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Short Communication

A gamma rays-induced novel fertile subsessile leaf mutant in cowpea [*Vigna unguiculata* (L.) Walp]

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ABSTRACT

Protein-rich legumes contribute to nutritional security by complementing cereals-based diets rich in carbohydrates. In cowpea, an arid legume with a narrow genetic base, we attempted to induce ideotype mutations using gamma rays for augmenting productivity and discovered a novel fertile, subsessile leaf mutant 'PLM211'. Despite the mutant and the parent exhibiting similar individual leaf area and node number, the rudimentary petioles, shortened rachis, little to no branching, and consequently fewer leaves greatly influenced the canopy structure of the mutant. Histological sections of the mutant's 0.55-1.01 cm short petioles showed reduced cell size and number as opposed to the parent's 12.64-14.55 cm long petioles. The potential for uncovering the underlying genes associated with petiole length, leaf morphogenesis, and branching highlights the significance of the mutant as a genetic treasure, opening up avenues for manipulating plant architecture, especially in legumes.

Key words: Cell size, Gamma rays, Ideotype, Mutation, Petiole length.

Pulses or legumes are inevitable components of Indian agriculture, as they primarily cater to the protein requirement of the nation's predominant vegetarian population. Cowpea (*Vigna unguiculata* (L.) Walp), one of the pulses and vegetables, is a major multifaceted legume cultivated in arid and semi-arid regions by farmers with limited resources (Dhanasekar *et al*. 2021). They play a prominent role in subsistence farming and substantially contribute to ensuring nutritional security. The narrow genetic base, prevalent in this crop, severely constrains the genetic improvement to augment its productivity. Amongst the various interventions available, ideotype breeding through induced mutagenesis is a potent tool (Souframanien and Dhanasekar 2023). In agriculture, plant types have been restructured and made more productive by ideotype breeding, which has significantly increased the physiological efficiency of crops (Murchie and Burgess 2022). The plant architecture is greatly influenced by its canopy type. Canopies appropriately articulate the positioning of leaf blades for maximal photosynthetic efficiency with minimal physiological losses (Chavarria *et al*. 2017, Mwanamwenge *et al*. 1997). Petiole length, petiole angle, and branching extent, potentially influence the spatial placement of leaf blades and, subsequently, the canopy (Niinemets and Fleck 2002). Besides, the petioles also take part in the movement of photosynthates, nutrients, and water, among other aspects (Huang *et al*. 2005). The knowledge of the genes governing petiole length becomes valuable for ideotype breeding and gaining insights into the process of leaf morphogenesis. The variability for petiole length in cowpea is limited or largely unexplored, and most leaf trait mutants are sterile. Therefore, the discovery of a fertile, subsessile leaf mutant in cowpea assumes significance.

The cowpea variety 'TC-901' was irradiated with 250 Gy of ^{60}Co-sourced gamma rays at a dose rate of 90 Gy/m in a gamma chamber GC5000. The M_1 population, entailing 1060 plants, was field grown in Kharif 2019 at the Bhabha Atomic Research Centre's Experimental Field Facility in Trombay, Mumbai. Progeny rows of the M_2 population, comprising about 22,365 plants, were raised during the summer of 2020. We identified a fertile, subsessile leaf mutant 'PLM211' with rudimentary petioles, established its true breeding nature in subsequent generations (M_3 to M_5), and evaluated the mutant (M) along with its parent (P) for various agronomical traits. For histological analysis, the middle part of the petioles at the fourth node, counted from the cotyledonary node, were collected from the 'M' and the 'P' plants. Longitudinal sections of the epidermis were obtained with a fine surgical blade, stained with 1%

safranin/1% iodine, observed, and photographed with a compound microscope (Quasmo-Digi Elite) at 100X magnification. Longitudinal lengths of the petiole epidermal cells were measured using Fiji (ImageJ) software (Schindelin *et al*. 2012). When necessary, a two-tailed Student's t-test was used to determine the statistical significance between means.

The subsessile leaf mutant exhibited extremely short petioles, with indeterminate plant type and little to no branching (Fig.1a,1b,1c). Table 1 summarizes the various morphological and anatomical features of the parent and the mutant. The observations on the bottom leaves (first to fourth from the cotyledonary leaves) showed that the petiole length of the wild type (12.64 cm) was almost 12.5 times that of the mutant (1.01 cm), widening to about 26.5 times in the upper leaves (14.55 cm vs. 0.55 cm). There were minor variations in the leaf area of different leaflets of the bottom leaves, albeit the total leaf area remained almost the same in both the parent (140.23 cm^2) and the mutant (140.82 cm^2). The marginal increase in the terminal leaflet area (57.92 cm^2 vs. 52.06 cm^2) offsets a similar decrease in the left lateral leaflet area (41.21 cm^2 vs. 46.85 cm^2) in the bottom leaves of the mutant. There were no discernible differences in the right lateral leaflet area in both cases (41.32 cm^2 in P vs. 41.68 cm^2 in M). The rachis of the mutant (0.47 cm) was about one-fifth that of the parent (2.61 cm) (Fig. 1c), and it also maintained the status quo in the upper leaves. The larger dimensions (l=12.17cm; b=7.85 cm) reflected the larger areas of the terminal leaflets of the mutant as compared to its parent (l=10.91 cm; b=7.50 cm). In the upper leaves, the scenario was contrasting. The total leaf area (+32.1%) and individual leaflet areas (+11.9 to +50.8%) were significantly larger in the wild type than in the mutant. Wild-type plants exhibited higher dimensions of the leaflets, except for the lengths of the terminal leaflets. Accordingly, mutants possessed longer (+4.3%) terminal leaflets than the parent. The leaves of the mutants were less green than the parents (Fig.1a). To this effect, the wild type contained significantly (α= 0.05) more chlorophyll a (M=1.20 mg/g), more chlorophyll b (M=0.39 mg/g), and more carotenoids (M=0.40 mg/g) than the mutant [t(10)=5.00, p<.001; t(10)=4.31, p=.002; t(10)=3.37, p=.007, respectively]. Preliminary histological studies ascribed the difference in the petiole lengths of the parent and the mutant to a smaller cell size and a lesser cell number (Table 1, Fig. 1d, 1e). There was a significant difference in the number of branches in the parent (3 to 6) compared to the mutant (0 to 2). Both the wild type and the mutant possessed the same number of nodes (mean=~15), while the parent had a greater number of leaves (mean=33) than the mutant (mean=21).

Plant architecture plays a determinative role in the plastic responses of plants to varying environmental stimuli. Petiole length is one of the prime factors affecting the canopy type and, thereby, the architecture. Calibrated tweaking of the petiole length enables the plant to orient the leaf lamina suitably for maximizing photosynthetic efficiency. It also contributes equally to minimizing the physiological losses and shading of the bottom leaves. Similar to any other developmental phenomenon, cellular processes like cell division and elongation control the petiole length (Weijschedé *et al*. 2008). Establishing the identity of the genes governing petiole length enables us to manipulate their expression to our benefit. In this regard, the novel fertile, subsessile leaf mutant identified in the present study is a valuable genetic treasure in legumes for conducting basic and applied research.

Novel mutations associated with leaf traits are usually sterile (Olasupo *et al*. 2018), and the uniqueness is unavailable for all practical purposes. Therefore, the mutant in the present study is unconventionally unique. Nonpetiolate or sessile leaf mutants reported previously in cowpea arose spontaneously in explicit crosses or through induced mutagenesis. They were, in contrast, ovate or orbicular shaped, unifoliate, and with malformed flowers (Olasupo *et al*. 2018, Rawal *et al*. 1976, Fawole 1988). Fawole (2001) identified two nonpetiolate and unifoliate leaf mutants in cowpea with ovate or orbiculate leaf morphologies. The nonpetiolate and unifoliate traits were each controlled by nonallelic single recessive genes (*pt-1* and *pt-3* for the former and *un-2* and *un-3* for latter) in the mutants. In the orbicular leaf shape mutant, the genes *pt-1*, *un-2* and the *orb* gene conditioning the leaf shape were found linked on the same chromosome, while the genes *pt-3* and *un-3* were linked on a different chromosome in the other mutant. These nonpetiolate genes *pt-1* and *pt-3* were also nonallelic to a nonpetiolate gene *pt-2* previously reported in a unifoliate cowpea mutant (Rawal *et al*. 1976). In other legumes, like soybean, single [*lps1* (Jun *et al.* 2009), *lps3* (Kilen 1983)] or two recessive genes [*spwp1, spwp2* (Kong *et al*. 2022); *dsp1, dsp2* (Liu *et al*. 2019)] with complete dominance or single gene with incomplete dominance (Cary and Nickell 1999) are known to control short petiole trait. In *Arabidopsis*, the petiole length was

Table 1. Data on various morphological and anatomical traits in the parent 'TC-901' and the cowpea mutant 'PLM211'

Traits	Bottom leaves				Upper leaves			
	Parent (TC-901)		Mutant (PLM211)		Parent (TC-901)		Mutant (PLM211)	
	Value	SE	Value	SE	Value	SE	Value	SE
RL (cm^2)	46.85	2.64	41.21	4.31	48.61	3.58	32.23	2.60
TL (cm^2)	52.06	1.72	57.92	1.38	54.90	2.94	49.05	5.84
LL (cm^2)	41.32	3.46	41.68	1.37	47.56	3.20	33.09	3.43
TLA (cm^2)	140.23	5.43	140.82	4.47	151.07	8.70	114.37	11.15
PL (cm)	12.64	0.77	1.01	0.09	14.55	1.03	0.55	0.02
LR (cm)	2.61	0.15	0.47	0.01	2.85	0.08	0.55	0.04
RLL (cm)	9.28	0.10	9.19	0.40	9.69	0.40	8.74	0.33
RLB (cm)	6.66	0.28	6.17	0.33	6.96	0.24	5.32	0.24
TLL (cm)	10.91	0.41	12.17	0.13	10.76	0.48	11.22	0.67
TLB (cm)	7.50	0.23	7.85	0.29	7.80	0.16	6.81	0.35
LLL (cm)	9.26	0.28	9.43	0.16	9.53	0.44	8.72	0.51
LLB (cm)	6.18	0.32	6.15	0.23	6.97	0.23	5.49	0.30

	Parent (TC-901)		Mutant (PLM211)		t value	p value
	Value	SE	Value	SE		
Chl. a (mg/g)**	1.20	0.06	0.90	0.02	5.00	<.001
Chl. b (mg/g)**	0.39	0.03	0.27	0.01	4.32	.002
Carotenoids (mg/g)**	0.40	0.02	0.33	0.01	3.37	.007
Cell length (μm)**	60.82	2.11	20.10	1.01	19.07	<<.001
Cell number**	2129.32	124.35	808.15	55.13	10.75	<<.001
No. of nodes	15.00	0.16	14.67	0.13	1.48	0.15
No. of branches**	3.83	0.27	1.08	0.21	7.33	<<.001
No. of leaves**	33.00	0.76	21.00	0.70	10.64	<<.001

RL: right lateral leaflet area; TL: terminal leaflet area; LL: left lateral leaflet area; TLA: total leaf area; PL: petiole length; LR: length of rachis; RLL: right lateral leaflet length; RLB: right lateral leaflet breadth; TLL: terminal leaflet length; TLB: terminal leaflet breadth; LLL: left lateral leaflet length; LLB: left lateral leaflet breadth; Chl.a: chlorophyll a content; Chl.b: chlorophyll b content; cell length: average longitudinal length of epidermal cells of petioles; cell number: estimated petiole cell number in a single longitudinal column (derived by dividing the petiole length by the longitudinal cell length); **: significant at 0.01 level of significance; SE: standard error

influenced by genes exclusively controlling cell elongation (*PHYB* and *ACL2*) or in combination with those affecting cell number (*GAI, GA1* and *ROT3*) (Tsukaya *et al*. 2002). Fawole (2001) reported that the unifoliate gene in cowpea had a pleiotropic effect on floral development, resulting in deformed flowers. However, in the current study, the subsessile leaf mutation had no deleterious effects on floral morphology, suggesting the non-existence of pleiotropy between the genes coordinating these traits. In addition, the subsessile leaf mutation did not alter the leaf morphology, substantiating the role of induced mutations in breaking linkages, if any, existing between the genes governing the petiole length and leaf morphology. The terminal leaflets of the cowpea subsessile mutant were longer and attributed to the space-restricted elongation caused by the highly reduced rachis and rudimentary petioles. In contrast to the pinnately trifoliate leaves of the parent, the mutants' drastically shortened leaf rachis further increased the compactness of the plant canopy and made the leaf morphology appear more palmately trifoliate (Fig. 1b). Upper leaves in the wild-type plants, as opposed to the bottom leaves, exhibited longer petioles and larger leaf areas. The larger leaves probably intercepted more sunlight for increased photosynthates production, while the longer petioles facilitated the dynamic placement of upper leaves, minimizing the shading effect on the bottom leaves. But in the mutant, the subsessile nature of the leaves limited its maneuverability. Perhaps, in its pursuit to alleviate the shadowing effects on the bottom leaves, the mutant, as observed in the present study, reduced both the leaf areas and

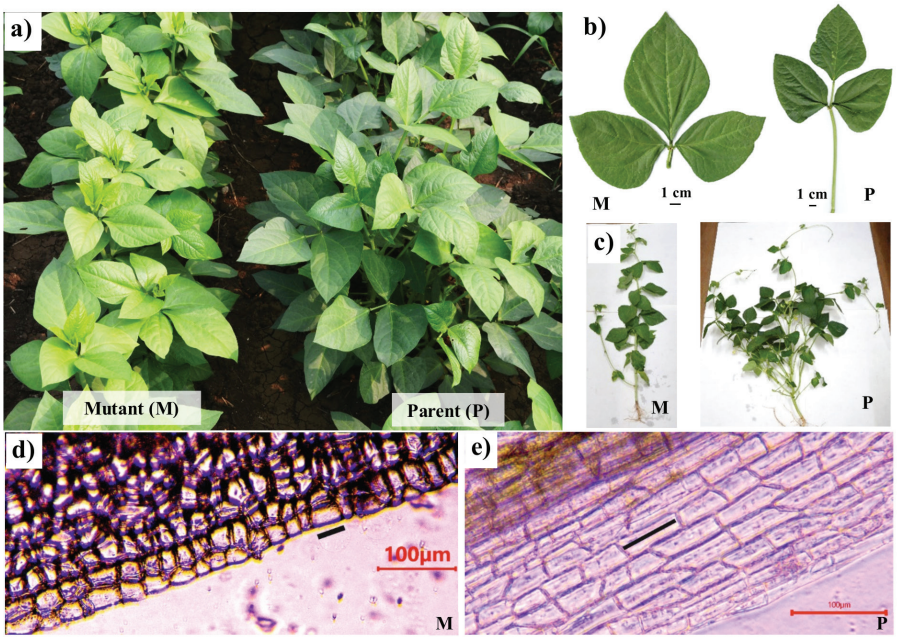

Fig.1. a) 'PLM211' cowpea mutant (M) with light green subsessile leaves, compact canopy and reduced rachis as opposed to parent 'TC-901'(P) with green petiolate leaves, open canopy and normal rachis; b) Subsessile leaf of M vs. long petiolate leaf of P. The scale bars are 1 cm; c) Mono branching in M vs. multiple branching in P; d,e) Longitudinal sections of the middle part of the petioles in M (d) vs. P (e). The scale bars are 100 µm and the black line shows the length of a representative epidermal cell.

petiole lengths of its upper leaves (Table 1). Despite the parent and the mutant having a comparable number of nodes, the greater branching extent in the former elucidates a higher number of leaves than in the latter. The number of branches in the mutant varied from zero to two, and by exploiting the non-branching types, one can gain insights into the genes governing branching. Additionally, it is envisaged that shorter petioles can boost crop productivity by modifying plant architecture and optimizing population canopy structure (Jun and Kang 2012, Liu *et al*. 2020). Under conditions of high-density plantations, the mutant with compact canopies resulting from reduced petioles and rachis can perform better by subduing inter-plant shading. However, this is subject to the actual performance of the mutant in agronomical trials for optimizing the inter- and intra-row spacing. Moreover, the mutant 'PLM-211' with reduced chlorophyll content could also potentially contribute to increasing photosynthetic efficiency and yield as observed in crops like rice and soybean (Wang *et al*. 2022, Gu *et al*. 2017, Slattery *et al*. 2017). In soybean plants with normal chlorophyll levels, one-fourth of the uppermost canopy absorb three-fourths of the incident light, much of which is wasted as a result of light saturation of photosynthesis, while the leaves in the lower canopy remain undersaturated for want of light (Long *et al*. 2006). Therefore, reducing the leaf chlorophyll content and thereby, the leaf absorbance, is envisaged as a strategy to improve photosynthetic light use efficiency by allowing deeper penetration of light into the canopy. This ensures that the leaves in the upper canopy in direct exposure to the sun are not oversaturated and the lower leaves in the shade aren't undersaturated, consequently, improving canopy photosynthesis. The excess of nitrogen and other resources going into the production of pigment-proteins could also be rerouted towards increased biomass production in plants with reduced chlorophyll content (Slattery *et al*. 2017). Thus, as contemplated in rice, reducing leaf chlorophyll content can potentially lead to reduced light absorption, increased PSII efficiency, higher electron transport rate, improved light distribution within the canopy and thereby enhanced leaf photosynthesis and yield (Gu *et al*. 2017).

Mutation breeding can effectively restructure plant ideotypes, and to this effect, the novel subsessile leaf mutant 'PLM211' discovered in cowpea emerges as a valuable genetic resource. The mutant could be used to identify and map underlying genes associated with petiole length, leaf morphogenesis, and branching, potentially

paving the way for genetic manipulation of plant architecture.

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