Co-regulation of Biomass Partitioning by Leafblade Morphology Genes *AFILA*, *MULTIFOLIATE-PINNA*, *TENDRIL-LESS* and *UNIFOLIATA* in Grain Pea *Pisum sativum*

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In grain pea *Pisum sativum*, 16 genotypes constructed by combining wild type and mutant alleles of *MULTIFOLIATE-PINNA* (*MFP*), *AFILA* (AF), *TENDRIL-LESS* (*TL*) and *UNIFOLIATA* (*UNI*) genes, which differed in leaf blade morphology, were quantitatively phenotyped for allometry. The biomass partitioning among root, stem, stipule, leaf blade and seeds was unique for these genotypes suggesting that *MFP, AF, TL* and *UNI* genes determined leaf blade and plant architecture. Gene actions were inferred on the basis of mutant phenotypes. Biomass of all the organs was found to be increased in the *tl* single mutant. The *af* mutation singly and in combination with *mfp, mfp tl, mfp uni-tac* or *mfp tl uni-tac* decreased biomass of all the organs. Allocation of biomass to leaves was increased at the expense of that to seeds or seeds and stems by a single *mfp* mutation or in combination with *uni-tac, af tl, tl uni-tac* and *af tl uni-tac* mutations. The AF and MFP functions are essential in pea cultivars for high yield of grains.The mechanism for simultaneous control of leaf blade and plant architecture suggested by mutant phenotypes has three elements. The *MFP, AF, TL* and *UNI* genes exercise control over meristematic activity in all the organs. Their determination of leaf blade morphology and size affect net photosynthesis or metabolite supply. The quantities of available metabolites determine numbers and sizes of organs or partitioned total biomass. The *tl* allele is identified as a genetic marker/determinant for breeding tendril-less prolific pea cultivars for obtaining herbage and grains in high yields.

Key Words: ***AFILA*; Leafblade Architecture; *MULTIFOLIATE-PINNA*; Plant Allometry; *TENDRIL-LESS*; *UNIFOLIATA***

Introduction

Similar to other seed plant species, leaves in pea are the principal sites of gas exchange (H_2O, O_2, CO_2), light reception, temperature control and photosynthesis. Pea leaves differ in their size and morphology within a single plant (heteroblasty) and between genetic resources [1-4]. The leaves in pea have two components, a semi-determinate compound leaf blade composed of upto 15 pinnae attached to stem by petiole and two sessile peltate stipules attached to stem, one on either side of the site of attachment of leaf blade to petiole, at each node. The leaf blade is imparipinnate; it comprises of upto 3 pairs of simple leaflets borne on rachis proximal to petiole (proximal domain of leaf blade), upto 4 pairs of simple tendrils borne on rachis distal to petiole (distal domain) and an apical tendril (terminal domain) [4-6]. The leafblades produced on the first flowering node and nodes immediately below and above it bear the most complex leaf blades. The late post-flowering nodes produce relatively less complex leaf blades. The first few nodes after the cotyledons bear least complex leaf blades. Natural and/or induced variation is known in both stipule and leaf blade morphologies. Stipules are small knife blade like in the natural mutant called *stipule reduced* (*st*) [7]. They have the same morphology as leaf blades in the natural mutant called *cochleata* (*coch*) [8]. Mutation at 5 loci/genes namely *UNIFOLIATA (UNI), STAMINA-PISTILLOIDA (STP), TENDRIL-LESS (TL), AFILA (AF)* and *MULTIFOLIATE-PINNA* (*MFP*) have been found to differentially alter the basic pattern/ architecture of pea leaf blades [6, 9-14]. The leaf blades of all nodes are less complex in the *uni* and *stp* mutants. Both natural and induced mutant alleles exist for the *UNI* gene. Among the two types of alleles, *uni* and *unifoliata tendrilled acacia* (*uni-tac*), the former exists in both natural and induced forms [3, 4,15-22]. The *uni-tac* mutants produce leaf blade that have normal proximal domain, fewer tendrils in distal domain and a simple leaflet at the terminal domain. The *uni* leaf blades are less complex than *uni-tac* leaf blades and can be simple. The *stp* leaf blades are like those of *uni-tac*, except that tendril occupies the terminal domain [13]. The *tl* leaf blades have simple leaflets in all the domains. Both natural and induced *tl* alleles are existent [3,4,21-25]. The leaf blades of natural *af* mutant bear branched tendrils in the proximal domain and simple tendrils in distal and terminal domains [3,4,22,26,27]. Leaf blades

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in the induced *mfp* mutant have normal proximal domain but the distal domain bears multifoliate pinna blades [6]. Several studies have compared the leafblades of *af, tl, uni* and *mfp* single mutants, *af tl, af mfp, af uni/uni-tac, tl mfp, tl uni/uni-tac, af tl uni-tac* and *af tl mfp* double and triple mutants with those of wildtype [3,4,14,21,22, 27-33]. Recently, the leafblade phenotypes of *mfp uni-tac, af mfp uni-tac*, *tl mfp uni-tac* and *af tl mfp uni-tac* have also become known. The wild type and 15 combinatorial genotypes of *af, tl, mfp, uni-tac* mutation have uniquely different leaf blades (Table 1). The related usefulness of the repertoire of above leaf blades forms in pea breeding needs evaluation.

A comparative morphological, anatomical and transcriptional studies carried out on the wildtype and mutant genotypes of pea have shown that proximodistal growth of the leaf primordium that forms rachis and initiates pinna sub-primordia requires the activity of *UNI* gene [3,4,18,20,22]. The *AF* gene is the repressor of any *UNI*-led mediolateral rachis growth associated with pinna compounding in proximal [19,20,22,26,34,35] and distal domains [35]. It is the determinant of the boundaries of proximal and distal domains and of determinate growth of rachis [35]. Besides, *AF* is the activator of pinna growth as leaflet in the proximal domain [19,20,22,26,34,35]. The *TL* and *MFP* genes are repressors of mediolateral rachis growth as well as abaxial-adaxial differentiation necessary for pinnae to grow as leaflets in the distal domain [35]. There are AF, TL- and MFP-independent pathways for pinna branching and formation of leaflets on termini of pinna branches, in mutant genotypes [6, 35]. To produce their effects the UNI, AF, TL and MFP must function in the meristems of leaf blade primordium and pinna subprimordia.

Table 1. Leaf architecture related genotypes and phenotypes and origins of the wild type and mutant accessions used in the study

S.No.	Designation	Relevant homozygous genotype in respect of stipule and leafblade genetic determinants				Features that are different from wild type[a] in imparipinnate leafblade[f]	Origin/Reference
		MFP	*AF*	*TL*	*UNI*		
1	SKP-1	+[b]	+	+	+	Domainilized rachis bears upto 3 pairs of simple leaflets (pinnae) in petiole proximal domain, 4 pairs of simple tendrils in distal domain and a terminal tendril	[4]
2	SKP-351a	-[c]	+	+	+	Multifoliate pinnablades comprising of tendrilled leaflets replace distal tendrils	[6]
3	SKP-11	+	-	+	+	Branched tendrils replace proximal leaflets	-do-
4	SKP-12	+	+	-	+	Simple leaflets replace distal and terminal tendrils	-do-
5	SKP-13	+	+	+	-[d]	Distal domain is simplified, a terminal leaflet replaces missing tendrils	-do-
6	SKP-352	-	-	+	+	Compound rachides that bear tiny leaflets at their ends replace all pinnae	-do-
7	SKP-353	-	+	-	+	Multifoliate pinnablades comprising of small normal leaflets and tendrilled leaflets replace distal tendrils	-do-
8	SKP-354	-	+	+	-	One or two pairs of pinnablades of tendrilled leaflets replace distal tendrils	Segregant from a SKP-107 x SKP-351a cross
9	SKP-101	+	-[e]	-[e]	+	Compound rachides that bear tiny leaflets apically replace all pinnae	[6]
10	SKP-102	+	-	+	-	Terminal tendril is replaced by leaflet, proximal pinnae are compound tendrils which may bear leaflets apically	-do-
11	SKP-103	+	+	-	-	Distal domain is simplified; both distal and terminal domains bear simple leaflets	-do-
12	SKP-354	-	-	-	+	Highly ramified rachides that bear lanceolated tiny leaflets apically replace all pinnae	-do-
13	SKP-355	-	-	+	-	Compound pinnablades of tendrilled leaflets and simple leaflet replace proximal leaflets and distal tendril and terminal tendril respectively	Segregant from a SKP-107 x SKP-351a cross
14	SKP-356	-	+	-	-	Distal domain has lanceolate and terminal domain normal leaflets	-do-
15	SKP-107	+	-	-	-	Compound small leaflet bearing pinnablades and simple small leaflets occupy proximal and distal and terminal pinnae positions respectively	[6]
16	SKP-357	-	-	-	-	Compound pinnablades of small simple normal and asymmetrical leaflets occupy distal and proximal domains and a leaflet occupies terminal position	Segregant from a SKP-107 x SKP-351a cross

a= Fully developed leaves formed on the first flowering node are described; b= Wild type allele; c= Mutant allele; d= The mutant allele used was of the *uni-tac* type [4]; e= The, *af* and *tl* alleles were from the collection of Blixt 1972 [52]; f= The stipules were wildtype with peltate morphology in all the genotypes.

Leaf morphology and metabolism are known to be coordinated; alteration in leaf primary metabolism can perturb both leaf and plant development [36]. Accordingly, it is hypothesized that the distinctness in leaf blade morphologies of the 16 combinations of wild type and mutant alleles *of MFP, AF, TL* and *UNI* must affect one or more metabolic properties of leaves of their plants and thereby the morphology/architecture of whole plants. Among *UNI, AF, TL* and *MFP* genes, the UNI and TL products have been identified as transcription factors [18,37]. UNI is orthologous to *LEAFY* (*LFY*) transcription factor of *Arabidopsis thaliana* [14,18,19]. The properties of *af* and *mfp* mutants indicate that the corresponding genes may also specify transcription factors (TFs). Often TFs regulate more than one gene or co-expressed genes. Consequently, the null mutation in TFs can produce pleiotropic phenotypes. The TFs affecting leaf morphogenesis have been shown to express in meristems of leaf primordia and related cell types [38,39]. The progenitors of meristematic cells present in leaf primordia have origin in the shoot apical meristem (SAM), from which leaf primordia are separated as lateral organs. Besides, meristematic cells are also present in inflorescence primordia and axillary buds that are also separated laterally from SAM and in root meristems. It is therefore hypothesized that the pea mutant genotypes differing in leafblade architecture must differ in whole plant architecture.

The above hypotheses prompted addressing of the following question: Whether in pea the altered leaf blade morphologies of single mutant and mutant combination genotypes affect plant development features or architecture. Another question asked was: which of the mutation(s) altered leaf blade morphology(ies) are suitable for incorporation in cultivar breeding programmes? To answer these questions the organ- wise distribution of the biomass was studied in the 16 genotypes possessing distinct leaf blades morphologies. Leaf blades and genetic architectures were indeed observed to impact plant architecture. Some leaf form mutations were identified to be valuable germplasm for evolvement of cultivars for high grain yield and /or herbage.

Materials and Methods

Plant Genetic Resource

The genotypes studied are listed in Table 1. The homozygous genetic constitution of the lines, their stipule blade and leaf blade phenotypes and information about their origin are summarized. The leaf blade phenotypes of the 16 combinatorial genotypes of the wild type and mutant alleles of the *MFP, AF, TL* and *UNI* genes are shown in Fig. 1. The source of mutant alleles of the *MFP, AF, TL* and *UNI* genes is also mentioned. The genotypes analyzed had origin in crosses between related lines and share much of their genetic background.

Growth Conditions

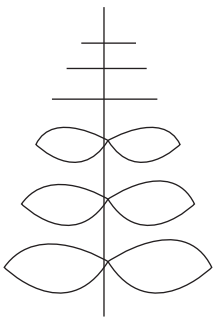
The genotypes were characterized for their leaf related features by growing them in the field plot of the experimental farm of the Institute at New Delhi during the winter season (October to March /April) of the years 2004 to 2008. The soil type of the field plot was sand loam and it was solarized before use. The crop cultivation and seed preservation conditions were the same as described earlier [4-6]. Plant architecture of different genotypes was studied during 2007-2008 season. Ten seeds of each of the 16 genotypes were sown line-wise in completely randomized design with five replications.

Observation and Methodology

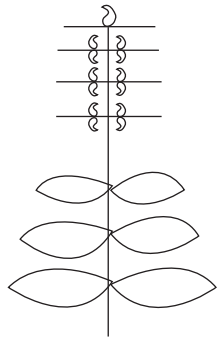
Quantitative measurements were taken on 14-weeks-old plants, two weeks before the crop harvest time. Three plants per replication per genotype were uprooted as samples. After recording the numbers of all nodes (I), inflorescences (J) and pods (K) on individual sample plants, the sampled plants of each genotype were pooled replication-wise. Stipules (A), leaf blades (B), stems (D), roots (E) and pods (F) were separated and dried for each of the 80 pools. The material to be dried kept in paper bags was exposed to 80^0C for 2h and later shifted to 37^0C incubators for drying. All 400 samples were weighed. After recording the dry weights, pods were shelled to record seed weights (G). The dry weight of leaves (C) was obtained at as C = A + B. The whole plant dry matter (C + D + E + F = H), stipules index (A/C X 100), inflorescence frequency or fertility index (J/I X 100), stem growth index (D/H X 100), leaf growth index (C/H X 100) and seed growth index (G/H X 100) were estimated from the primary data. The data were statistically analyzed to obtain genotype-wise estimates of mean and variation for each of the quantitative trait studied.

Results

The genotype-wise primary observations, on biomass in whole leaves (and in stipules and leaf blades, separately), stem, root, whole pods and seeds and number of nodes, inflorescence and pods and calculated percent (%) biomass of whole leaves in stipules and percent distribution of biomass in major organs are given in Table 2. Whole plant biomass was 23.1g when all genotypes were considered. The relative distributions of this biomass among root, stem, whole leaves, empty pods and their pedicels and seeds were 0.9, 24.3, 33.1, 15.3 and 26.2%, respectively. The corresponding biomass respectively of stipules and leafblades was 9.0



MFP UNI AF TL



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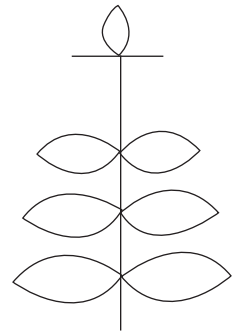
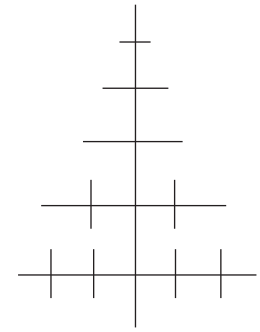
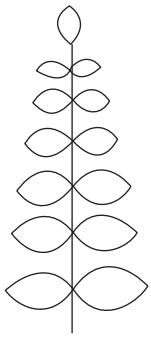


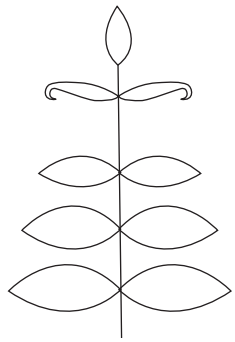
MFP uni-tac AF TL



MFP UNI af TL



MFP UNI AF tl



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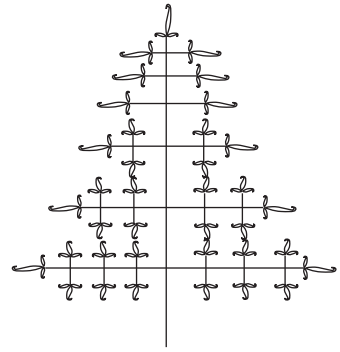
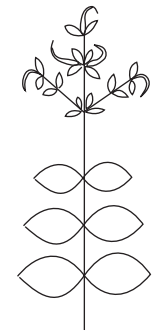


mfp UNI af TL



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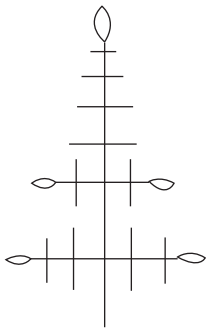

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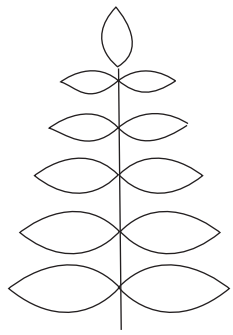

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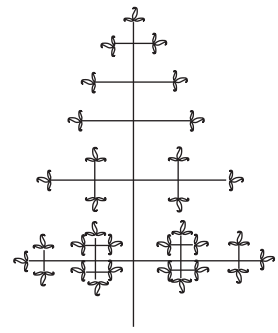

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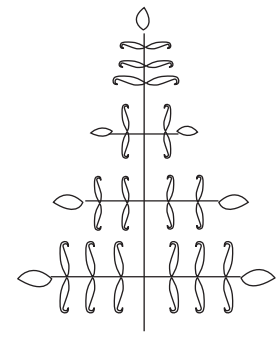

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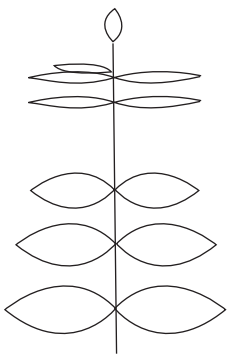

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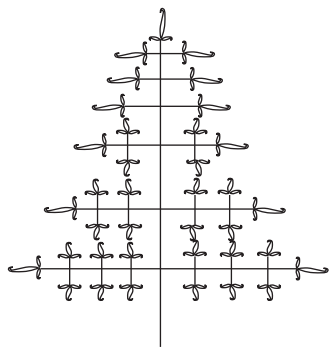

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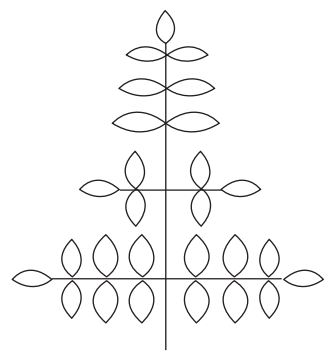

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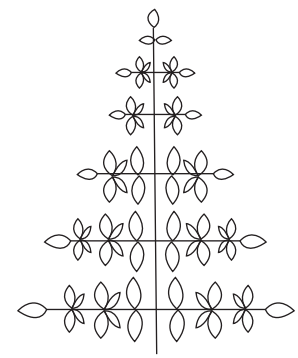

mfp uni-tac af tl

Fig 1: *Diagrammatic representation of leaf blade morphologies of combinatorial genotypes of the wild type and mutant alleles of MULTIFOLIATE-PINNA (MFP), UNIFOLIATA (UNI), AFILA (AF) and TENDRIL-LESS (TL) genes in grain pea Pisum sativum. The phenotypes are briefly described in Table 1.*

Table 2. Effect of leaf blade mutations on biomass partitioning among organs in mature grain pea *Pisum sativum*

S.No	Genotype				Biomass (g) in								Stipule index (A/C x 100)	Number of			Biomass partitioning index		
	MFP	*AF*	*TL*	*UNI*	Stipules (A)	Leafblades (B)	Leaves (A+B=C)	Stem (D)	Root (E)	Pods (F)	Seeds (G) (G)	Whole plant (C+D+E +F=H)		Nodes (I)	Inflores-cence(J)	Pods (K)	Leaf growth index (C/Hx 100)	Stem growth index (D/Hx 100)	Seed growth index (G/Hx 100)
1	+[a]	+	+	+	2.2±0.8	5.1±1.9	7.3±2.6	6.8±3.3	0.21±0.04	13.2±4.5	9.7±3.3	27.5±10.0	30±1	63±29	26±12	35±13	26.2±0.4	23.9±4.7	35.8±1.9
2	–[b]	+	+	+	1.7±0.2	4.5±0.3	6.2±0.3	4.9±0.8	0.16±0.02	10.0±2.6	5.3±2.4	21.3±2.3	30±4	37±7	15±3	23±4	29.3±2.6	23.4±5.3	23.9±9.3
3	+	–	+	+	0.7±0.2	2.2±0.5	2.9±0.7	1.4±0.4	0.11±0.02	3.9±0.1	2.7±0.3	8.4±1.2	26±1	35±4	7±1	8±2	34.2±3.4	16.7±2.3	33.0±4.5
4	+	+	–	+	6.7±2.1	16.6±4.2	23.3±6.2	17.4±4.3	0.60±0.15	34.7±10.0	24.0±7.6	76.5±18.3	29±2	129±31	42±8	68±17	30.8±4.0	23.3±1.8	31.2±5.6
5	+	+	+	–	1.8±0.5	4.1±0.6	5.9±1.0	5.8±1.3	0.21±0.06	9.8±4.3	6.3±3.2	21.7±6.5	29±5	48±12	17±6	23±10	27.9±4.6	27.5±3.8	27.6±8.6
6	–	–	+	+	0.5±0.1	1.3±0.3	1.8±0.4	0.7±0.2	0.11±0.03	2.3±0.5	1.8±0.4	4.9±1.1	28±4	33±9	5±2	6±2	36.6±1.5	13.7±2.3	36.4±1.4
7	–	+	–	+	3.5±1.8	9.8±6.0	13.3±7.8	11.4±4.7	0.39±0.11	19.5±10.3	11.5±6.4	44.6±22.6	28±2	90±20	39±17	57±22	29.8±3.4	26.7±3.1	25.3±1.8
8	–	+	+	–	1.0±0.4	2.3±1.0	3.2±1.3	3.2±1.0	0.21±0.03	3.1±2.5	1.5±2.3	9.7±3.3	29±4	46±8	9±3	12±4	34.2±9.1	33.8±6.4	12.9±16.1
9	+	–	–	+	2.0±0.4	11.4±5.3	13.4±5.8	4.5±1.3	0.17±0.03	8.5±3.5	5.7±2.8	26.6±4.3	17±6	47±5	9±2	12±3	48.7±15.1	16.8±3.0	22.5±13.0
10	+	–	+	–	2.4±0.5	3.9±0.6	6.4±0.8	6.4±1.2	0.22±0.03	13.3±3.4	7.6±2.3	26.2±5.0	38±5	60±19	27±7	44±14	24.4±2.3	24.8±3.9	28.7±6.0
11	+	+	–	–	3.2±0.8	7.1±2.6	10.3±3.4	9.2±2.7	0.25±0.06	8.6±4.9	5.8±3.5	28.3±10.4	32±2	50±18	11±6	16±8	37.4±5.7	33.5±3.8	19.0±7.4
12	–	–	–	+	0.9±0.3	5.0±1.4	5.9±1.7	2.1±0.7	0.15±0.03	3.9±1.8	1.4±0.7	12.1±3.5	14±6	43±16	10±5	14±6	49.4±7.4	17.5±1.2	11.6±3.5
13	–	–	+	–	1.5±0.6	3.6±1.1	5.1±1.7	4.5±1.7	0.15±0.06	8.4±4.6	5.5±3.8	18.2±6.4	29±3	42±17	14±7	20±12	29.0±6.2	25.5±8.2	28.8±13.1
14	–	+	–	–	1.9±0.2	4.1±0.3	6.1±0.4	3.9±0.7	0.19±0.02	4.9±0.1	3.1±0.3	15.0±1.2	32±2	47±5	10±2	12±3	40.2±0.9	25.8±2.6	20.4±0.9
15	+	–	–	–	2.5±1.3	5.8±2.7	8.3±3.9	5.2±3.7	0.27±0.09	7.9±6.1	4.3±3.5	21.7±13.1	30±5	53±25	15±10	18±13	41.9±11.4	23.6±5.6	18.5±10.2
16	–	–	–	–	0.9±0.1	2.1±0.3	2.9±0.5	2.5±0.4	0.07±0.02	1.4±0.2	0.8±0.1	6.9±0.9	30±1	43±11	4±1	4±1	42.4±3.3	35.9±2.8	12.3±1.5
	Mean				**2.08**	**5.55**	**7.64**	**5.61**	**0.21**	**9.60**	**6.06**	**23.10**	**28.20**	**54.12**	**16.25**	**22.80**	**35.15**	**24.52**	**24.24**

a= +, wild type allele; b= –, mutant allele

and 24.1%. The range of inter-genotype differences for biomass allocated to stipules, leafblades and whole leaves (stipules + leaf blades), root, stem, pods and seeds and node, inflorescence, pod number and total biomass were about 13-, 9-, 26-, 25-, 30-, 4-, 10-, 17- and 16-fold, respectively. Simultaneous evaluation of all the studied features has revealed uniqueness in the allometry (biomass distribution among organs) of each of the 16 genotypes, suggesting that the leaf structure related functions affected plant architecture. The inter-genotype comparisons showed gene effects on the whole plant growth and allocation of total biomass among specific plant organs.

Study of inter-relationships between certain organ parameters among 16 genotypes have revealed some features of interaction between *MFP, AF, TL* and *UNI* genes in plant growth and development. The inter-relationships between the stipule biomass and leaf blade biomass in 16 genotypes are shown in Fig. 2A. Generally, the genotypes displayed a near linear relationship or interdependence between the biomass of two sub-components of pea leaf. The two genotypes that deviated from this trend were *MFP af tl UNI* and *mfp af tl UNI*. In these genotypes the leaf blade biomass by stipule biomass ratio was about 80% higher, ≥5.5 in comparison with ≤3.1 for all other genotypes. These observations indicated that leaf blade growth in the absence of AF and TL functions, presence of UNI function and presence or absence of MFP function suppressed stipule growth.

The co-relationships between leaf biomass and inflorescence frequency/fertility index depicted in Fig. 2B showed a broad distribution. Both the parameters of plant growth and development were expressed at high levels in *MFP AF tl UNI* and *mfp AF tl UNI* genotypes. This observation indicated suppressive effect of MFP and TL functions on leaf growth/biomass and inflorescences formation on post-flowering nodes. High inflorescence frequency despite average level of leaf biomass in *MFP AF TL UNI, mfp AF TL UNI, MFP AF TL uni-tac, MFP af TL uni-tac*, and *mfp af TL uni-tac* genotypes indicated that TL function mediated high inflorescence frequency in these genotypes. Two pathways of gene interactions appear to be reflected by these observations. There must be a TL-dependent pathway for high fertility associated with average growth in leaf biomass. Additionally, a TL-independent pathway is expected, which expresses in presence or absence of MFP function but requires AF and UNI functions, that permits both hyper-increased leaf biomass and inflorescence formation.

The leaf biomass is plotted against seed biomass for 16 genotypes in Fig. 2C. The *MFP AF tl UNI* and *mfp AF tl UNI* are seen as elite genotypes in that their plants were richer in leaf biomass and seed biomass than all other genotypes. Correlation of total biomass and seed growth index in all the genotypes are plotted in the Fig. 2D. Here too, positions of *MFP AF tl UNI* and *mfp AF tl UNI* genotypes demonstrate that their plants produce more biomass than plants of all other genotypes and allocate 25 to 30% of it to seeds. The *MFP AF TL UNI* genotype emerged as the most efficient in the allocation of total biomass to the seeds as its plant allocated greater than 35% of total biomass to the seeds. The above idea of TL-dependent and TL-independent pathways for biomass growth and partitioning in organs is supported by observations depicted in the Figs. 2C and 2D. While the TL-dependent pathway is low in biomass production/accumulation, it is expansive in biomass partitioning to seeds. Contrastingly, the TL-independent pathway is prolific in biomass production, but conservative in the allocation of biomass to seeds. The results presented above also showed that the allocation of biomass to seeds was improved when the TL-independent pathway conceived above functioned in the absence of MFP function.

Some more features of *MFP, AF, TL* and *UNI* gene effects were revealed by inter-genotype comparisons of parameters given in Table 2. The biomass accumulated in all the organs of *tl* single mutation plant was more than that in plants of each of 14 genotypes, except in the double mutant *mfp AF tl UNI* which carried *tl* mutation. The results suggested that *tl* increased the biomass allocations, or overall sizes (bins), of all the organs studied. The *MFP af TL UNI, mfp af TL UNI, mfp AF TL uni-tac, mfp af tl UNI, mfp AF tl uni-tac* and *mfp af tl uni-tac* genotypes accumulated whole plant biomass less than wild type. Among these, in *MFP af TL UNI, mfp af TL UNI* and *mfp af tl uni-tac* genotypes, biomass was less than in wild type in all the organs. These results suggested that *af* mutation singly and in combination with *mfp, mfp tl, mfp uni-tac* or *mfp tl uni-tac* mutations decreased the overall mass of all the organs. It is noteworthy that the relative allocations of total biomass to different organs in *tl* mutant (*MFP AF tl UNI* genotype) were like in wild type. By contrast, the biomass allocation among organs were disproportionate to these in wild type in the *mfp AF TL UNI, mfp af TL UNI* and *mfp af tl uni-tac* genotypes.

The *mfp* mutation (in *mfp AF TL UNI* genotype) reduced the allocation of biomass to seeds and increased it for leaves. Among the other genotypes carrying *mfp* mutation, decrease in allocation of biomass to seeds was related to increase in allocation to leaves (as in the plants of *mfp af tl UNI)* or both stem and leaves (exemplified by *mfp AF TL uni-tac* and *mfp AF tl uni-tac* double and triple mutant genotypes). An extreme example was the quadruple mutant genotype *mfp af tl uni-tac* in which

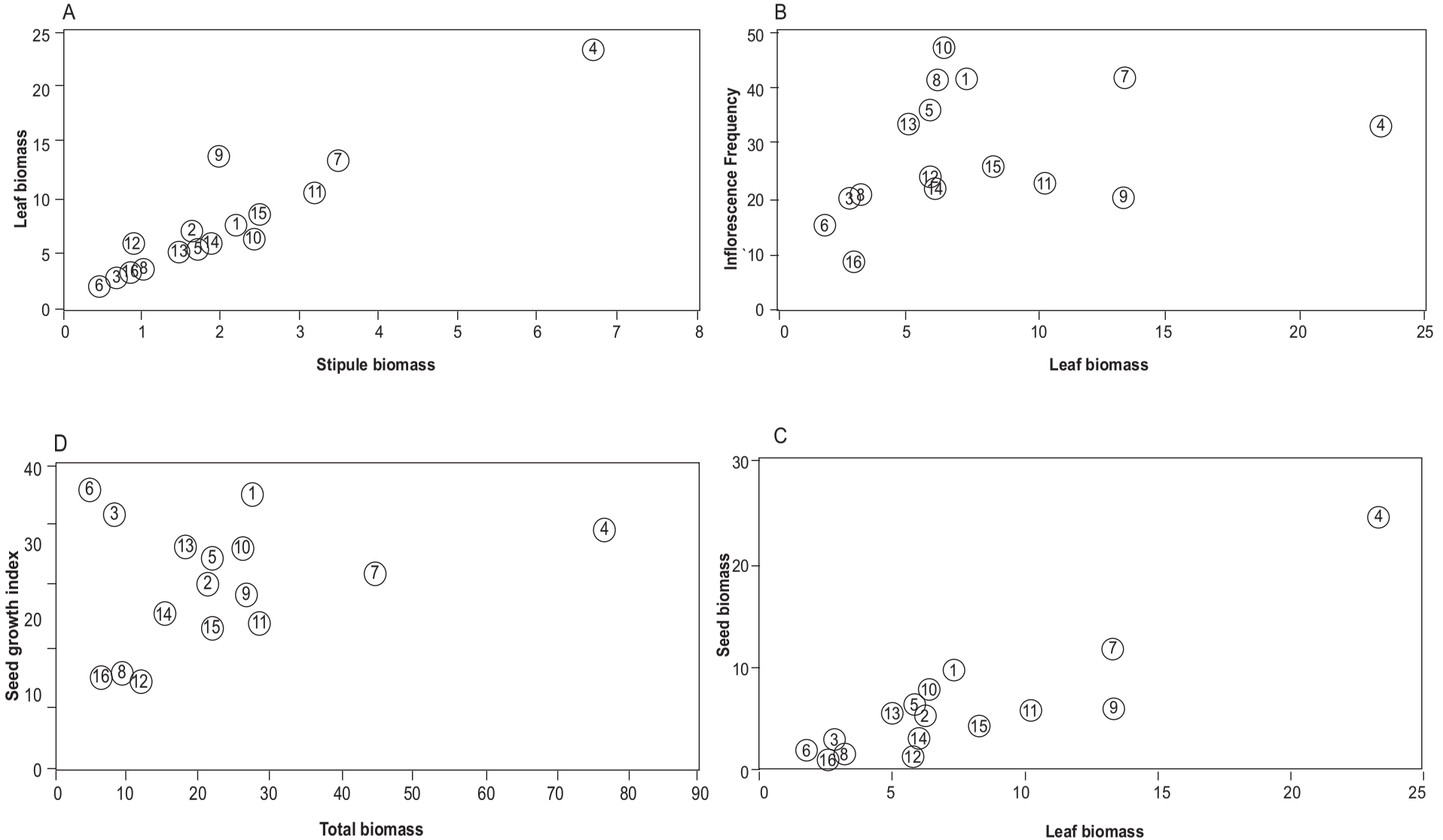

Fig 2: *Relationships between stipule biomass and leaf biomass (A), leaf biomass and inflorescence frequency (B), leaf biomass and seed biomass (C) and total biomass and seed growth index (D) among 16 combinatorial genotypes of wild type and mutant alleles of MFP, AF, TL and UNI genes listed in Table 1. The numbers within circles are the genotype numbers as given in the Table 1.*

12.3% of total biomass was in seeds and 78.3% in leaves and stem, as compared to 35.8% in seeds and 50.5% in leaves and stem in wild type. These results indicate that *mfp* mutation increases the overall sizes of leaf and stem biomass at the expense of allocation of biomass to seeds. It should be noted that in the genotypes identified above, presence of *tl* mutation did not overcome the impact of *mfp* mutation thereby leading to shrunken seed biomass. The total biomass accumulated in *af* single mutant plant (*MFP af TL UNI* genotype) was about 2 fold less than that in wild type. In this genotype, the accumulation of biomass in the seeds was also similarly affected. About three fold less biomass was accumulated in the *mfp af TL UNI* double mutant plant. The *tl* and *uni-tac* mutations somewhat rescued the effect of *af* mutation or *af mfp* mutations, as seen in the *MFP af tl UNI, MFP af TL uni-tac, MFP af tl uni-tac, mfp af tl UNI* and *mfp af TL uni-tac* double and triple mutants genotypes. The total biomass in the quadruple mutant *mfp af tl uni-tac* was about 2.3 fold less than that in wild type. In the *MFP af TL UNI* and *mfp af TL UNI* plants leaves were partitioned more biomass at the expense of stem and in *mfp af tl uni-tac* plants, stems and leaves were partitioned more biomass at the expense of seeds. The above results reveal that AF and MFP functions are necessary for the optimal transfer of biomass adding metabolites from vegetative parts to seeds.

Discussion

Relationship between Leafblade Morphology and Biomass Partitioning

Present study showed co-variation in leaf blade structure, total biomass and in allocations of biomass to root, stem, leaves and seeds. These results support the idea that *MFP, AF, TL* and *UNI-TAC* determine leaf blade and whole plant architectures. It appears that the observed inter-relationships between leaf blade structure and plant allometry can be understood at least partly in terms of the role of shoot apical meristem (SAM) in growing of stem and lateral appendages.

More biomass than in wild type was accumulated in the entire root system, whole of stem, all of leaves and in seeds, in the *MFP AF tl UNI-TAC* and *mfp AF tl UNI-TAC* genotypes. Since plant growth continues until meristematic cells of root and shoot get consumed in the latest organs produced by them, the relative abundance of meristematic cells in shoot and root meristems is expected to produce plants of larger size in these genotypes. Organs had more biomass on account of their larger cumulative size.

The *MFP af TL UNI-TAC, mfp af TL UNI-TAC* and *mfp af tl uni-tac* plants accumulated less biomass in their root, leaf and stem systems and seed produce than wild type. Although the amount of total biomass was similar in plants of these genotypes, the pattern of allocation to organs was different; it apparently reflected the ontogenic, structural and/or functional relationships between the organs. The *MFP af TL UNI-TAC* and *mfp af TL UNI-TAC* plants bore highly ramified leaf blades. In such leaf blades, pinna subprimordia undergo subdivision with each cycle of rachis branching. To meet the demands of daughter subprimordia, the pinna subprimordium should have a larger supply of meristematic cells to begin with. The over-supply of meristematic cells to lateral organs will be at the expense of stem growth. The consequences will be pleiotropic - smaller plant with more biomass in leaves than in stem. These plants accumulated biomass in seeds in the same proportion as that in wild type, despite lower inflorescence frequency. The pleiotropy therefore included enlarged sink for biomass in seeds. The *mfp af tl uni-tac* quadruple mutant exemplified highly pronounced deficiency in inflorescence frequency. This characteristic decreased the allocation of biomass to seeds, and increased that to leaves and stem. The major leaf blade structural and plant allometric differences between the genotypes could thus be consistently explained in terms of relative differences in the allocation of meristematic cells for initiation of lateral organs and degree of proliferation of meristematic cells retained in SAM.

In a recent report on the relative biomass allocations to vegetative organs of pea plant, it has been shown that in comparison to wild type, *af tl* accumulated more biomass in leaves than in stem and *af* accumulated more biomass in leaves than in root [40]. Although in present study, allocation to seeds has also been considered, the earlier observations that *af* and *af tl* mutants allocate more biomass to leaves than in wild type is confirmed.

Mechanism of Variant Biomass Partitioning

The differential partitioning of biomass to both vegetative and reproductive organs in mutant genotypes may be related to alterations in the (a) shoot and root meristematic activity, and (b) metabolic properties of leafblades. The two mechanisms may jointly produce the observed variation.

a) To drive its ontogenic development, the meristematic stem cells in pea plant are expected to be located in the vegetative root and shoot organs and in embryos in the post-reproduction organs. The meristematic cells are maintained in SAM and some from this population serve as founders of all stipules, leaf blades and inflorescences. Likewise, the meristematic cells present in the root apical meristem (RAM) are the progenitors of the entire root system. The pea plant development must be impacted by the degree of cell proliferation in the meristematic

regions of SAM and stipule, leaf blade and inflorescence primordia and their sub-primordia, sizes of the meristematic regions founded for stipules, leaf blades and inflorescences, corresponding meristematic activity for the growth of root system and proliferation capacity of the meristems initiated in the seed embryonic growth. The observed allometric changes in the mutants imply that the genes *MFP, AF* and *TL* hitherto known to have leaf-specific functions also function in SAM. It was already known that *UNI* is involved in inflorescence and flower development. The new observation permits the suggestion that *MFP, AF, TL* and *UNI* determine number and size of lateral organs via effect on meristem size. A detailed genetic analysis of meristem dynamics in SAM of *Arabidopsis thaliana* has revealed that a large number of genes, that specify a variety of TFs on the one hand and proteins/peptides on the other hand, govern the size of meristem and allocation of meristematic cells to leaves and inflorescences [38, 41-44]. The *A. thaliana* mutants in some of these genes, such as *WUSCHEL* (*WUS*), *CLAVATA* (*CLV1*), *CURLY LEAF* (*CLY*) and *DAWDLE* (*DDL*) mimick the properties of pea leaf blade mutants of the present study. The *cly* mutants have brief leaf lamina because of deficiency in cell proliferation and elongation during leaf development [45]. SAM is enlarged in *clv1* mutant [46]. The *wus* mutants have smaller meristem and are unable to proliferate meristematic cells to the same level as that in *WUS* plants [47, 48]. In *ddl* mutant the root, stem, leaf and seed development is deficient in comparison to *DDL* plants [49]. In all the above mutants wherein homozygotes survive to produce seeds, the mutations affect meristematic activity such that the numbers and sizes of lateral organs are altered. By analogy it appears that in pea *tl* and *mfp* mutant forms of *TL* and *MFP* genes and *UNI* gene increase the proliferation of meristematic cells and *AF* gene optimizes the meristematic activity for the balanced growth of leaf blade and whole plant. Future experiments that compare the gene expression patterns of genotypes for the growing SAM, lateral primordia and embryos, will be helpful in definition of the interactions of *MFP, AF, TL* and *UNI* genes in the development of pea plant architecture.

b) Biomass is a measure of vigour of each plant organ [40, 50, 51]. In the present experiment, the total biomass of a pea organ is the product of its number and average biomass or size. The size of an organ is reflected in its growth, which is dependent on several growth determining factors such as photosynthetic efficiency, retention of locally synthesized metabolites, capacity to serve as sink for acquiring metabolites in transport from other organs and morphology. Although, all green organs of plant, including leaves, stem, pedicel, calyx, pod and seeds photosynthesize, but leaves constitute the principal photosynthesizing organs. It is presumed that other organs that fail to meet their requirements for metabolites from local photosynthesis will depend on leaves to fulfill the deficit. The net photosynthesis in pea leaves is a sum of that which occurs in stipules, petiole, leaflets, rachis and tendrils. Photosynthetic activity in organs with adaxial-abaxial differentiation (stipules and leaflets) is greater than in petiole, rachis and tendrils, the organs of radial symmetry; experimentally too is observed to be much higher in *MFP AF tl UNI* leaf blades than in *MFP af TL UNI* mutant leaf blades (unpublished observations). The relative photosynthetic efficiencies of different organs in wild type and various mutants will help in the assessment of relative contributions of metabolites by different organs in the pool available for the expression of growth in all organs. There were considerable differences in the leaf sizes of wild type and mutant genotypes. The average leaf size (leaf biomass/node number) among the 16 genotypes varied between 55 to 206 mg. The genotypes could be arranged in the following increasing order for leaf size: *mfp af TL UNI, mfp AF TL uni-tac* and *mfp aftl uni-tac* (≥80 mg) < *MFP af TL UNI, MFP af tl UNI* and *MFP af TL uni-tac* (81-110 mg) < *MFP AF TL UNI, MFP AF TL uni-tac, mfp af tl UNI, mfp AF tl uni-tac* and *mfp af TL uni-tac* (111-140 mg) < *mfp AF TL UNI, mfp AF tl UNI* and *MFP af tl uni-tac* (141-170 mg) < *MFP AF tl UNI* and *MFP AF tl uni-tac* (≥171 mg). The leaf size was smaller than wild type in mutant genotypes that were missing the AF function and it was usually larger than wild type in mutants missing the TL function. In an earlier study too, where leaf blade sizes had been measured in terms of both weight and surface area, the *MFP af TL UNI* leaf blades were observed to be smaller (408±41mm^2; 34 mg) and *MFP AF tl UNI* of the largest category (3720±385 mm^2; 136 mg) [4]. The interactions between *MFP, AF, TL* and *UNI* functions appeared to be affecting the photosynthetic potential of leaves by determining the size of leaf blade and proportion in it of the non-laminated rachis (petiole + rachis + tendrils) versus laminated leaflet tissues.

The above discussed results allow the conclusion that *MFP, AF, TL* and *UNI* functions affect the plant architecture in at least two ways, by determining the meristem activity in SAM, RAM and primordial and subprimordia of stipules, leafblades and inflorescences and embryos and by determining the patterns and sizes of the leaf blades and thereby photosynthetic potential and metabolite contribution to organs that are dependent or serve as sinks.

Identification of Leafblade Mutant Allele for Evolving Prolific Cultivars

The existing field pea cultivars grown in India or elsewhere are tendrilled, with wild type leaf blades or

afila leaf blades. There are few if any non-tendrilled cultivars of pea in wide cultivation. Present study show that *tl* mutant allele is highly beneficial to pea plant for its vegetative and reproductive growth. The *tl* genotype out yielded all other genotypes in herbage and grain yields while maintaining the wild type biomass partitioning. The herbage and grain yields of *MFP AF tl UNI* were about 2.5 and 2.8 times higher than the corresponding yields of the *MFP AF TL UNI* genotype. This work suggests that the introduction of *tl* allele should be attempted towards the improvement of pea cultivars to evolve dual purpose varieties, with increased vegetative matter and harvest in the pods for vegetable production or for dry seeds. It may be noted that production of tendrils is not a common feature of the crop plants in the family Fabaceae to which pea belongs. The crop plants *Cicer arietinum* (Bengalgram), *Glycine max* (soybean) and *Arachis hypogea* (groundnut) bear pinnately compound leaves bereft of tendrils. Thus, the *tl* varieties of pea may perform well and give high yields like the cultivars of non-tendrilled leguminous grain crop species.

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