Role of *LLD*, a new locus for leaflet/pinna morphogenesis in *Pisum sativum*

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Properties of a mutant at the *LLD* (*LEAF-LET DEVELOPMENT*) locus in pea *Pisum sativum* L. are reported in this paper. Plants homozygous for the Mendelian recessive mutation *lld* bear leaves in which a few to many leaflets are incompletely developed. Opposite pinnae of rachis nodes often formed fused incompletely developed leaflets. The *lld* mutation was observed to abort pinna development at almost all morphogenetic stages. The *lld* mutation demonstrated high penetrance and low expressivity. The phenotypes of *lld* plants in *tl*, *tac*, *tl tac*, *tl af* and *tl af tac* backgrounds suggested that LLD function is involved in the separation of lateral adjacent blastozones differentiated on primary, secondary and tertiary rachides and lamina development in leaflets. The aborted development of tendrils and leaflets in *lld* mutants was related to deficiency in vascular tissue growth. The morphological and anatomical features of the leaflets formed on a *tl lld* double mutant permitted a model of basipetal leaflet development. The key steps of leaflet morphogenesis include origin of the lamina by splitting of a radially symmetrical growing pinna having abaxial outer surface, opposite to the vascular cylinder, through an invaginational groove, differentiation of adaxial surface along the outer boundary of split tissue in the groove and expansion of the lamina ridges so formed into lamina spans.

1. Introduction

In angiospermic plants, leaves are the primary organs for gas exchange, light capture, photosynthetic carbon fixation and heat transfer. The size, shape and structure of leaves in different species of plants is related to their ecological and evolutionary success in diverse environments (Sinha 1999; Pyankov *et al* 1999). Leaves are products of proliferation and differentiation of the leaf specific meristems initiated laterally by the shoot apical meristem (SAM). The SAM and axillary SAMs develop in the adaxial bases of cotyledons during embryogenesis and that of leaves in later stages of plant life respectively (Laux and Jurgans 1997) and serve as the progenitor of lateral organs. A SAM has a population of stem cells distributed in its central (CZ), peripheral (PZ) and rib (RZ) zones. The descendants of PZ cells generate the primordial meristems for the leaf and flower organs and those of RZ elongate the stem (Lenhard and Laux 1999). Spatial differences in the patterns of cell multiplication and gene expression in meristems are responsible for the sizes and shapes taken by the individual leaf, flower and embryo organs. From among a network of a large number of plant genes that may determine formation and maintenance of SAM and organ development generally and specifically, the roles of some have been identified.

In *Arabidopsis thaliana*, while the function of *SHOOT MERISTEMLESS* (*STM*) gene is required in the process of initiation of SAM (Barton and Poethig 1993), those of *STM*, *WUSCHEL* (*WUS*) and *PINHEAH* (*PNH*) and/or

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Abbreviations used: *af*, *afila*; *AGO*, *ARGONAUTE*; *Cu*, curl; *CUC*, *CUP-SHAPED COTYLEDON*; *LBL*, *LEAF BLADELESS*; LP, leaf primordium; Me, mouse ears; *NAM*, *NO APICAL MERISTEM*; *PHAB*, *PHABULOSA*; *PIN*, *PIN FORMED*; *PNH*, *PINHEAH*; SAM, shoot apical meristem; *STM*, *SHOOT MERISTEMLESS*; *tl*, *tendriless*; *uni*, unifoliata; WUS, WUSCHEL.

ARGONAUTE (*AGO*) and *REVOLUTA* keep the cells of growing SAM in meristematic state (McConnell and Barton 1995; Talbert *et al* 1995; Laux *et al* 1996; Lynn *et al* 1999). The *STM*, *PNH*, *AGO1* and *REVOLUTA* are also required for the axillary SAM initiation and maintenance and *AGO* and *REVOLUTA* in leaf development (McConnell and Barton 1995; Talbert *et al* 1995; Bohmert *et al* 1998). The down regulation of *STM* appears to be a requirement for the normal development of the organ primordia outgrown from *SAM* (Long *et al* 1996). Over expression of the *STM* analogues *KNOTTED* and *KNAT-1* genes of maize and *A. thaliana* respectively in homologous and/or heterologous plants is associated with ectopic meristem formation in leaves (Chuck *et al* 1996; Sinha *et al* 1993). The *CUP-SHAPED COTYLEDON-1* and *-2* (*CUC1* and *CUC2*) gene functions affect growing embryos such that in the mutants SAM-less and cup-shaped cotyledon bearing embryos are formed (Aida *et al* 1999). These and *UNUSUAL FLORAL ORGANS* gene (Levin and Meyerowitz 1995) in *A. thaliana* and its homologue *FIMBRIATA* (Ingram *et al* 1995, 1997) in *Antirrhinum majus* are involved in the initiation and maintenance of separate identity of primordial domains for leaf and floral organ development. The expression of *CLAVATA* (Lauf *et al* 1998) and *AINTEGUMENTA* (Mizukami and Fischer 2000) in SAM and/or primordia outgrown by SAM affect organ number and size at all stages of plant development, in *A. thaliana*. In all the angiosperms, leaf primordial meristems are also called blastozones (Hagemann and Gleissberg 1966). These arise in succession from PZ of SAM (Vernoux *et al* 2000) during their vegetative growth. As a result, a shoot is formed which bears leaves on stem and flower bearing inflorescences in the axils of leaf nodes in the reproductive phase of plant growth.

Leaves consist of a stem proximal petiole and a distal blade. In simple leaves the blade consists of a single lamina which may be lobed or dissected. The blade in compound leaves has many laminar units/leaflets, borne on a rachis. The leaf lobes in simple leaves are equivalent of leaflets in compound leaves. Leaf or leaflet morphogenesis involves meristematic activities in the proximo-distal, dorsi-ventral and centro-lateral directions (Szymkowiak and Sussex 1996; Tsukaya 2000). The compound leaf may have basipetal, acropetal or mixed patterns of leaflet initiation. The developmental programmes that leaf primordial meristems follow to form simple, basipetal compound and acropetal compound leaves may be mutually exclusive (Sinha 1997) or share certain features (Byrne *et al* 2001).

In *Arabidopsis* and maize, the leaf primordium establishment and its growth and expansion into simple mature leaf requires a reduction in levels of expression of class-I *KNOX* genes, including *KN-1*, *STM* and *KNAT-1* in SAM periphery, initiating leaf meristems and growing leaves (Vollbrecht *et al* 1990; Taylor 1997; Schneeberger *et al* 1998; Waites *et al* 1998). The *KNOX* genes that encode homeodomain-containing proteins appear to be present in diverse angiosperms (Bharathan *et al* 1999). The over-expression of *KNOX* genes in transgenic plants of tobacco and *Arabidopsis* leads to lobing in simple leaves (Lincoln *et al* 1994; Chuck *et al* 1996). This same phenotype is demonstrated by *KN-1* transgenics of *LA* lanceolate simple leaf bearing mutant of tomato (Dengler 1984; Hareven *et al* 1996). However, *KN-1* transgenics of wild type compound leaf bearing tomato strains form compound leaves having higher levels of ramification (Hareven *et al* 1996). Tomato plants having dominant mutations *Curl* (*Cu*) and *Mouse-ears* (*Me*) in which the inborn class I *KNOX* gene *KN-2* is expressed abundantly also have similar leaf morphologies (Parnis *et al* 1997; Janssen *et al* 1998). The above phenotypes and absence of compound leaved mutants in simple leaf bearing species has been used to give the following scheme for the basipetal compound leaf formation. Primary leaf meristem grows to give rise to lateral blastozones which in turn form higher levels of lateral blastozones. The lamina expansion from diffused meristems initiates after rachial blastozones development gets arrested (Hareven *et al* 1996).

Some of the genes that may promote and limit leaf compounding have been identified in *Pisum sativum*. A normal (wild type) leaf of pea is composed of a rachis bearing two or more pairs of proximal leaflets, two or more pairs of distal simple tendrils and a simple terminal tendril (Hofer and Ellis 1996). In the plants homozygous for the recessive *afila* (*af*) mutation (Kujala 1953), a compound tendril is produced in place of each leaflet (pinna). Acacia like leaves are formed by production of leaflets in place of tendrils in the homozygotes of *tendriless* (*tl*) mutation (White 1917). In plants homozygous for tendrilled acacia (*tac*) mutation (Sharma and Kumar 1981; Marx 1987), a leaflet is formed in place of a tendril at the terminal position of rachis; the proximal positions are occupied by leaflets and distal by tendrils. The leaves of the double mutant homozygous for *af* and *tac* mutations are tendrilled like those of *af* mutant but bear a leaflet at the terminus of each tendril (Marx 1987). The *af tl* homozygotes have compound pinnae that terminate in small leaflets and there are no tendrils (Marx 1987). Tendril-less leaves like those on *tl* mutant, bearing leaflet pairs in smaller numbers are formed on *tl tac* double homozygotes (Marx 1987). The triple mutant homozygous for *tl*, *af* and *tac* have no tendrils and highly compound system of pinnae in the proximal segment (Marx 1987).

In the homozygotes of *unifoliata* (*uni*) mutation, a simple leaf is borne on petiole and rachis and tendril(s) are absent; the leaves are lobed and bi- or tri-foliate in *uni tl*, *uni af* and *uni af tl* homozygotes respectively (Hofer and Ellis 1998). The *FALSIFLORA* gene of tomato,

FLORICAULA gene of snapdragon (Coen *et al* 1990) and *LEAFY* gene of *Arabidopsis* (Blazquez *et al* 1998) appear to be homologues of the pea *UNI* gene (Hofer *et al* 1997). Since the F_1 hybrid of *uni* and *tac* mutants has *uni* phenotype (Marx 1987) and the flower abnormalities of the kind noted in *uni* mutant(s) are not found in *tac* mutants, *UNI* gene may function upstream to *TAC* gene in the pathway of pea leaf morphogenesis. Thus both *UNI* and *TAC* genes appear to be involved in the process of blastozone development for acropetal growth in pea leaves. *AF* and *TL* genes are negative regulators of *UNI* and *TAC* gene expression (Lu *et al* 1996; Villani and DeMason 1999; Gourlay *et al* 2000; Prajapati S and Kumar S, unpublished results). It has been demonstrated that leaflet/pinna development in pea occurs by *AF*-dependent and -independent pathways. While *TAC* and *AF* are thought to complement the *AF*-dependent lamina dominated pinna growth, *TL* and *TAC* are identified as antogonists of *AF* – independent second pathway (Prajapati S and Kumar S, unpublished results).

The relative positioning of pinnae on the rachial axis and differentiation of each pinna along its three axes are the additional features that determine compound-leaf shape. The differentiation of midrib and of lateral dimensions in laminated pinna may occur sequentially or concurrently. The cell division number and degree of cell expansion are expected to regulate pinna size. The relative sizes of the differentiated epidermis, mesophyll and vascular system also appears to determine the pinna shape (Lloyd *et al* 1998). Although little progress has been made in the compound leaf bearing dicot plants, a few mutants affecting such functions are known in simple leaved dicot plants. *Arabidopsis* plants bearing *curly leaf* (*cu*) mutation bear narrow leaves in which number and size of cells is reduced (Kim *et al* 1998), cell expansion is affected in transverse, longitudinal or both the directions in *ANGUSTIFOLIA*, *ROTUNDIFOLIA-3* and *DIMINUTO* gene mutants respectively, in *Arabidopsis* (Takahashi *et al* 1995; Tsuge *et al* 1996).

In this study, we report the characteristics of a spontaneous novel mutant, the wild type allele of which is involved in the normal origin and development of pinnae for leaflets and tendrils in pea *P. sativum*. The effects of the highly penetrant and variably expressible mutation in pea on the three-dimensional structure and development of pinnae of acropetally-compound leaf are reported.

2. Materials and methods

2.1 Pisum sativum *leaf variants*

An abnormal plant, in which several leaflets of many leaves showed aborted growth, was observed in the field pea experimental crop grown at the Indian Agricultural Research Institute, New Delhi in the rabi (winter) season of 1986–1987. The plant was discovered in the F_2 generation of a cross involving the accession SKP-2 bearing *tl tl* acacia type leaves and Pusa-Harbhajan (Kumar and Sharma 1986) that has normal leaves. All the progeny of this plant raised in the next season bore leaves in which several leaflets occurred in the form of rudimentary pinnae. This accession was called SKP-200. The true breeding nature of the line suggested that it might be homozygous for a genetic locus which was called *lld*, an acronym for the lesion in leaflet development gene *LLD*. SKP-200 was backcrossed to Pusa-Harbhajan and a plant bearing the tl lld phenotype was picked up in F_2 generation. This process was repeated 5 times to obtain *tl lld* double mutants in the background of Pusa-Harbhajan. A plant bearing the tl lld phenotype in the F_2 generation of the final back cross was called the SKP-201. The SKP-201 *tl tl lld lld* was hybridized with Pusa-Harbhajan on one hand and an accession having *af af tac-2 tac-2* genotype in Pusa-Harbhajan background to recover various *lld lld* bearing segregants. The following genotypes could be identified and have been maintained: *lld lld*, *tac tac lld lld*, *tl tl af af lld lld*, *tac tac tl tl lld lld* and *tl tl af af tac tac lld lld*. The *af af tac-2 tac-2* had been derived by crossing an *af af tl tl* line with the *tac-2 tac-2* mutant isolated in Pusa-Harbhajan (Sharma and Kumar 1981).

2.2 *Field technique*

The experiments described here were carried out in a field plot of this institute at Lucknow during 1997 to 2001 (Prajapati 2001). Lucknow is located in the Indo-Gangetic plains at 26·5°N, 80·5°E and 120 m above the sea level and has three seasons: monsoon (from July to September); winter (from October to March) and summer from (April to June). Pea crop is grown in the area in the winter (rabi) season. Each season, the sandy-loam field plot was ploughed, levelled, irrigated, harrowed and applied 20 tons/ha of farmyard manure and 80, 50 and 50 kg/ha of N, P and K fertilizers respectively and harrowed and levelled. The field was cut into plots of 3 m × 5 m. The pea seeds were sown in 3 m rows spaced 75 cm apart, with a seeding rate of about 50 seeds per row for maintenance 20 seeds per row for parents to be used for hybridizations, 5–10 seeds per row for F_1s and 30 seeds per row for F_2 progeny. The seeds were sown in late October or early November and crop was harvested from middle of March to middle of April, as the cultures matured. Whole pods were harvested plant-wise and later threshed to obtain seeds. The growing crop was applied one spray each of 0·1% metacid and 0·1% dithane M-45

to prevent insect pests and fungal diseases respectively. The field was given two or three irrigations, one at the time of initiation of flowering in early January, second in early February and third in late February or early March. The crop was weeded as and when required. The entire field was protected from birds by erecting a net over it. The seeds were stored in trunks at room temperature. Tablets of Parad (Zandu Pharmaceutical Works Ltd., Mumbai) were spread among the envelopes in the trunk to limit growth of any stored grain pests.

2.3 *Morphological and growth studies*

The plants to be examined were individually labelled, and were observed three to six times, three weeks after the seeds were sowed to the time of harvesting. Up to 30 leaves were examined from each plant for the expression of various leaf shape determining markers used, at each rachis position.

2.4 *Inheritance studies*

All the accessions were grown in 4 rows that were randomly dispersed in the field. To hybridize the genetic stocks, flower buds of the female parent containing as yet undehisced anthers were emasculated in the evening and labelled. Late in the next morning, pollen was collected from the nascently opened flowers of the male parent and applied to the stigma of emasculated flowers. The hybridization for the inheritance studies on the *lld* marker were done in the rabi season of 1997–1998. The F_1 seeds from each cross and those of the parents were sown in the field in late October 1998. The F_1 plants were compared with the parent plants throughout their life cycle until they were harvested in March–April 1999. The F_2 seeds collected from F_1 plants and seeds of parents were sown in field in November 1999. The F_2 plants of each cross were individually labelled and notes were taken on their traits several times until they were harvested in April 2000. F_3 seeds were harvested from selected F_2 plants to confirm the phenotype recorded for their F_2 parents, in the 2000–2001 rabi season. c^2 tests were used to measure the goodness of fit of ratios of F_2 plants for the studied traits, considered singly or together, at the 0·05 probability level.

2.5 *Anatomical studies*

Leaves borne on node numbers 15 to 20 were studied. Samples were taken from *tl tl* and *tl tl lld lld* plants. Random samples of rachis, leaflet petiolules, leaflet lamina of *tl tl* leaves and rachis and petiolules and lamina of normal looking and aborted leaflets of *tl tl lld lld* leaves were sectioned transversely. Cylinders of about 2·5 cm length and 0·5 cm thickness were carved out of radish roots to serve as support for the experimental material to be sectioned. The radish cylinder was slit in the middle at one end and material to be sectioned was placed between the two strips of slit end of radish. A razor blade was used to cut sections. At least three samples of each kind of material were sectioned thrice. The fresh sections were stained with saturated saffranin, washed and mounted in 70% glycerine and examined using a Nikon Optiphot-Pol Inverted light microscope at 10 × and 40 × magnifications. The photomicrographs were taken with Nikon FX-35A camera using Nikon UFX-II photographic system and Kodak's coloured film roles of 100 × were used.

3. Results

3.1 *Isolation and genetic analysis of* lld *mutation*

The *lld* mutant line was isolated from among F_2 segregants of a cross between an accession carrying *tl* mutation in homozygous condition used as the female parent and a dwarf early maturing land race of field pea used as the male parent. The spontaneous *lld* mutation has been maintained in the *tl* background now for about 14 years. In *lld tl* double mutant plant, the lld phenotype is clearly visualized by the presence of abnormal leaflets on the leaves formed in adult vegetative and reproductive phases (figure 1A). The wild type leaflets (pinnae) borne on *tl tl* plants have obovate/ovate/oval-shaped fully formed lamina connected to rachis by short petiolule. Among the laterally paired leaflets formed on rachis the proximal ones have obovate to ovate shape and distal ones ovate to oval shape. The opposite leaflets at a rachis position develop from two separate meristems differentiated by acropetally growing leaf primordium. In the mutant (*tl tl lld lld*), many of the affected pinnae are represented by very small, slender needle/pin shaped structures to almost fully formed cup/spoon shaped laminated leaflets (figure 1B). Often a single cup shaped leaflet of variable size or two laterally fused normal sized leaflets are formed at proximal and distal rachis positions (figure 1C). The number of leaves formed was normal as was the distichous phyllotoxy. The morphologies of root, flowers, floral organs, pods and seeds also appeared to be normal in the mutant.

Several crosses were performed to determine the inheritance of *lld* mutation. In the reciprocal crosses between

Figure 1. Leaflet morphologies of *tl LLD* and *tl lld* leaves of *P. sativum*. (**A**) Wild type leaflets borne on *tl LLD* leaf (left), several leaflet pinnae are seen aborted in growth demonstrating lld-phenotype in *tl lld* leaf (right). (**B**) Spoon-/cup-shaped leaflets arrested in growth at different stages of development, seen on *tl lld* leaves. (**C**) Fused and funnel-/cup-shaped leaflets, formed from lateral opposite meristems of leaf rachis, arrested at different stages of development, seen on *tl lld* leaves.

lld mutant and *LLD* wile type leaf bearing parents (table 1), the leaflet development was generally normal in the F_1 plants, indicating that the *LLD* allele was dominant over *lld* allele. However a very small number of leaflets in a few F_1 plants were observed to be incompletely developed. The presence of anamolous leaflets indicated (i) partial dominance of *lld* over *LLD* in *LLD lld* heterozygotes, or (ii) somatic recombination based homogenenization leading to *lld lld* constitution in the concerned leaflet meristems of *LLD lld* heterozygote. The segregation pattern of plants possessing *LLD* and *lld* phenotypes in F_2 generation of these crosses confirmed that the *lld* allele was most likely inherited as a Mendelian recessive at the concerned locus. The phenotypes of F_1 and segregation patterns in F_2 progeny of crosses involving *lld* locus on one hand and *tl* or *tac* locus in *cis* or *trans* on the other hand (table 1) indicated that the *lld* locus assorted independently of the other two Mendalian recessive loci (White 1917).

3.2 *Expressivity of* lld *mutation at different phase of plant life cycle*

A large number of *lld tl* homozygotes were observed for leaf morphology from the stage of emergence of cotyledons in seedlings to plant maturity (figure 2). All the plants carried one or more abnormal leaves, typical of lld phenotype. The cotyledons and juvenile leaves borne on all the plants had normal morphology. There was progressive increase in the incidence of leaves bearing lld phenotype, as the plant progressed from juvenile, through vegetative adult to reproductive phases (table 2). The lld phenotype expression reached its peak as the plant entered its reproductive phase. The average incidence of lld expressing leaves in the post-juvenile to pre-flowering stages was about 58%. With the onset of flowering about 90% of the plants demonstrated lld phenotype in their leaves. Thus while the penetrance of lld mutation was absolute, the lld expressivity varied with the leaf position.

3.3 *Pinna-wise expressivity of* lld

The leaves borne on fifth to fifteenth node in *lld tl* homozygotes were examined pinna-wise through proximal to terminal positions of leaf rachis, for lld phenotype. The results summarized in table 3 show that pinnae at all the positions were affected by *lld* mutation, although with varying frequencies. The leaflets produced at the most proximal and most distal or terminal positions on leaf rachis were least affected by *lld* expression (about 3%), whereas average expressivity for all the positions taken together was about 8-fold higher (26·5%). The proximo-distal rachis positions could be placed in the following order in terms of *lld* expressivity: 3 > 4 > 2 and 5 > 6 > 1 and 7, where 1 is the most proximal and 7 is the most distal/terminal position.

Table 1. c^2 tests for 3 : 1 F_2 segregation of the *tac* and *lld* loci and 9 : 3 : 3 : 1 F_2 segregation for *tl* and *lld* and *tac* and *lld* loci in field pea *P. sativum*.

Relevant genotype/ cross configuration	Generation	Total number of plants studied	Number of plants (phenotype)								c^2	*P*
			TL LLD[a]	TL lld[b]	t l LLD	tl lld	TAC LLD	tac LLD	TAC lld	tac lld		
Wild type (P1)	Parent	100	100									
tl tl (P2)		100			100							
tl tl lld lld (P3)		100				100						
tac tac (P4)		100						100				
lld lld (P5)		100							100			
P1XP3	F_1	5	5									
P2 X P3		4			4							
P3X P2		29			29[b]							
P1XP4		20					20					
P5XP4		13					13					
P1XP3	F_2	102	61	19	15	7					1·0	> 0·95–0·50
P2XP3		93			68	25					0·2	0·95–0·50
P3XP2		651			473	178					1·8	0·50–0·10
P1XP4		245					180	65			0·3	0·95–0·50
P5XP4		257					140	56	47	14	1·7	0·95–0·50

[a]Empty space in the column indicates that the marker(s) was/were irrelevant; [b]there was indication of partial dominance of *lld* over *LLD*; as 4 leaflets out of a total of 2412 examined showed lld phenotype.

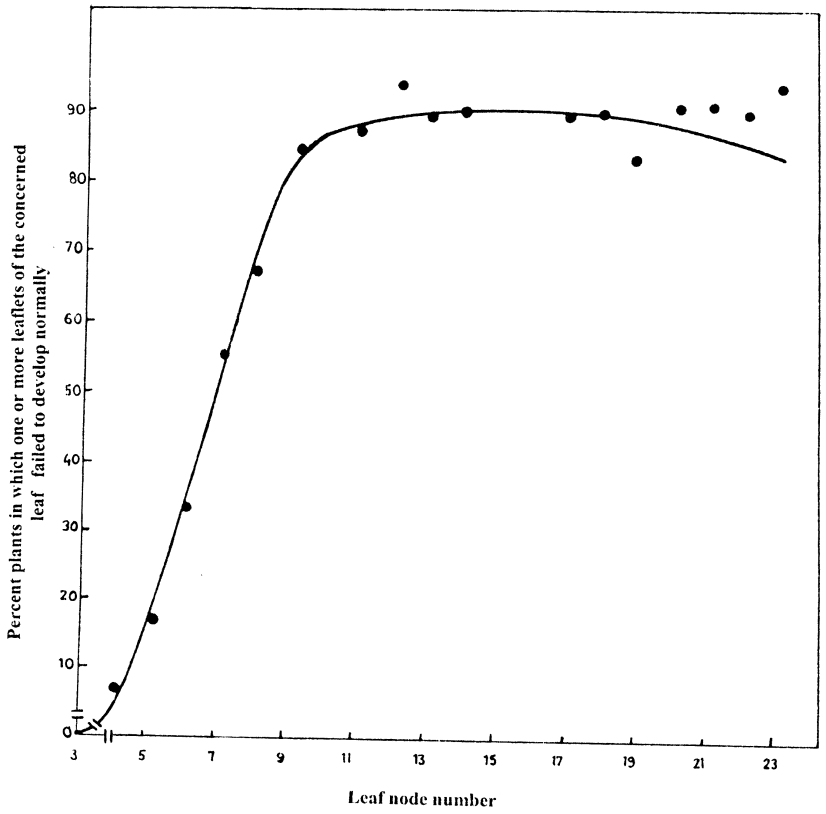

Figure 2. Expressivity of lld-phenotype in the shoot ontogeny of *tl lld* plants.

3.4 *Leaf/leaflet anatomy of* lld *mutant*

In both *LLD tl* and *lld tl* leaves, the rachis had radially symmetrical similar anatomical features (figure 3A). However, there were striking anatomical differences between *LLD tl* and severely affected *lld tl* leaflets (table 4, figures 3 and 4).

The affected single lld leaflets had narrower petiolule than LLD leaflets (figure 3B, C). The narrowness of petiolule in lld leaflets was associated with smaller size of parenchymatous cells in the cortex and central vascular bundle. In lld-leaflets that were fused, the petiolule possessed two discrete vascular bundles (figure 3D).

The lamina of normal LLD leaflets was bilaterally symmetrical on the two sides of midrib and spanned a few centimeters (figure 4A). The severely aborted lld leaflets had a radially symmetrical lamina having a diameter roughly equal to the width of midrib in LLD leaflets. There were 1 or 2 well developed vascular bundles in the midrib of lamina of LLD-leaflets (figure 4A). The vascular bundle was rudimentary or absent in the lld leaflets which were highly aborted in their growth and development. The epidermis and cortical paranchymatous cells that filled the lld-leaflet were generally of smaller size than the cells in LLD leaflets (figure 4D, E). In the incompletely developed lld-leaflets that developed some

Table 2. Expressivity of the genetic defect at the *LLD* locus on the leaflet development in leaves borne at different early to late nodes of the *tl tl lld lld* plants in field pea *P. sativum.*

Leaf number from cotyledon end	Number of plants observed	Number of plants in which one or more leaflet(s) were incomplete in development	Stem node-wise expressivity of lld phenotype
4	40	0	0
5	41	3	7·3
6	46	8	17·4
7	53	18	33·9
8	54	30	55·6
9	58	39	67·2
10	62	52	83·9
11	65	57	87·6
12	64	54	84·3
13[a]	66	62	93·9
14	67	60	89·5
15	64	58	90·6
16	58	51	92·2
17	51	47	92·2
18	46	41	89·1
19	40	36	90·0
20	38	32	84·2
21	33	30	90·7
22	25	23	92·0
23	22	21	90·9

[a]First flower bearing node.

cup-shaped lamina in the rachis distal or leaflet apical region, the anatomy in the laminated portion was similar to that of LLD lamina in that the midrib, chloroplast containing ground tissue and rudimentary to well developed vascular bundles of veins were present (figure 4B). The non-laminated rachis proximal parts of such leaflets had anatomical similarity with somewhat curtailed midrib or petiolule of normal leaflets.

The degree of anatomical complexity in the fused, incompletely developed cup-shaped leaflets varied like in single incompletely developed leaflets, according to the size of the lamination achieved in rachis distal part of the affected leaflet. The lamina lobe was round on account of fusion, and two major endarched vascular bundles were visualized diagonally opposite to each other, in a section cut below the apex where lamina was contiguous in the cup-shaped leaflet. In the section cut in between the petiolule and base of the cup, again two well developed vascular bundles were seen that grew into midribs of the fused laminae (figure 4C).

3.5 *Expression of* lld *mutation in leaf shape complexity variants*

About 20–50 adult vegetative/reproductive stage leaves of each of the following genotypes were examined for the expression of lld phenotype: *lld* (figure 5A), *tac lld* (figure 5B), *tl tac lld* (figure 5C), *tl af lld* (figure 6A) and *tl af tac lld* (figure 6B) homozygotes. The observations are diagrammatically summarized in figure 7. In all the genotypes studied, incompletely developed or lld-pinnae were observed at almost all of the rachis positions. Aborted development of the distal tendril was not recorded in the *tac lld* homozygotes. Incompletely developed pinnae, representing the morphogenetic products of primary rachis borne fused opposite blastozones, were observed in all the genotypes, specially at distal positions in *lld*, *tl af lld* and *tl af tac lld* homozygotes. In the *tl af lld* homozygotes, incompletely developed pinnules were produced singly or conjointly at different positions of the secondary rachides of proxial pinnae. In this genotype, occasional single secondary, tertiary or quartenary leaflets at distal and proximal positions demonstrated aborted lamina development.

In the *tl af tac lld* homozygotes, secondary leaflets of proximal pinnae and primary leaflets of distal and terminal rachis positions showed both single and conjoint aborted lamina growth and development.

The expressivity of *lld* mutation in *lld* homozygotes is shown quantitatively in table 5. It will be seen that like in *lld tl* homozygotes, the *lld* expression is lower at the most proximal and terminal positions of rachis. The proximal leaflets forming positions show higher level of *lld* expressivity than the distal tendril forming rachis positions.

4. Discussion

Above, a novel Mendelian recessive *lld* mutation which affects leaf morphogenesis and does not apparently

Table 3. Expressivity of genetic defect at *LLD* locus on the leaflet development at different positions on rachis in imparipinnate acacia type leaves borne on *tl tl lld lld* plants in the field pea *P. sativum.*

Leaflet position on rachis from leaf petiole end	Number of leaves: Examined	In which the concerned rachis position bore: Only one fused incompletely developed leaflet	Two leaflets on which one on left (L) or right (R) was incompletely developed: L	R	Leaf-rachis position-wise nodal expressivity of lld phenotype[b]
1	1076	15 (1·4)[a]	10 (0·9)	26 (2·4)	3·0
2	962	234 (24·3)	110 (11·4)	110 (11·4)	35·7
3	950	384 (40·4)	91 (9·6)	91 (9·6)	50·0
4	698	247 (35·4)	62 (8·9)	52 (7·4)	43·5
5	510	129 (25·3)	50 (9·8)	37 (7·3)	33·8
6	237	14 (5·9)	28 (11·8)	19 (8·0)	15·8
Apical	1076	in which imparipinnate leaflet was incompletely developed 37 (3·4)			3·4

[a]Values in parenthesis represent events in terms of per cent of total for the concerned rachis position; [b]expressivity = (number of leaflet meristems producing incompletely developed leaflets/total number of leaflet meristems) × 100.

modify root, flower, pod and seed morphology of *P. sativum* is described. The characteristics of *tl* and *tl lld* homozygotes have been compared in some detail. The *lld* mutation was highly penetrant, with low expressivity. It caused fusion of opposite leaflets and aborted morphogenesis of single and fused leaflets at various stages of their development. The action of *LLD* gene was required for normal pinna development from pea plants juvenile phase to its maturity. The *lld* mutation caused defect in leaf development was detectable in *tl*, *tac*, *tl tac*, *tl af*, and *tl af tac* backgrounds as summarized in figure 6. The phenotypes of *lld lld* plants in the various genetic backgrounds suggested that *LLD* is involved in the separation of lateral adjacent blastozones (primordia) differentiated on the primary, secondary and tertiary rachides and lamina development in leaflets. The implications of the observed phenotype(s) of the mutation in terms of overall compound leaf development are discussed below.

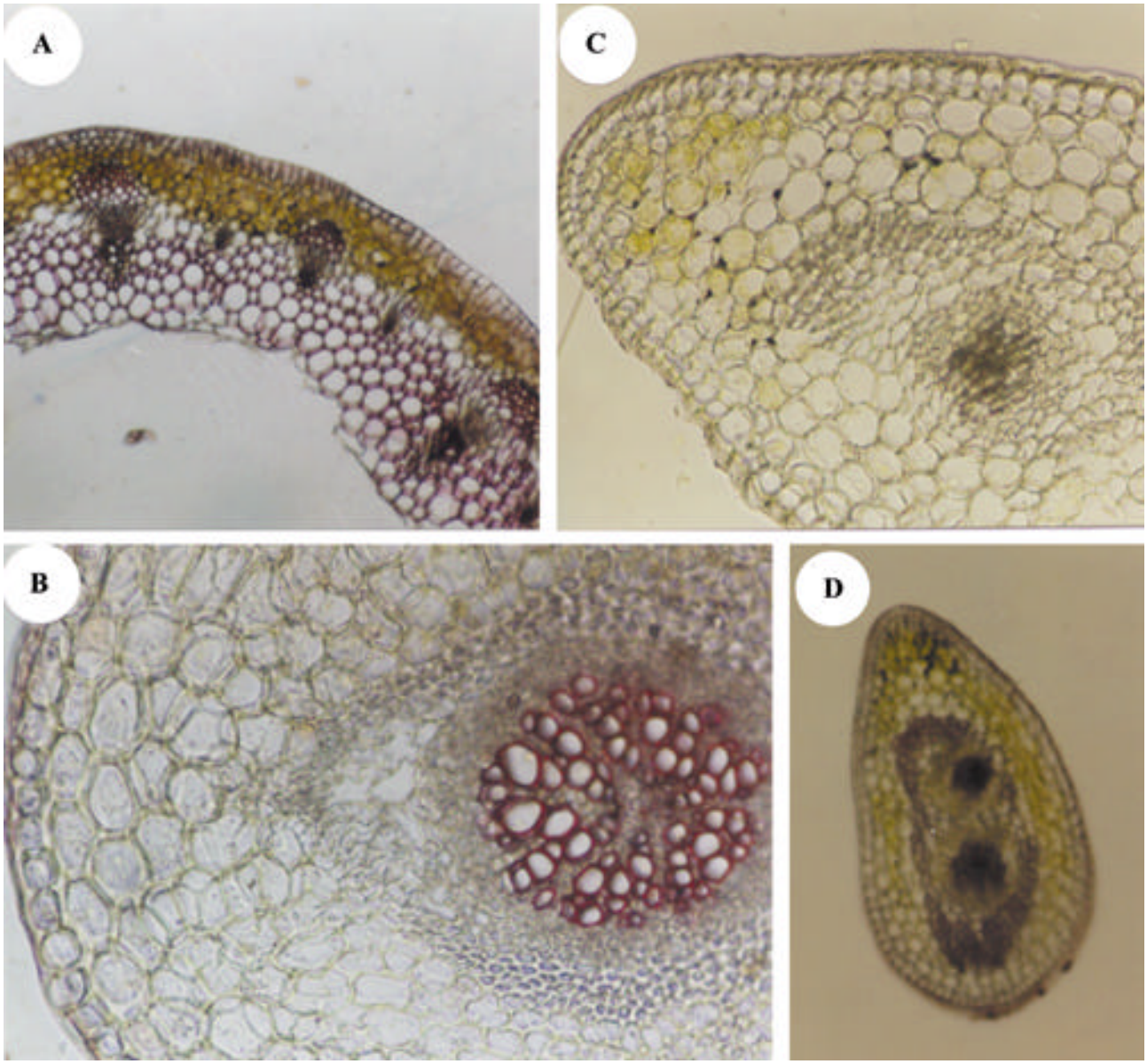

Figure 3. Anatomy of the rachis and leaflet-petiolules of *tl LLD* and *tl lld* leaves of *P. sativum*, visualized in transverse sections. (**A**) Rachis of a LLD leaf (those of lld leaves looked similar) (× 10). (**B**) Petiolule of a leaflet from *LLD* plant (those of normal looking leaflets from *lld* plants looked similar) (× 40). (**C**) Petiolule of an incompletely developed leaflet arising from a meristem of rachis node in a *lld* plant (× 40). (**D**) Petiolule of an incompletely developed conjoint leaflet produced by two meristems of a rachis node in a *lld* plant (× 10).

4.1 *Impairment of leaflet separation by* lld *mutation*

The opposite leaflets of the leaves formed on *tl lld* (homozygous) plants were often fused wholly or partially along both their sides or on one side resulting in funnel-/ cup-shaped uni- or bi-lobed leaflets or two, almost separate, leaflets joined proximally and having a common petiolule. The morphological features, venation patterns and internal tissue organization characteristics of the abnormal leaflets showed that these consisted of two leaflets.

In the *tl* leaf, two leaflet primordia (blastozones) are formed close to each other at each rachis node while the primary leaf primordium (LP) elongates to form a paripinnate rachis (Lu *et al* 1996). In *LLD tl* leaves, the discretely formed members of blastozone pairs develop into full fledged leaflets pairs, with individual leaflets of a pair placed at roughly 180° to each other (Villani and

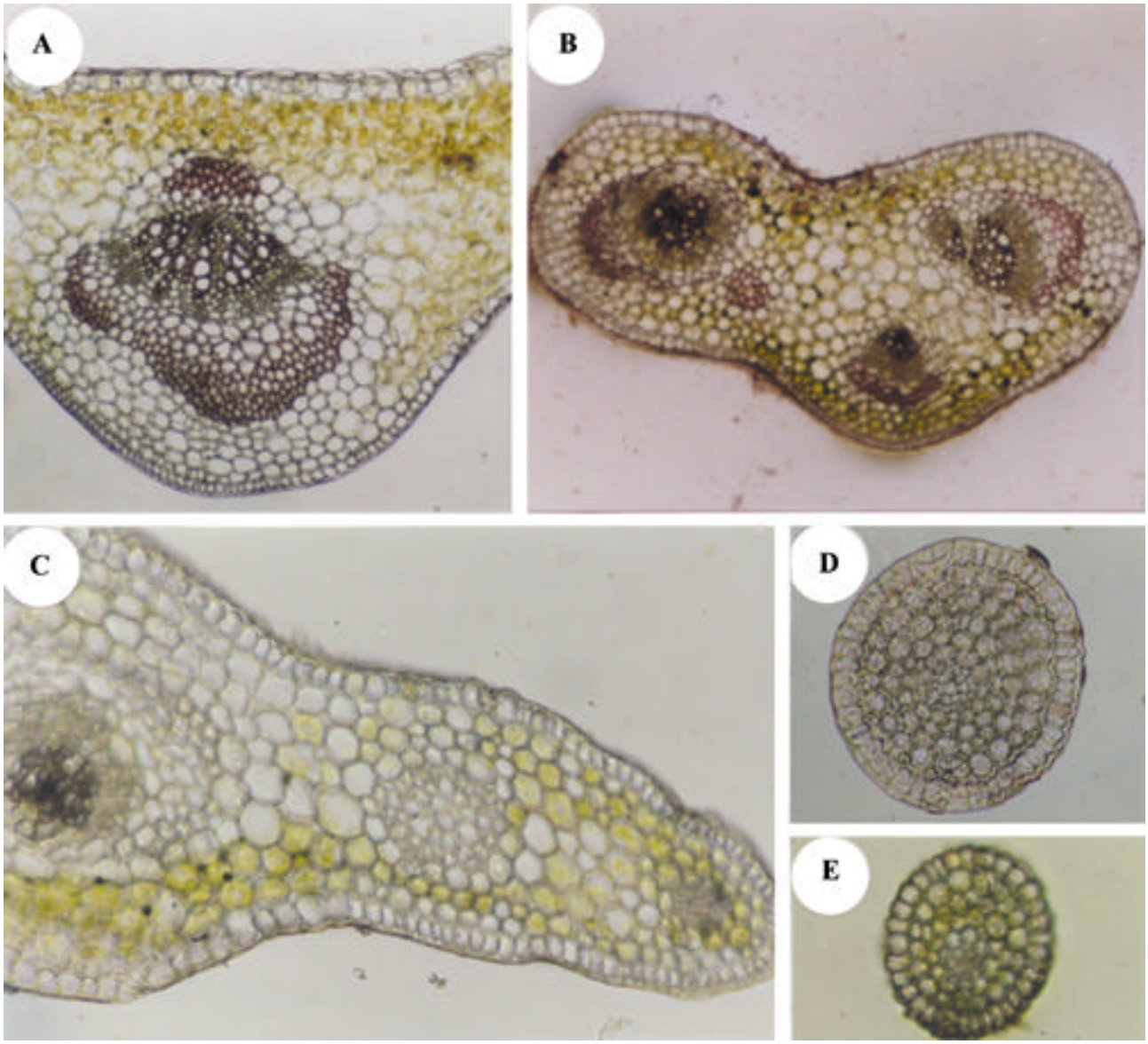

Figure 4. Anatomy of the leaflet pinnae borne on *tl LLD* and *tl lld* leaves of *Pisum sativum*, visualized in transverse sections. (**A**) Midrib zone of a leaflet lamina of *LLD* plant (normal looking leaflets of *lld* plants had similar structure) (× 10). (**B**) An incomplete cup-shaped leaflet product of two meristems at a rachis node in *lld* plant, visualized just below the cup whereform the rachis proximal lamina began to assume petiolule like internal structure (× 10). (**C**) Midrib zone of lamina of an incompletely developed leaflet produced by one meristem at a rachis node in a lld leaflet (× 40). (**D**) and (**E**) Stub/pin like structures representing lamina in aborted leaflets in lld leaves.

Figure 5. The leaf morphologies of *LLD*, *lld*, *tac LLD*, *tac lld*, *tl tac LLD* and *tl tac lld* homozygotes of pea *P. sativum*. (**A**) *LLD* homozygote (left), several leaflet pinnae and a few tendrilled pinnae showing aborted pinna lld phenotype in two leaves of *lld* homozygote (right). (**B**) *tac LLD* leaf (left), a pinna is seen aborted in development in *tac lld* leaf (right). (**C**) *tac tl LLD* leaf (left), a leaflet is seen in aborted form in *tac tl lld* leaf (right).

Demason 1999). Since the opposite leaflets are often fused in *lld tl* leaves, the *lld* mutation appears to repress or in some other way fails to achieve separation of blastozones into autonomous units of leaflet development. The fused leaflets could also be thought to arise as a result of fusion of separately initiated blastozones some time later during cell expansion/differentiation phases(s) of leaflet development. However, this also does appear to be the case since fused leaflets that were separated distally and fused proximally were often observed; the fused leaflets were always completely fused proximally to rachis. It is therefore visualized that normally the small number of cells that lie between the nascent blastozones undergo bulging to distance the blastozones. The process of boundary formation between the two leaflet blastozones is therefore promoted by the *LLD* function. In the *lld* mutant, the two blastozones and cells located between them together form a joint blastozone that develops into a fused leaflet. The LLD function seems to be required after leaflet primordia have been initiated by LP for the former's establishment as an autonomous leaflet primordia (LLP), as illustrated in the figure 8.

Recessive mutations in the *STM*, *CUC-1* and *-2* and *PIN-FORMED* (*PIN*) genes of *A. thaliana* (Aida *et al* 1999) and *NO APICAL MERISTEM* (*NAM*) gene of *Petunia* (Souer *et al* 1996) have been shown to be involved in the process(es) of organ separation in plants. In the *stm*, *pin* and *nam* mutants, the embryos display variously fused cotyledons in place of two distinct normal bilateral cotyledons. The cotyledons in embryos and sepals and stamens in flowers are often found fused in *cuc* mutants; the *lld* mutation affects leaf organ/tissue separation. Since *STM*, *CUC*, *NAM*, *PIN* and *LLD* genes are involved in plant organ separation, their protein products may perform similar roles in tissue specific manner. The NAC domain (N-terminal region of *Petunia* NAM and *Arabidopsis* ATAF and CUC proteins) has been shown to be conserved in the *CUC* and *NAM* genes in heterologous plants; the functions of their protein products remain unknown (Souer *et al* 1996; Aida *et al* 1999). The *STM* gene encodes a KNOTTED 1 class of homeodomain transcription regulation protein. There is evidence that CUC and STM products are required for SAM formation and cotyledon separation (Barton and Poethig 1993; Aida *et al* 1999).

4.2 *Defective leaflet morphogenesis in* lld *mutant*

Lateral blastozones, formed on the main rachis of pea compound leaf, grow and develop into leaflets by the regulated processes of morphogenesis – cell division in different plains, cell elongation and cell differentiation. In the *lld* plants, one or more leaflets fail to develop fully, in many leaves. The leaflet morphogenetic process gets aborted at various steps in different leaflets.

Many of the leaflets develop as very small needle/pin like structures, a few millimeters in length. Histological examination of such structures at various levels of longitudinal axis revealed that they were radially symmetrical over most of their length. They possess single well-differentiated vascular strand at rachis proximal end, which was of much diminished differentiation or absent at the distal end. Vascular strands comprising of xylem, phloem and some other cell types are source of water, nutrients and signal molecules that are essential for the growth of vascular and other tissues of plants (Raven *et al* 1976). Vascular tissue deficiency may be related to poor lamina growth in aborted leaflets (Berlath *et al* 2000). As the *LLD* gene product appears to be directly or indirectly involved in growth and differentiation of vascular strand components, the aborted development of leaflets in *lld* mutant may be partly related to the unavailability of critical concentrations of auxin and cytokinin (Aloni 1995) necessary for the vascular tissue development (figure 4 C–E). Since pro-cambium is the precursor of

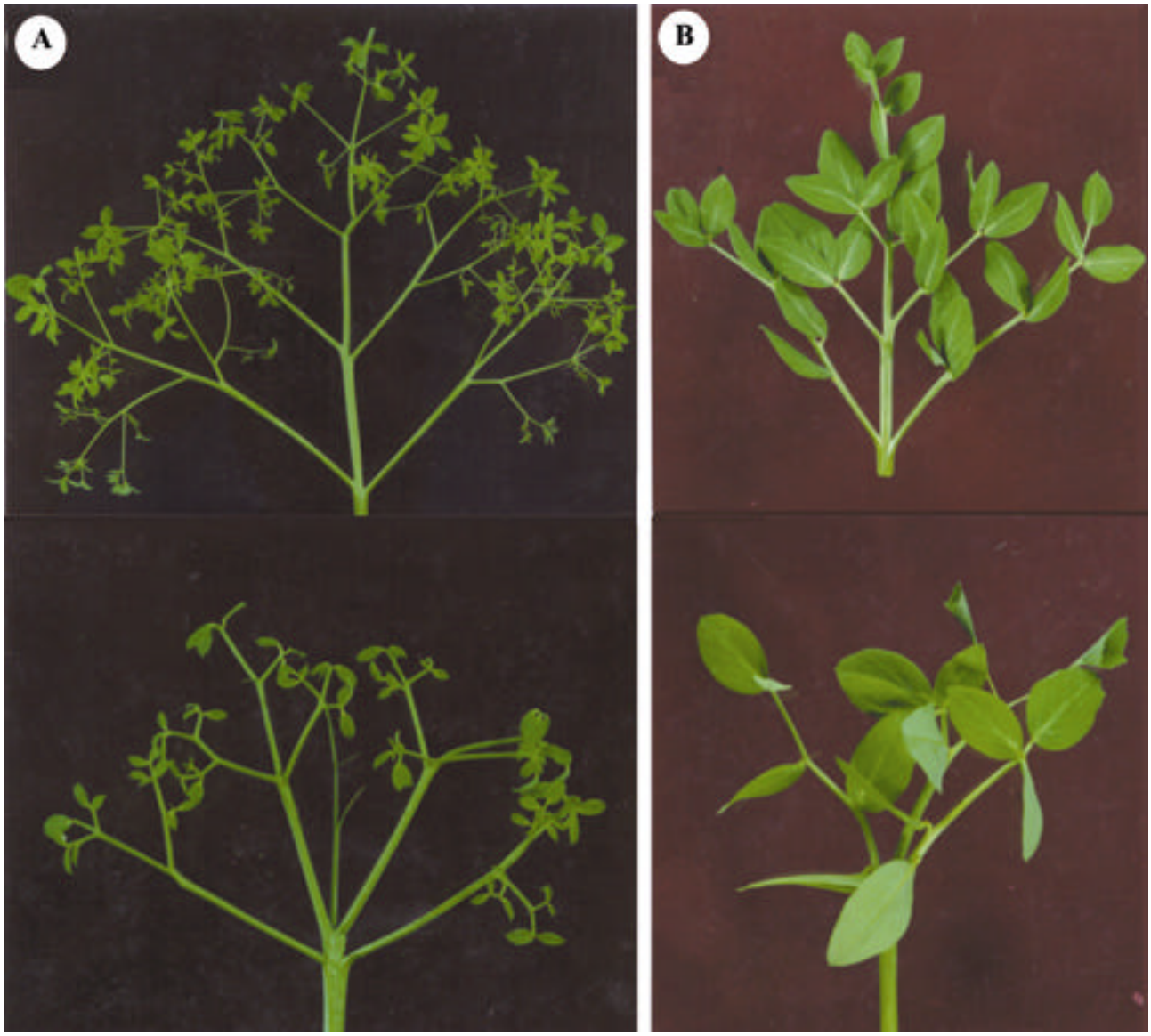

Figure 6. The leaf morphologies of *af tl LLD*, *af tl lld*, *af tl tac LLD* and *af tl tac lld* homozygotes of pea *P. sativum*. (**A**) *af tl LLD* (top left), opposite pinnae at third and fourth positions of rachis are represented by pin like fused leaflets and a rachide of second (left) pinna is seen similarly aborted in *af tl lld* leaf (bottom left) of pea *P. sativum*. (**B**) *af tl tac LLD* (top right), a leaflet borne on the left compound pinna at position 1 of rachis is seen aborted in growth and two funnel shaped fused pinnae are seen at the third and fourth terminal position of rachis in *af tl tac lld* leaf (bottom right).

mature vascular tissue (Sachs 1991), the vascular defect may be related to faulty procambial strand pattern maintenance (Carland *et al* 1999; Dengler and Kang 2001).

Another frequent type of incomplete leaflet observed on *lld* mutant is funnel-/cup-shaped leaflet (figure 1) in which distal cup-shaped lamina blade, cleaved on one side, is borne on a radially symmetrical stem shaped lamina that extends into the petiolule. Both parts of the lamina in the above shaped leaflets have variable sizes. The two edges which are laterally free in a normal (LLD) flat leaflet are fused to give a cup-shape to an otherwise normally developed lamina in the distal region of *lld* cup-shaped incompletely developed leaflet. The lack of dorso-ventral and lateral growth, reticulation of vascular tissue and separation for adaxial-abaxial patterning appeared to

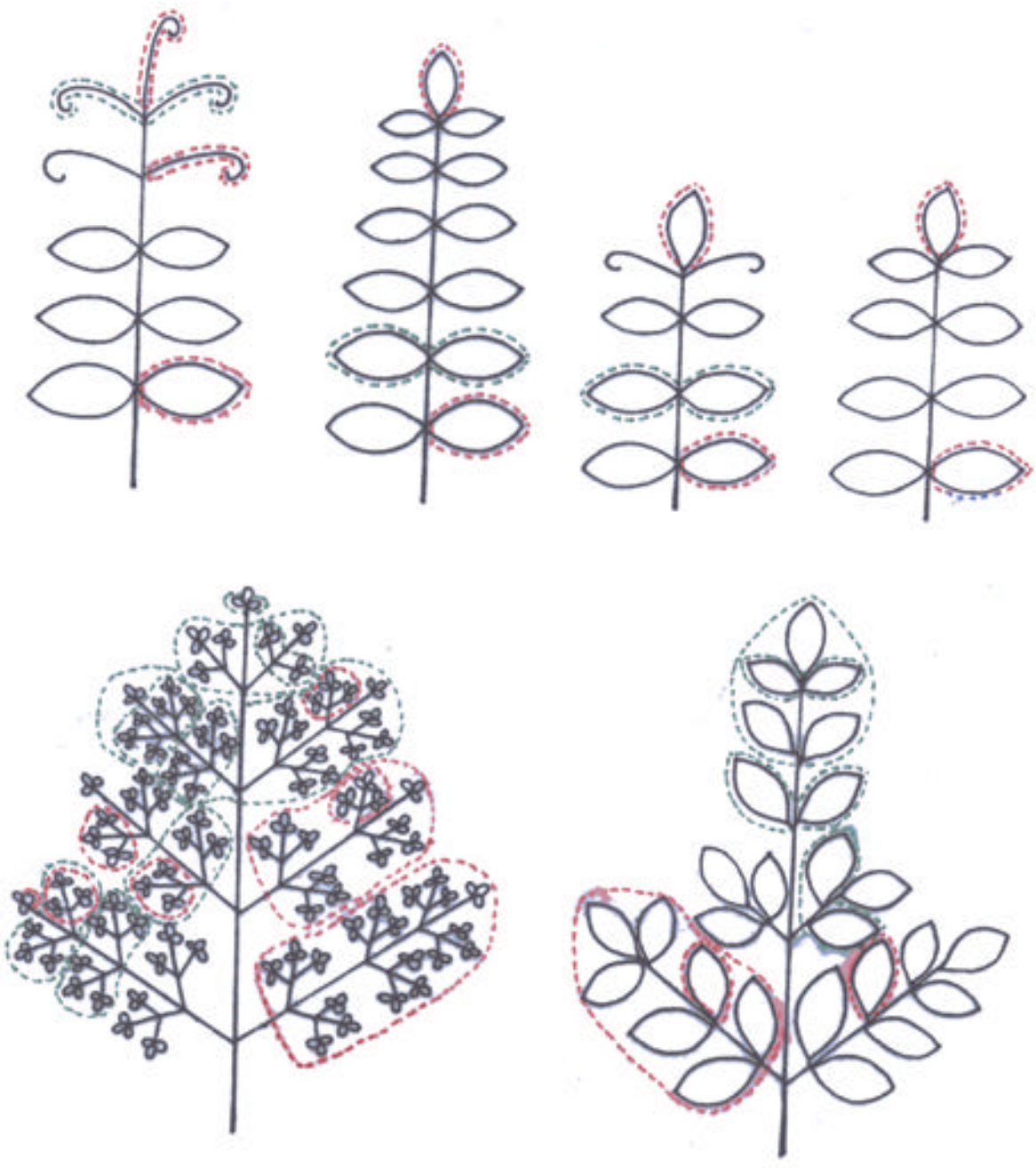

Figure 7. Diagrammatic representation of lld phenotype in 6 genotypes of pea *P. sativum;* the fusion events accompanied by incomplete development is shown with green colour and single event accompanied by incomplete development by red colour.

Table 4. Anatomical characteristics of leaf organs visualized, in transverse sections, in rachis and leaflets of *tl LLD* and rachis and severely aborted leaflets of *tl lld* plants of field pea *P. sativum.*

		Characteristics of rachis	
Organ	Tissue	Normal looking leaflets in *tl tl lld lld* plants and those in *tl tl* plants	Leaflets borne on *tl tl lld lld* plants and expressing lld phenotype intensely
Rachis	Girth	7·51 ± 0·44 mm	Like in LLD
	Epidermis	Single layer of round cells measuring 25 ± 2 μm in length (L) and 20 ± 2 μm in width (W)	-do-
	Chlorophyllous parenchyma	3 to 6 layers of round cells of 49 ± 23 μm in L and 41 ± 15 μm in W	-do-
	Sclerenchyma	5–6 layers of hexagonal cells of 28 ± 9 μm in L and 30 ± 14 μm in W	-do-
	Vascular bundle	Number 20 ± 1 of variable size; large (500 ± 10 μm L and 180 ± 12 μm W): medium (254 ± 11 μm in L and 98 ± 10 μm in W): small (98 ± 10 μm W and 38 ± 5 μm in W) :: 1 : 1 : 1	-do-
	Sheath	Sclerenchymatous, 3–4 layers of hexagonal cells of 36 ± 13 μm L and 16 ± 8 μm W	-do-
	Phloem	Compactly arranged small cells placed between the bundle sheath and xylem	-do-
	Xylem	Metaxylem topped by protoxylem on adaxial and abaxial sides	-do-
	Pith	Hollow of 1·36 ± 0·03 mm diameter	-do-
Leaflet petiolule	Girth	3·32 ± 0·12 mm	1·61 ± 0·13 mm
	Epidermis	Single layer of round cells 23 ± 8 μm L and 21 ± 2 μm W	Single layer of elliptical cells of 25 ± 10 μm L and 16 ± 10 μm W
	Cortex	Parenchymatous-chlorophyllous, 4–8 layers of cells of 73 ± 10 μm L and 49 ± 7 μm in W	Parenchymatous with chlorophyll, cells round/oval of 34 ± 2 μm L and 33 ± 2 μm W
	Vascular bundle	Central of 1·06 ± 0·05 mm girth	Central of 0·31 ± 0·03 mm girth (two bundles were present in the leaflets formed by two meristems at a rachis position conjointly)
	Sheath	Small thick walled fibrouss phloem cells seen around the vascular bundle	Small thick walled, sclerenchymatous tissue seen around the vascular bundle(s)
	Phloem	Small cells, compactly arranged, above the xylem	Like in LLD leaflets
	Xylem	Ring shaped, metaxylem in the centre, protoxylem present both inside and outside	Central
Leaflet lamina	Span	Bilaterally symmetrical lobes on either side of midrib, 2·59 ± 0·19 cm span and 212 ± 10 μm W	Round, 289 ± 1 μm in diameter
	Epidermis	At midrib level single layer of round cells of 57 ± 5 μm L and 34 ± 5 μm W	Single layer of elleptical cells of 8 ± 1 μm L and 5 ± 1 μm W
	Mesophyll	At midrib level chlorophyllous-round cells of 85 ± 15 μm L and 67 ± 7 μm W	Parenchymatous-round cells of 16 ± 5 μm diameter
	Vascular bundle(s)	At midrib level 1 or 2 of 370 ± 80 μm L and 270 ± 24 μm W	Absent or indistinguishable
	Sheath	Above and below the vascular bundle(s) of thick walled hexa/polygonal cells; 33 ± 15 μm L and 14 ± 10 μm W	Absent
	Phloem	Small, compactly arranged cells	Absent
	Xylem	Metaxylem in the centre, protoxylem present adaxially and abaxially	Absent

Table 5. Expressivity of genetic defect at *LLD* locus on leaflet development at different positions on rachis in imparipinnate leaves borne on *lld lld* plants in the field pea *P. sativum.*

Leaflet position on rachis from leaf petiole end	Whether the position was occupied by laminated leaflet (Ll) or tendril (T)	Number of leaves: Examined	Concerned rachis position bore: Only one fused incompletely developed structure	Two leaflets on which one on left (L) or right (R) was incompletely developed: L	R	Leaf-rachis nodal expressivity of lld phenotype
1	Ll	35	0 (0)[a]	7 (20·0)	6 (17·1)	18·5[b]
2	Ll	24	1 (4·2)[a]	16 (66·7)	17 (70·8)	72·8
3	Ll	9	0 (0)	8 (88·9)	6 (66·7)	77·8
4	T	35	3 (8·6)	6 (17·1)	7 (20·0)	27·2
5	T	28	7 (25·0)	4 (14·3)	6 (21·4)	60·5
6	T	27	4 (14·8)	2 (17·4)	1 (3·7)	25·3
Apical	T	29	in which imparipinnate leaflet was incompletely developed 4 (13·8)			13·8

[a]Values in parenthesis represent events in terms of per cent of total for the concerned rachis position; [b]expressivity = (number of leaflet meristems producing incompletely developed leaflets/total number of leaflet meristems) × 100.

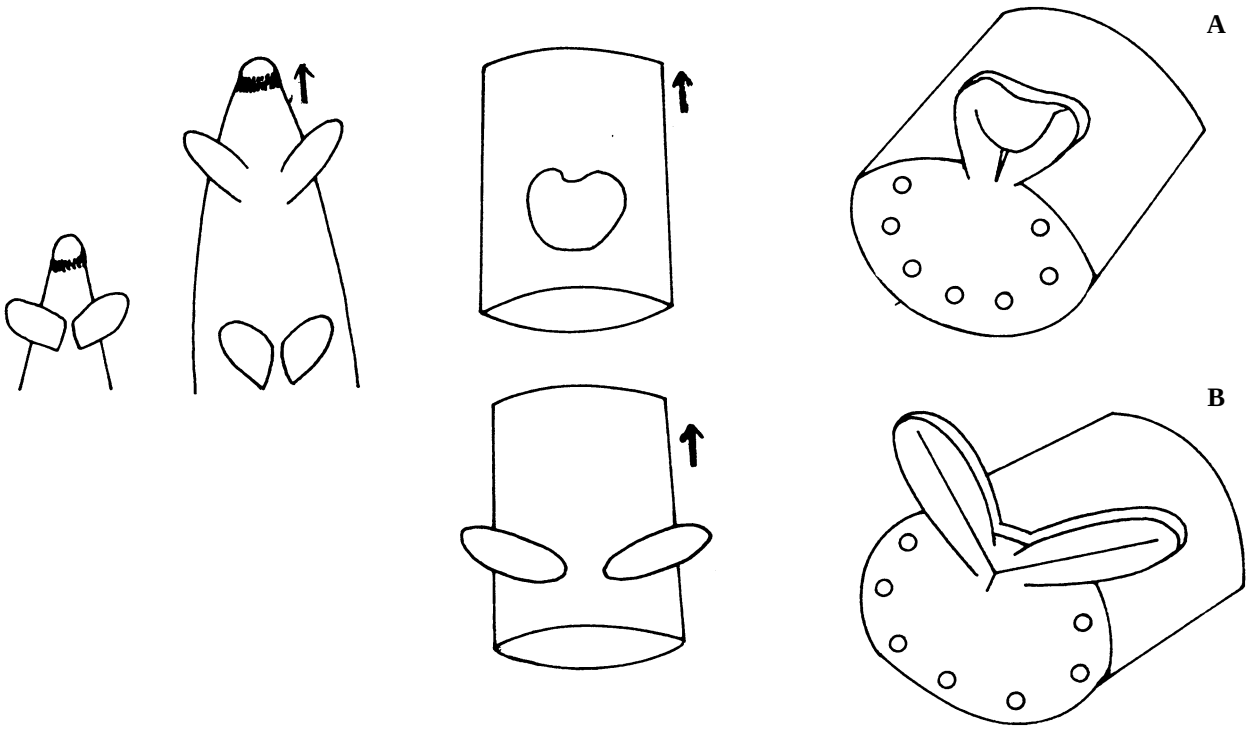

Figure 8. Schematic diagram for lateral leaflet blastozone pattern formation on rachis of pea *P. sativum* leaf. Frontal and radial section views of rachis nodes of leaves of *lld* (**A**) and *LLD* (**B**) homozygotes. Arrow indicates the direction of acropetal growth in rachis. Stages in development are shown from left to right and a trasverse section of the last stage is shown on the far right. The first stage is shown on the far right. The first stage is the same in **A** (mutant) and **B** (wild type).

be responsible for the incomplete growth in the proximal parts of *lld*- single and fused leaflets.

There was large variation in the size of incomplete *lld* leaflets. In *lld* mutant leaflet is initiated but not completed. It appeared that the growing leaflets lost their *LLD* function at different times. The leaflet size may have been proportional to the time for which *LLD* functioned in leaflet blastozone from the time of its differentiation on the rachis. The shapes of the aborted leaflets suggested that the distal part of the leaflet develops before the proximal part.

The *PHANTASTICA* gene mutant (*phan*) of *Antirrhinum* produces leaves that are radialy symmetrical and almost completely abaxialized (Waites *et al* 1998). Based on the phenotype of *phan* mutants, PHAN has been shown to play a role in the separation of adaxial and abaxial domains of a growing leaf blade. Since *PHAN* specifies a Myb family transcription factor (Waites *et al* 1998), certain other gene(s) regulated by *PHAN* may be performing the role(s) attributed to *PHAN*. Mutants in *PHABULOSA* (*PHAB*) and *AGO* gene in *Arabidopsis* (McConnell and Barton 1998) and *LEAF BLADELESS* (*LBL*) in *Zea mays* also (Timmermans *et al* 1998) have similar phenotypes. In the present work it has been shown that some *lld* mutant leaflets in the pea compound leaf are wholly and partially radially symmetrical. It is possible that in the absence of LLD function, one or more of the functions analogous to PHAN, PHAB, AGO and LBL in pea are not expressed normally. It can be further speculated that the *LLD* gene product may have a variety of interactions with *STM* and *PHAN* like genes and those interacting with the latter genes, in the process of morphogenesis of compound leaf. Since cell division and cell differentiation processes concerned with growth in proximo-distal, dorsi-ventral and marginal directions are arrested in leaflets of pea *lld* mutant, the LLD may exercise its role early in the hierarchy of actions of various genes involved in pinna blade morphogenesis.

4.3 *Role of LLD in leaflet morphogenesis*

Leaflets of *tl* pea plant are akin to simple leaves. The simple leaf or leaflet morphogenesis has been considered complex and the mechanism by which leaflet/pinna lamina expands is not understood (Poethig 1997). In the leaves of *tl lld* double mutants of pea, the morphogenesis of leaflets gets aborted at different stages of development. The morphologies of the leaflets aborted in growth at various steps of morphogenesis due to *lld* mutation permit a model of leaflet morphogenesis whose main features are illustrated in figure 9. The leaflet development occurs gradually from the pinna/leaflet blstozones (primordia) that are differentiated by the primary leaf primordium pair-wise acropetally on the rachis (figure 8) (Villani and Demason 1999). In the initial stages, the growing blastozone is radially symmetrical and has a vascular cylinder in the centre. The pinna primordium grows in the longitudinal direction basipetally, as well as diagonally to increase girth and acquires a bifacial shape. Veinal strands begin to reticulate from the main vascular cylinder which is now peripherally placed due to lateral growth in opposite direction. The outer layer(s) of this structure will form the abaxial side of fully formed leaflet. The developing pinna further elongates in the distal direction, further increases in the girth and begins to develop laminal ridges. The process of laminal ridges formation involves separation of tissue from outside to within, opposite to the main vascular bundle that will form the midrib of leaflet. The groove becomes deeper as the pinna expands laterally along the two ridges. The septation process in the groove generates the adaxial surface of the leaflet. Finally, a flat leaflet blade develops by unfolding of the laminar ridges whose span has now increased considerably lateral to the middle vascular bundle. The leaflet has oval-obovate shape on account of the relative restriction on lateral lamina growth at the proximal and distal ends of the growing pinna.

It is visualized that LLD function is required at all stages from blastozone initiation to its development into a full-fledged pinna. In the process a very large number of genes (Takahashi *et al* 1995; Tsuge *et al* 1996; Kim *et al* 1998) concerned with cell differentiation, direction of cell division, cell division and elongation, must function in concert with *LLD* gene. The pattern of leaflet lamina expansion is controlled by LLD gene function.

4.4 *Effect of* lld *mutation on tendrilled and compound pinnae*

In *lld* mutant, wild type for *TL*, *AF* and *TAC* genes, tendrils were observed to be aborted in growth, forming short hook like single and fused incompletely development pinnae. The incomplete morphologies of tendrils like those of leaflets may also be related to atrophy of their vascular tissue in the *lld* mutant. The *tl af* and *tl af tac* homozygotes of pea produce at different rachis nodes, pinnae that are variously compounded. The pinnae at the distal rachis nodes were often aborted singly or jointly to form hook like structures in *lld tl af* homozygotes and cup-/funnel-shaped leaflets in *lld tl af tac* homozygotes. In the proximal nodes, pinnae were aborted in growth at various orders of compounding, at the primary level, like on the distal nodes, or at secondary and higher levels of branching. It is possible to conclude that although *lld* mutation did not affect growth of primary rachis, *lld* mutation affected growth and morphogenesis of rachides

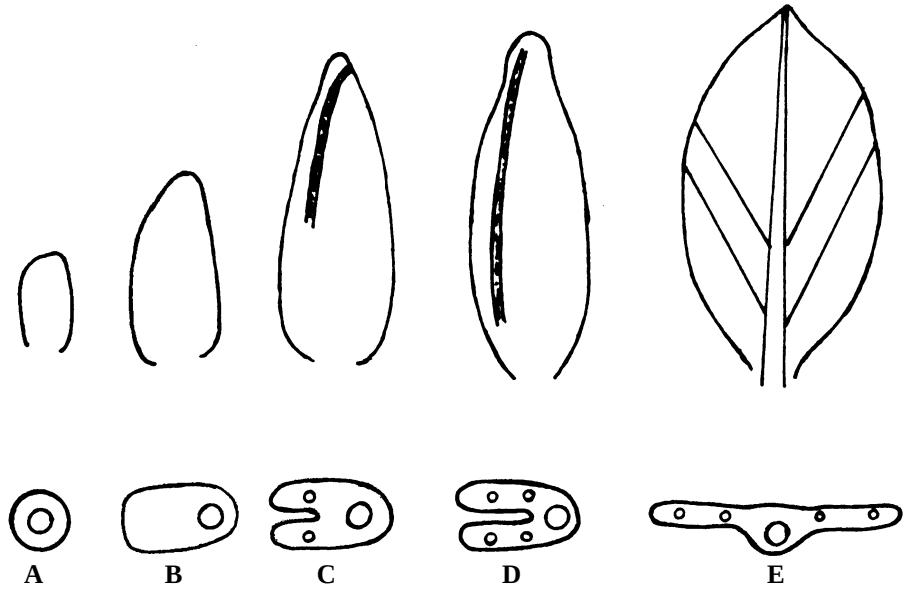

Figure 9. Model of lamina expansion during pinna leaflet morphogenesis in *P. sativum*. The pinna form at various stages is depicted frontally and by a view of transverse section. (**A**) Pinna primordium or blastozone that is radially symmetrical around a central vascular cylinder. (**B**) Distally elongated and diagonally expanded bifacial juvenile pinna in which the vascular bundle has assumed peripheral position along the abaxial side to later form the midrib of leaflet. (**C**) Growing pinna; reticulation of vascular tissue has initiated and laminar ridge formation by septation along inward growing groove opposite to the abaxial vascular bundle has begun. (**D**) Basipetally elongated pinna, deeply grooved by lateral expansion of lamina ridges; adaxial surface has formed inside the groove to delimit the lamina growth in ventrodorsal direction. (**E**) Fully formed flat leaflet in which the lamina spans on two sides of midrib vascular bundle have opened exposing the adaxial surface dorsally.

of compound pinnae as well as of leaflets laminal/blades borne on different levels of rachis. The morphogenesis of both simple and compound pinnae of pea is determined by LLD function.

4.5 *Nature of* LLD *gene*

The inheritance and phenotypic expression of LLD locus demonstrated that the *lld* mutation examined was viable and behaved in a Mendelian recessive manner. Although *lld* mutation was highly penetrant, its effect was manifested only in a small number of leaflets only. These observations can be explained as follows. It is hypothesized that *LLD* is an essential gene in pea and null mutations in the gene may be lethal. The *LLD* gene is linked to or is a part of a stably inserted DNA element. It is known that plant and animal genomes have a variety of transposons inserted on them and some of these may be involved in the regulation of host genes (Frank *et al* 1997; Yang 1998; Kidwell and Lisch 2000). There are two copies of *LLD* gene on the homologoues of one of the chromosome of the main genome of pea. The expression of *LLD* gene occurs from transcription promoter of the concerned insertion/element (transposon). In the *lld* mutant the insertion element has become unstable and excises with some frequency; thus *LLD* is lost from its chromosomal site upon such an excision. The *lld* mutant is homozygous for the instability of the insertion element controlling the *LLD* gene. The mutation related to the instability of insertion element is recessive. Since there are two copies of the *LLD* gene, leaflet abortion is visualized only when both the *LLD* gene copies have been excised. The two excision events required for the expression of lld mutation are not synchronous. Due to the different timings of the two events, the degree of abortion of morphogenesis in different leaflets is different. The *lld* mutants survive because the aberrant excision character of the concerned insertion element is expressed in a tissue specific manner in the pinnae/leaflets only. The gametes produced on the *lld* plants carry normal gene product and therefore the *lld* mutants complete their life cycle and the effects of mutation are manifested in leaflets that are vegetative parts of plant body.

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