1* point mutants (figures 10 and 11). Although the requirement of compound→simple→compound leaf is met in principle, there is a complication that *uni* mutant of *P. sativum* and *sgl1* of *M. trucatula* are sterile. In *P. sativum*, *uni-tac* mutants that are deficient in *UNI* transcription and possess compound leaves, of much reduced complexity than wild-type, are fertile. It is visualized that there could be *uni* mutants (of *uni-tac* kinds) in which *UNI* product occurs in amounts sufficient for fertility but insufficient for leaf compounding. *KNOX1* class of genes that are responsible for leaf compounding in *C. hirsuta* and *S. lycopersicon* are also present in *P. sativum* but are not involved in leaf compounding. It is known that in *S. lycopersicon*, *G. max* and *L. japonicus*, *KNOX1* and *LFY* orthologs interactively determine leaf compounding (Busch and Gleissberg 2003; Champagne *et al.* 2007; Blein *et al.* 2008). It is hypothesized that mutation(s) activating the involvement of *KNOX1* gene may serve as extragenic suppressor(s) of *uni* mutants. AT1 treatments that affect *PIN1* activities were found here to induce simple leaves on *uni-tac* nodes. A combination of two mutations, one like *uni-tac* and another affecting *PIN1* activity could give rise to simple leaved fertile *P. sativum* mutant plants. Reversion mutation in any one of the two forward mutations would restore the compound leaf condition. If simple leaved angiosperms had origin in compound leaved angiosperms(s), explanation is required as to why transgenics of simple leaved *A. thaliana* overexpressing its *KNOX1* do not produce compound leaves. It is thought that a combination of deletion mutations in *COCH* and *ST* and a dominant deletion mutation in *UNI*, that reduces availability of altered *UNI* such that *UNI* led flowering occurs normally in company of simple leaf development, could produce a simple leaved genotype such as represented by *A. thaliana*. This hypothesis will require experimental support in *P. sativum*. At this time, of the two possibilities, the one that identifies compound leaf to be the ancestral leaf has more experimental support than simple leaf as the ancestral type (Sinha 1997; Busch and Gleissberg 2003). Further, it appears that forward or reverse mutation or duplication in the promoter or structural part of gene(s) leading to underexpression or overexpression / availability of product(s) of one or more of a small number of gene families (say 12) can provide a wide spectrum of morphogenetic variation in stipules and leaves of a species like *P. sativum*. Taking into consideration all the information about gene regulatory network for simple and compound leaved model plants it is surmized that there was an ancestral regulatory gene network for leaf development, comprising of a small number of genes in the early angiosperms. It was present in compound leaved earliest species and was much like that exists in *P. sativum*. Forward, reverse and extragenic suppressor mutations in the component genes of this network have in the main produced the simplest to highly compound stipule and leaf variation visualized in the extant angiosperms.

Concluding remarks

Auxin transport inhibition treated shoots of *P. sativum* of defined genotype were observed to have produced nodes that were devoid of leaf or possessed leaves of low

complexity such as simple, binate and trifoliate etc in comparision to multifoliate control leaves. The treated nodes produced all the known kinds of laminated angiosperm stipules. In addition amplexicaul, perfoliate and connate shaped stipules devoid of accompanying leaf were also produced. These observations together with genetic variation in leaf and stipule forms, known in *P. sativum* and in other model systems, was used to arrive at the following inferences. (i) Amplexicaul, perfoliate, connate and adnate sessile leaves have origin in foliaceous stipules and accompanying leaf of *P. sativum*-like ancestral angiosperm. (ii) Compound leaf of *P. sativum* type gene regulatory network may have been the ancestral leaf.

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